CURRENT STUDIES IN HEALTH AND LIFE SCIENCES 2023

Editors
Prof. Dr. MEHMET OZASLAN
Dr. MUHAMMAD SAFDAR

ISRES Publishing

Current Studies in Health and Life Sciences 2023

Editors

Prof. Dr. Mehmet OZASLAN

Dr. Muhammad SAFDAR

This book was typeset in 10/12 pt. Times New Roman, Italic, Bold and Bold Italic.

Current Studies in Health and Life Sciences 2023

Published by ISRES Publishing, International Society for Research in Education and Science (ISRES).

Includes bibliographical references and index.

ISBN

978-625-6959-18-7

Date of Issue

December, 2023

E-mail

isrespublishing@gmail.com

Address

Istanbul C. Cengaver S. No 2 Karatay/Konya/TÜRKİYE

www.isres.org

CURRENT STUDIES IN HEALTH AND LIFE SCIENCES 2023

EDITORS

PROF. DR. MEHMET OZASLAN

DR. MUHAMMAD SAFDAR

ISRES Publishing

PREFACE

Welcome to "Current Studies in Health and Life Sciences 2023." This compilation stands as a testament to the ever-evolving landscape of scientific inquiry, offering a panoramic view of the latest advancements, breakthroughs, and critical inquiries in the fields that define our understanding of life and well-being. As we navigate the complex terrain of health, veterinary, and life sciences, this book serves as a comprehensive guide to the forefront of contemporary research.

In the dynamic realm of health and life sciences, the pursuit of knowledge is relentless. Researchers, scholars, and practitioners continually explore new frontiers, pushing the boundaries of our understanding of the human body, the environment, and the intricate interplay between the two. This book encapsulates the spirit of this intellectual journey, presenting a mosaic of studies that illuminate the multifaceted aspects of our existence.

The twenty book chapters written by different authors within this collection span a diverse range of topics, reflecting the interdisciplinary nature of modern scientific exploration. From cutting-edge medical and veterinary interventions to ecological studies that underscore the delicate balance of our ecosystems, each contribution offers a unique perspective on the challenges and opportunities that define the current state of health and life sciences.

In an era marked by unprecedented advancements in technology and data analytics, the methodologies employed by researchers are as varied as the subjects they investigate. As you delve into the pages of this book, you will encounter a tapestry of methodologies, from rigorous clinical trials to innovative computational models, all aimed at unravelling the mysteries of life and health.

The collaborative nature of contemporary research is evident throughout these pages. Many of the studies presented here are the result of interdisciplinary collaboration, highlighting the importance of bringing together diverse expertise to tackle the intricate problems facing humanity. The interconnectedness of health, veterinary, and life sciences is a central theme, emphasising that progress in one area often relies on insights from another.

As we embark on this intellectual journey together, let us celebrate the dedication and ingenuity of the researchers who contribute to the collective knowledge of humanity. "Current Studies in Health and Life Sciences 2023" is more than a compilation of selected papers; it is a testament to the indomitable human spirit that drives us to explore the unknown and seek solutions to the complex challenges that shape our world.

May this book inspire curiosity, spark new ideas, and foster a deeper appreciation for the remarkable strides we are making in understanding and enhancing life in all its forms.

December, 2023

Prof. Dr. Mehmet Ozaslan & **Dr. Muhammad Safdar**

TABLE OF CONTENTS

SECTION I: MEDICAL SCIENCES

SECTION II: LIFE SCIENCES

Section

I

MEDICAL SCIENCES

ANTIMICROBIAL RESISTANCE (AMR): A GLOBAL CHALLENGE IN DISEASE PREVENTION AND CONTROL

Muhammad SAFDAR
Shafeeq ur REHMAN
Minal HUSSAIN
Muhammad Naveed NAWAZ
Muhammad Abdullah MUEEN
Fazal e SUBHANI
Salman NAZIR
Mehmet OZASLAN

Introduction

Antimicrobial resistance (AMR) is the capability of microorganisms such as parasites, fungi, viruses, and bacteria to acclimate and thrive in the occurrence of drugs that formerly adversely impacted them. AMR is seen as a severe hazard to public health systems everywhere, not only in underdeveloped countries (Haque; Karthik et al., 2014). Since infectious illnesses cannot be treated with antibiotics, the future of medical care is unclear. Significant illnesses, long hospital admissions, larger healthcare charges, advanced second-line drug prices, and failure of treatment are all results of AMR infection (Chokshi et al., 2019; Dixit et al., 2019). For example, it is predicted that the yearly cost of antibiotic resistance only in Europe is more than nine billion euros (Dixit et al., 2019; Llor & Bjerrum, 2014). Furthermore, the CDC predicts that antibiotic resistance increases direct healthcare costs in the US by an extra $20 billion per year, not considering the anticipated $35 billion in yield losses (Control & Prevention, 2017).

Antimicrobial resistance poses a scary threat, which is especially important in the context of bacterial drug resistance (Dixit et al., 2019). Antibiotic-resistant infections affect more than two million Americans annually, the CDC estimates and at least 23,000 of them die (Control & Prevention, 2017). The human immune system's ability to combat infectious infections is weakened by antibiotic resistance, which increases the risk of a number of problems in susceptible individuals undergoing surgery, joint replacement, dialysis, and chemotherapy (Control & Prevention, 2017). Patients who suffer from chronic conditions including asthma, rheumatoid arthritis, and diabetes will also be significantly harmed by antibiotic resistance (Haque). Doctors should utilize last-resort classes of medications like carbapenems and polymyxins since the efficacy of antibiotics will be lowered as a result of persistence in patterns of AMR (Organization, 2019; WHO, 2015). Methicillin resistance in *Staphylococcus aureus* (MRSA), one of the most well-known examples of AMR, has been linked to high death rates each year all around the world (Karthik et al., 2014). Additionally, the treatment of several illnesses, including pneumonia and urinary tract infections, has become more challenging due to multi-drug resistance gram-negative bacteria (MDR-GNB) (Bassetti et al., 2019; Ramírez-Castillo et al., 2018).

Numerous antibacterial medicines have been created and commercialized for therapeutic use since penicillin was first introduced in the 1940s, reducing the high rates of human mortality formerly related to bacterial illnesses before the "antibiotic era." However, as soon as reports of resistance started to appear, the preliminary hope that all bacterial illnesses could be effectively administered with these new drugs was immediately dashed (Furuya & Lowy, 2006; Levy, 1994; Livermore, 2003). Studies conducted in the 1950s and 1960s showed that susceptible recipient cells might acquire multiple drug resistance as a result (Salyers & Amabile-Cuevas, 1997). All

known antibiotics that are presently used clinically in human and veterinary medicine have been linked to antimicrobial resistance pathways.

Over the last numerous decades, antimicrobials have significantly enhanced animal output and health in addition to the apparent benefits for people (Adesiyun, 1992; Johnston, 1998; Piddock, 1996). However, its utilization of food animals, whether to promote growth or the avoidance and treatment of infectious disorders, has the potential to breed resistant bacterial pathogens and commensal organisms. Even while a few significant zoonotic Gram-negative bacterial pathogens are still largely vulnerable to the bulk of therapeutic antimicrobials, developing resistance traits have been seen in these pathogens (Gold & Moellering Jr, 1996; Kruse & Sørum, 1994; Salyers & Amabile-Cuevas, 1997; Witte, 1998). For instance, the incidence of human infections caused by multidrug-resistant Salmonella enterica serotype Typhimurium Definitive Type 104 (DT 104) grew from 0.6% in the United States in 1979 to 34% in 1996. The resistance of DT 104 to tetracycline, chloramphenicol, streptomycin, ampicillin, and sulfamethoxazole is one of its distinguishing characteristics (Dechet et al., 2006; Glynn et al., 1998). Sadly, there are still gaps in our understanding of how bacteria might become resistant to antimicrobial treatments and spread in different animal production contexts.

There are just a few ways through which these resistance characteristics are developed, despite the multitude of resistance patterns found in bacteria. Antimicrobial resistance-related genes may be located on chromosomes, where daughter cells inherit them, or they can be horizontally transmitted on mobile DNA elements like plasmids and transposons (Stefani, 2005). Due to bacterial populations' capability to adapt to dangerous circumstances and their capability to exchange DNA, antibiotic resistance is an inevitable biological phenomenon that will likely always be a chronic public health concern. It is essential to manage current antimicrobials efficiently and to continue developing new ones to successfully protect human and animal health against bacterial diseases (Morley et al., 2005). Furthermore, plasmids found in Gram-negative bacteria that were isolated before the widespread use of antibiotics in medical practice were very comparable to newly characterized plasmids, with the difference that the original isolates missed any resistant gene (Datta & Hughes, 1983; Hughes & Datta, 1983).

Recent research has shown that a range of soil-dwelling actinomycetes is resistant to natural antimicrobial agents, synthetic byproducts, and entirely synthetic antimicrobials. A few of these actinomycetes also show resistance traits that are unusual in bacterial infections in humans. Every isolate they looked at, according to the researchers, was resistant to at least six to eight different antimicrobial agents, and in some cases as many as twenty (D'Costa et al., 2006). The concept that resistance is not a recent phenomenon is emphasized by the fact that these organisms have evolved specific resistance mechanisms and that they are not subject to the same intense antimicrobial selection pressures as clinical infections (D'Costa et al., 2006). The discovery of this new resistance gene pool raises concerns about the potential future horizontal transmission of new antimicrobial resistance factors to bacteria, which might have serious effects on both human and animal health (D'Costa et al., 2006).

AMR may lead to treatment failures and the escalation and severity of ordinarily mild illnesses (Lishman et al., 2018). Longer hospital admissions, higher risks for individuals with repeated infections, such as those with cystic fibrosis, and the requirement for more toxic medicines, if previous treatments fail due to resistance, are all consequences of increasing complications. According to a review of studies (Smith

& Coast, 2013) that attempted to determine the costs of AMR per patient episode, the extra expenses ranged from less than $5 to more than $55,000. However, these studies solely calculated the direct expenses of healthcare, such as increased hospital stays (Roope et al., 2019).

Globally, the impact of AMR is still not well quantified. With the remarkable exception of England & Wales (Carter, 2009), no national death registry has yet to document "deaths caused by antimicrobial-resistant illness," hence it is technically impossible for anybody to have ever passed away from it. AMR increases the likelihood of chronic illness or bacterial infection-related mortality in both people and animals (Lambert et al., 2011). The requirement for more complicated and ineffective antimicrobial therapies for illnesses with multiple drug resistance contributes to some of this increased mortality. The issue may be exacerbated by virulence factor genes that travel on mobile genomic elements with AMR genes (Pereira et al., 2015).

1. Genetics and genes involved in AMR

Antibiotic resistance genes can be transmitted from cell to cell by conjugation, transformation, or transduction and are frequently found on plasmids or transposons. Through this gene transfer, the resistance can quickly spread across several bacterial species and within a population of bacteria. We refer to this as horizontal transmission. For doctors treating infectious infections, this kind of antibiotic resistance presents serious challenges.

Salmonella is one of the most common bacteria that cause foodborne illnesses globally, and it has been extensively documented that it is resistant to tetracyclines and sulfonamides. The findings showed that Salmonella had significant rates of resistance to sulfonamide and tetracycline, *tetA* and *tetB* were the genes present in Salmonella species that show resistance to tetracycline. *SulA* and *sulB* are the most frequent genes that present in Salmonella which show resistance to sulfonamides. these genes are associated with plasmid or transposon which transfer to other non-resistant bacteria through conjugation (Pavelquesi et al., 2021).

Staphylococcus aureus is a gram-positive bacteria that has shown resistance to the first antibiotic methicillin. Many of the genes that are present show multiple of drug resistance including:

- Phenicol Exporter Gene *Fexa*,
- Multiple Drug Resistance Gene *Cfr,*
- Resistance To Tetracycline Gene *Tet*(L),
- Trimethoprim Resistance Gene *Dfrk*,
- Macrolide Resistance Gene *Erm*(T),
- lincosamide–streptogramin A–pleuromutilin resistance genes *vga*(C) and *vga*(E
- apramycin resistance gene *apmA*

enterotoxigenic E.coli are the most common species that belongs to enteric fever and have developed resistance against most of the antibiotics *(Pavelquesi et al., 2021)*. Many of antimicrobial resistance genes are present on plasmids and transposons which are further transported to other strains through conjugation. Resistance genes include (Boerlin et al., 2005):

- *tetA* and *tetB*
- *strA* and B

- *sul1* and *sul2*
- *aadA*

Pseudomonas aeroginosa bacterial species that cause cystic fibrosis in humans that was not possible to treat due to the involvement of resistance genes. AmpC β lactamase enzymes that break the action of β-lactam antibiotics and MexXYOprM, a three-protein efflux pump that extracts aminoglycoside drugs from bacterial cells. antibiotic resistance genes are expected to play an important role in antibiotic resistance. Genes involved in resistance are *ampC* and *mexX* (Martin et al., 2018).

Table 1. Genes, resistant drugs and their target microorganisms.

Genes	**Drug**	**Genera**
tet(A),*tet(B)*	Tetracycline	*Pseudomonas, Proteus, Salmonella, Klebsiella, Vibrio, Escherichia, Shigella*
tet(v)	Tetracycline	*Mycobacterium*
*qnr*B9, *qnr*B10 *qnr*B11, *qnr*B12 *qnr*B13	Quinolines	*Citrobacter*
*qnr*B31		*Klebsiella*
*qnr*S1		*Enterobacter, Escherichia, Klebsiella, Proteus, Salmonella,*
aac(6′)-Ib, aac(6′)-Ib-cr	Ciprofloxacin	*Candida*
*dfr*A21	**Trimethoprim**	*Klebsiella, Salmonella*

How antimicrobial resistance developed?

Acquisition of foreign resistance genes, mutations in native cellular genes, or a combination of these two methods can all lead to acquired bacterial antibiotic resistance. The most frequent bacterial resistance mechanisms include.

- Active efflux
- Mutation target site,
- Enzymatic destruction of the antimicrobial
- Reduced cell wall permeability.

The fast development of antibiotic resistance across different species of bacteria of interest to humans and animals has been significantly aided by the transmission of mobile genetic elements including plasmids, transposons, and genetic elements (Harbottle et al., 2006).

Permeability changes in the bacterial cell wall which restricts antimicrobial access to target sites:

- Alteration in metabolic pathways
- Mutation at antibiotic target sites
- Alternate enzymes

All of this resistance is achieved by microorganisms through horizontal gene transfer and continuous mutation in chromosomal DNA and extrachromosomal DNA structure plasmid (Maiden, 1998; Spratt, 1994).Tetracycline was first introduced in 1948. It was

used as a broad-spectrum antibiotic by acting multiple actions. it breaks the bacterial cell wall and inhibits protein production or ribosomal function. Later in 1953, the first resistance to tetracycline was detected in some microbes. Three mechanisms were detected.

- ATP –dependent efflux pump
- Alternative ribosomal protection proteins
- Alternatives enzymes (Levy et al., 1999; Speer et al., 1992).

Streptothricin antibiotics were used against gram-negative bacteria. Later *E.coli* were isolated it were found resistant to the strepothiricin. *Sat2* and *sat4* resistant genes were present on the plasmid they showed resistance to acetylation of the drug by acetyltransferase enzymes (Tschäpe et al., 1984).

2. Clinical signs and symptoms:

There are some important causes of Intra-abdominal infections (IAI) like cholecystitis and cholelithiasis which arise from the biliary tract. The Patient undergoes cholecystectomy due to antibiotic-resistant bacteria in bile (Claesson et al., 1984; Claesson et al., 1986; Csendes et al., 1994; Galili et al., 2008). Different bacteria have been identified in patients who suffered Acute cholecystitis (AC). Acute cholecystitis is swelling of the gallbladder. The main symptom of acute cholecystitis is a sudden sharp pain in the upper right side of your abdomen that spreads towards your right shoulder (Fuks et al., 2013).

Patients before empiric antimicrobial therapy have clinical signs and symptoms like sepsis or severe sepsis or septic shock. Septic shock is a life-threatening condition that happens when your blood pressure drops to a dangerously low level after an infection. Any type of bacteria can cause an infection. At first, the infection can lead to a reaction called sepsis (Castellanos-Ortega et al., 2010; Ferrer et al., 2009; Puskarich et al., 2011). Due to a vast variety of different pathogens more specifically antibiotic-resistant pathogens, patients with IAI suffer from uncomplicated appendicitis to fecal peritonitis. Its common symptoms include bloating, diarrhea, nausea and vomiting, fever, loss of appetite, abdominal pain, fatigue, confusion, thirst, and little urination. Due to infection, some people feel chills and fluid in the abdomen (Menichetti & Sganga, 2009).

Methicillin-resistant Staphylococcus Aureus (MRSA) causes so many infections in humans due to mecA gene which is resistant to beta-lactam antibiotics (Shahkarami et al., 2014). MRSA causes Skin and Soft tissue infections (SSTI) in a more invasive way than non-MRSA due to multidrug resistance. This infection clinically leads to cellulitis, Necrotizing fasciitis, and diabetic foot ulcers (Khan et al., 2018). Infection caused by MRSA shows other clinical symptoms like bacteremia, endocarditis, chemical burns, impetigo, juvenile idiopathic arthritis, Kawasaki disease, leptospirosis, pediatric bacterial endocarditis, and pediac osteomyelitis. MRSA is the main cause of necrotizing pneumonia which is characterized by severe respiratory symptoms, high fever, hemoptysis, and hypotension. The rapid progress of this infection leads to sepsis and septic shock with leucopenia. As MRSA is resistant to Oxicilline, it causes bone and joint infections which are characterized by osteomyelitis of spines and septic arthritis of native and prosthetic joints (Baddour et al., 2015).

Neisseria gonorrhea is related to sexually transmitted infections (STIs) and is resistant to many antimicrobial drugs like Sulfonamides, early-generation Cephalosporin, Penicillin, Tetracycline, Marcolides, and fluoroquinolones (!!! INVALID CITATION !!! (Ohnishi, Golparian et al. 2011, Cámara, Serra et al. 2012, Fifer, Natarajan et al. 2016)). Urogenital gonorrhea leads to urethritis, epididymitis, reduced fertility, and urethral stricture in men. In women, symptoms are not specific and include abnormal veginal discharge, dysuria, lower abdominal discomfort, and dyspareunia (Handsfield et al., 1974). Gonorrhea is also related to pregnancy complications and leads to chorioamnionitis, preterm birth, premature rupture of membrane, ectopic pregnancies, and spontaneous abortion (Arora et al., 2013; Maxwell & Watson, 1992; Tsevat, 2017). If the mother has gonococcal infection then the infant can be infected during delivery, which results in neonatal conjunctivitis. If it remains untreated, the baby will suffer scarring and blindness. Both sexes (males and females) will suffer Extra-genital infections, no matter whether urogenital infection is present or not (Koedijk et al., 2012; van Liere et al., 2014).

3. Involvement of pathogenesis in AMR

Sexually transmitted infection(STI) gonorrhea is still a worldwide health concern that eventually requires more effective medications and international attention (Organization, 2012). *N.gonorrhea* can adapt to antibiotic resistance by altering its genetic material more efficiently and that's why it's known as a competent or transformation/ transfer of half or complete gene during its entire life. Mutation also supports the changing of its genome more effectively. By all these processes, it survives in the often hostile environments in all sites of human hosts. This is a suitable way for gonococcus to develop all physiological mechanisms of AMR against all the antimicrobials used as a treatment (Ashford et al., 1976).

In a hostile environment also in the host body, bacteria deal with environmental stressors and pressures i.e. for nutrients, bacteria want to become superior and also want to cope or resist the attack of different molecules produced by different rival organisms and the immune system of the host. They also want to establish in biological niche. For all these accommodations, bacteria groom themselves by avoiding stressful conditions like the cell synthesis process and membrane homeostasis through the host immune system by incredible mechanism and results in resistance to different molecules i.e some resistant phenotypes of bacteria are resistant to Dopamycin and vancomycin produced by host immune cells (Pogliano et al., 2012).

There are some important causes of Intra-abdominal infections (IAI) like cholecystitis and cholelithiasis which arise from the biliary tract. Patient undergoes cholecystectomy due to antibiotic-resistant bacteria in bile (Claesson et al., 1984; Csendes et al., 1994; Morris-Stiff et al., 2007; Truedson et al., 1983). Due to different antibiotic-resistant strains, this condition ranges from appendicitis to peritonitis. The efficacy of pathogenesis depends upon the antimicrobial resistance. If the causative agent of any infection is not resistant to its related antimicrobial then it will be cured easily and the infection can be controlled by the time but due to resistance, the infection reaches its prime condition (Pieracci & Barie, 2007; Sartelli et al., 2014; Truedson et al., 1983). Cholecystitis and cholelithiasis are infections associated with the biliary tract caused by different antibiotic-resistant bacterial strains. Due to this resistance, bacteria oblige patients to undergo cholecystectomy and cause acute cholecystitis. This is how pathogenesis is involved in AMR (Fuks et al., 2013).

Urinary tract infections (UTIs) has a close relation with antimicrobial resistance due to different resistance bacterial strains which oblige patients to deal with invasive and acute infections. just like, a child dealing with pyelonephritis with sepsis and renal damage which can be controlled easily if the causative agent is not resistant. Female UTIs rise to pre-term birth and complications due to frequently using antibiotics against resistant species. if we do not treat it, we will not mitigate the infection. That's why empiric antimicrobial therapy is recommended for the treatment and control of the involvement of resistant bacteria.

As death and incidence rates rise, germs are becoming more resistant to antibiotics in both community and hospital settings. According to estimates, antibiotic-resistant bacteria result in 33,000 fatalities in Europe and 700,000 worldwide each year. In the worst-case scenario, bacterial infections would cause 10 million deaths worldwide year by 2050, surpassing the 1.8 million cancer fatalities. Our capacity to treat common infectious illnesses is under jeopardy due to the growth of bacterial resistance, which can lead to prolonged sickness, disability, and death. Antimicrobial resistance has a negative impact on healthcare expenditures since it leads to longer hospital admissions, which are predicted to add 2.5 million days to hospital stays each year throughout the European Union, costing roughly 900 million euros (Glasner et al., 2013).

After the discovery of cephalosporins in 1968, the research of novel antibiotics with novel modes of action stalled. The majority of the antibiotics created thereafter are derivatives of the current classes and are referred to as "new-generation" antibiotics. Unfortunately, the creation of an antibiotic is always followed, sooner or later, by the appearance of bacterial strains that are resistant to it (Saga & Yamaguchi, 2009). There is a relationship between disease pathogenesis and resistance that depends on the bacterial species, the specific mechanisms of resistance and virulence, the ecological niche and environmental conditions, and the immune system of the host. Although virulence and resistance develop at different times—virulence from the start of host colonization and resistance from the appearance of antibiotics—they are not independent characteristics. Diet, age, and stress, in addition to immunological function, affect a host's vulnerability to infection. Therefore, opportunistic pathogens can infect immunocompromised patients but not healthy people.(Nguyen et al., 2016)

Since severe instances of acne are frequently treated with topical antibiotics and formerly, systemic medicines as well, it is not surprising that C. difficile has developed resistance. Despite normally being a rather sensitive organism, the widespread emergence of acne has resulted in the invasion of more than 50% of antibiotic-treated patients with erythromycin- and clindamycin-resistant strains individuals and >20% of these patients had tetracycline-resistant bacteria colonizing them. Unsurprisingly, resistant C. Currently, acne strains are discovered in different forms of infections, such as infections connected to the use of various medical equipment, in addition to acne patients receiving antibiotic treatment for their condition (Achermann et al., 2014).

4. Diagnostic Methods of AMR

AMR is often diagnosed with traditional AST techniques including broth micro-dilution, disc diffusion, gradient tests, agar well diffusion, agar dilution, and break-point tests (Figure 1).

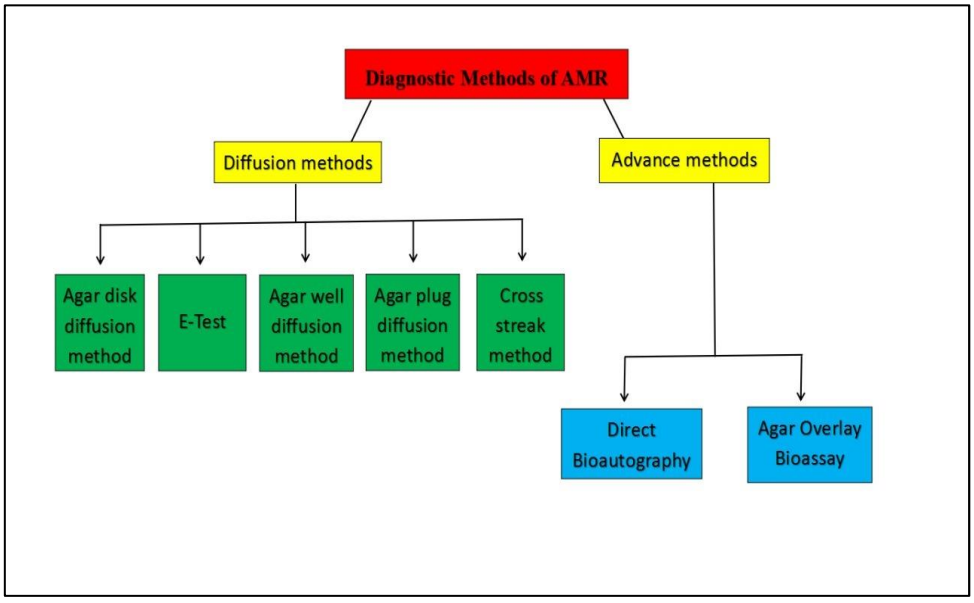


Figure 1. Diagnostic methods of AMR

4.1.Broth Micro-dilution

Small amounts of broth are used in this test, which is conducted using sterile micro-dilution dishes with conical well bottoms. There should be 0.1 ml of broth in each well.

1. Fill each well with 0.1 ml of antibiotic-containing broth. Include a sterility control and a growth control well (uninoculated well).
2. Place the plates in plastic bags and freeze them at -20°C, preferably -60°C.

3. On the day of the test, vaccinate the panels with the recommended dose of 5 x 105 CFU/ml.

4. To avoid drying during incubation, seal the plate in a plastic bag.

5. Before determining MICs visually, incubate for 16–20 hours at 35 2°C.

4.2. Disk diffusion D-zone tests

1. Prepare agar according to the directions above. Instead of lysed horse blood, however, use 5% sheep blood.

2. Place bacterial colonies on the plates that have attained the 0.5 McFarland turbidity threshold.

3. Set clindamycin (2 g) and erythromycin (15 g) discs 12 mm apart.

4. For 20 to 24 hours, incubate the plates at 35 °C with 5% CO2.

5. Check the plates. The clindamycin zone next to the erythromycin disc is flattened with erythromycin-resistant isolates, indicating a positive D-zone test (Bowling et al., 2010; Engelkirk & Duben-Engelkirk, 2008; Wikler, 2006).

4.2.1. Disk Diffusion Materials

- Mueller-Hinton broth, pH between 7.2-7.4
- Lysed horse blood
- Sheep blood

- pH meter
- Micro-dilution plates with conical bottom wells
- Distilled water
- Plastic bags
- Paper disks
- Antibiotics

Preparation of Mueller-Hinton agar:

- Prepare broth from a dehydrated medium according to the manufacturer's instructions.
- Autoclave and chill overnight at 2-8°C or in an ice bath.
- Ensure the pH falls between 7.2-7.4.
- Add additional cations if necessary.
- Add 2.5-5% (v/v) lysed horse blood.
- Check pH and confirm that it remains between 7.2-7.4.

Preparation of lysed horse blood

- Freeze and thaw Defibrinated horse blood until the blood is lysed (5-7 freeze-thaw cycles).
- Aseptically mix equal volumes of lysed blood and sterile, distilled water.
- Centrifuge at 12, 000 g for 20 minutes.
- Decant the supernatant and re-centrifuge if necessary.
- Add to the broth for a final concentration of 2.5-5% lysed horse blood (Jorgensen et al., 2011; Runyoro et al., 2006).

4.3. Antimicrobial gradient method (Etest)

1. The MIC value is calculated using the antimicrobial gradient approach, which combines the principles of diffusion and dilution procedures. It is predicated on the potential for establishing a gradient in the concentration of the antimicrobial agent examined in the agar medium. This method has a commercial application in The Etest (BioMérieux). The process involves depositing a strip on the agar surface that has been previously inoculated with the microorganism under test and coating it with an antimicrobial agent that has an increasing concentration gradient from one end to the other. Using this technique, the MIC of antibiotics, antifungals, and antimycobacterial may be determined.
2. At the point where the growth inhibition ellipse and the strip connect, the MIC value is calculated. It is frequently utilized to satisfy the needs of physicians since it is straightforward to apply. However, each Etest® strip costs between $2 and $3. Therefore, if several medications are examined, this strategy becomes expensive (Reller et al., 2009).

    - A high connection between the MIC values calculated by Etest and those acquired by broth dilution or agar dilution technique has been demonstrated in several earlier investigations (Baker et al., 1991; Berghaus et al., 2015; Gupta et al., 2015).
    - This method may also be used to examine the antibacterial interactions between two different medications (White et al., 1996).

3. An Etest strip impregnated with a first antibiotic is placed on an inoculated agar plate surface to examine the combined impact of the two antibiotics. The strip is taken out and replaced with a new one that has been treated with a different antibiotic after an hour. When the MIC of the combination is at least two dilutions lower than that of the most potent antibiotic tested alone, this indicates the presence of synergy (Denes & Hidri, 2009).

4. The Etest strips can be placed on the agar medium for the same purpose in a cross formation with a 90° angle at the intersection between the scales at the corresponding MICs for the tested microorganism (Gülmez et al., 2010).

Incubation is followed by the following calculation of the fractional inhibitory concentration index (FICI):

$$\sum \text{FICI} = \text{FIC(A)} + \text{FIC(B)}$$

where FIC(A)=MIC(A) in combination MIC(A)alone and FIC(B)=MIC(B) in combination MIC(B)alone. Synergy was defined by FICI ≤0.5 and antagonism by FICI >4. The FICI between 0.5 and 1 was interpreted as addition and between 1 and 4 as indifference (Bassolé & Juliani, 2012).

4.4.Agar well diffusion Method

5. To assess the antimicrobial activity of plant or microbial extracts, the agar well diffusion technique is frequently utilized. The agar plate surface is infected using a process similar to the disk-diffusion approach in which a volume of the microbial inoculum is dispersed across the whole agar surface. Next, a volume (20-100 L) of the antimicrobial agent or extract solution is put into the well by aseptically drilling a hole with a diameter of 6 to 8 mm using a sterile cork borer or tip. The test microorganism is then placed on an appropriate agar plate, and the incubation process is continued. The antibiotic ingredient spreads across the agar media and stops the tested microbial strain from growing (Magaldi et al., 2004; Valgas et al., 2007).

*4.5.*The agar plug diffusion method

The method of agar plug diffusion is frequently used to emphasize the competition between microorganisms and also follows a similar process to the disk-diffusion approach. Making an agar culture of the target strain using tight streaks on the plate surface requires using the strain's proper culture media. Microbial cells release chemicals that spread across the agar media as they proliferate. Once the test microorganism has been incubated, an agar plot or cylinder is sliced aseptically using a sterile cork borer and placed on the agar surface of another plate. The chemicals spread out into the agar media from the plug. The formation of the inhibition zone around the agar plug serves as a further indicator of the antibacterial activity of the chemicals released by the microbes (Elleuch et al., 2010; Jiménez-Esquilín & Roane, 2005).

4.6. Cross streak method

To quickly check for antagonism, the crosssstreak technique is employed with microorganisms. A single streak in the middle of the agar plate seeds the target microbial strain. The plate is seeded with the microorganisms examined by a single streak perpendicular to the center streak after an incubation period dependent on the microbial strain. By measuring the size of the inhibitory zone after additional incubation, the antimicrobial interactions are examined (Lertcanawanichakul & Sawangnop, 2008).

4.7. Poisoned food method

Most commonly, the poisoned food approach is used to gauge an antifungal's effectiveness against mould. The extract or anti-fungal agent is added to the molten agar at the appropriate final concentration and well stirred. The medium is then added to Petri dishes. After an overnight pre-incubation, inoculation may be made using a mycelia disc that is implanted in the plate's center and ranges in size from 2 to 5 mm. The diameters of fungal growth in the control and sample plates are measured after additional incubation under circumstances appropriate for the tested fungus, and the anti-fungal effect is evaluated using the method below:

$$\text{Anti-fungal activity (\%)} = ((Dc - Ds)/Dc) \times 100$$

Where Ds is the diameter of growth in the plate containing the tested anti-fungal agent and Dc is the diameter of growth in the control plate. Comparing sporulation to the control is also possible. The researcher must typically carry a positive control with a recognized antibacterial molecule to compare the results discovered and prove the experimental strategy is correct when standardization of the procedure employed fails (Ali-Shtayeh & Abu Ghdeib, 1999; Mukherjee & Raghu, 1997).

4.8. Agar diffusion

It is the least used of the procedures and is sometimes referred to as the agar contact method. It entails the antimicrobial agent being transferred by diffusion from the chromatogram (PC or TLC) to an agar plate that has already been infected with the pathogen under investigation. The chromatogram is removed after a few minutes or hours to allow for diffusion, and the agar plate is then incubated. Where the antimicrobial chemicals come into contact with the agar layer is where the growth inhibition zones occur (Kumar et al., 2014).

4.9. Direct bioautography

The most often used technique among these three is direct bioautography. A microbiological suspension is dipped or sprayed over the prepared TLC plate. The bioautogram is then incubated for 48 hours at 25 °C in a humid environment (Marston, 2011).

Tetrazolium salts are commonly used to visualize microbial development. The dehydrogenase in live cells converts these salts into the matching highly colored

formazan, p-Iodonitrotetrazolium violet is the most suitable detection reagent. These salts are sprayed onto the bioautogram, which is re-incubated at 25 °C for 24 h or at 37 °C for 3–4 h. It has been advised to use Mueller Hinton Broth supplemented with agar to provide a medium with enough fluid to provide the greatest possible adhesion to the TLC plate and to maintain the proper humidity for bacterial growth. Either fungus or bacteria may be used for direct bioautography. It is the simplest method for identifying anti-fungal compounds and provides reliable findings for fungi that produce spores, including *Aspergillus*, *Penicillium*, and *Cryptosporidium*. *E.Coli*, *Staphylococcus aureus*, and *Bacillus subtilis* strains are widely tested to find antibiotic chemicals for microorganisms (Brantner, 1997; Choma & Grzelak, 2011; Dewanjee et al., 2015; Grzelak et al., 2011; Silva et al., 2005).

4.10. Agar overlay bioassay

A combination of the two earlier techniques, is sometimes referred to as immersion bioautography. The seeded agar media used to cover the TLC plate is molten. The plates might be kept at a low temperature for a few hours before incubation in order to facilitate a proper diffusion of the tested chemicals into the agar medium. Tetrazolium dye can be used for staining once the test microorganism has been incubated under the proper conditions. This technique, like direct bioautography, applies to all microorganisms, including mould and *Candida Albicans*. It has clearly defined growth restriction zones and is not contaminated-sensitive (Dewanjee et al., 2015; Marston, 2011; Mehrabani et al., 2013).

Overall, TLC-bioautography is a straightforward, efficient, and affordable method for separating a complicated mixture while simultaneously localizing the active components on the TLC plate. Consequently, it may be carried out in both advanced labs and smaller laboratories with a limited amount of equipment. TLC-bioautography offers a quick method for screening a lot of samples for bio-activity and in the bio activity-guided fractionation despite having sophisticated on-line high-performance liquid chromatography coupled bio-assay, which is becoming more and more popular as the method of choice for a final clean-up of extractive fractions to obtain pure compounds. It may be used to find novel antimicrobial medications as well as to identify antimicrobials in food and environmental samples (Balouiri et al., 2015).

Table 1. Country wise diagnostic methods.

Sr#	Country	Locations	Strains	Sign & Symptoms	Diagnosis method	Mob %	Mor%
1	Canada	Alberta and Ontario	Escherchia Coli &Coliforms	1.Urinary Tract Infection. 2. Meningitis 3.Endocarditis	1.Active screening 2.Antibiotic Susceptibility Testing 3. PCR.	20%	7.5%.
2	United Kingdom	United Kingdom	Neisseria Gonorrhea	1. Urethritis 2.Epididymitis 3.Reduced fertility 4.urethral stricture 5.Abnormal vaginal discharge 6. Dysurea 7. Disparunia	1.Active screening 2.Antibiotic Susceptibility Testing 3. PCR.	38.1%	8.3%

3	India	Sikkim	MRSA	1. Red bumps on skin 2.Skin and soft tissue infections (SSTI): 3. Cause bone and joint infection 4. also causes pneumonia and endocarditis 5.Osteomalytis 6. Cellulitis	1.Active screening 2.Antibiotic Susceptibility Testing 3Detection of penicillin binding protein 2a (PBP2a)orPCR.	31.21%	6.87%
4	South Africa	Angola	Pseudomonas Aeruginosa	1.Urinary tract infection.	1.Antimicrobial susceptibility testing (AST) 2. PCR	28%	7.7%
5	Latin America	Brazil	*Klebsiella pneumoniae*	1.Urinary Tract Infection	1.Antimicrobial susceptibility testing (AST) 2. PCR	45%	4.2%
6	India	New Dehli	*Enterococcus Faecalis*	1.Urinary Tract Infection 2.Endocarditis	1.Antimicrobial susceptibility testing (AST) 2. PCR	10%	1.3%

5. Future Recommendations

The challenge of antibiotic resistance is an expected result of how antibiotics have been created and utilized since their introduction. The outcome of the resistance issue is clear if we keep creating, utilizing, and safeguarding antibiotics in the same manner that we have for the previous 80 years. We will face an ongoing rise in antibiotic resistance, a reduction in available treatments, and the post-antibiotic age for a growing variety of illnesses. We ought to think unconventionally and question long-held, and occasionally treasured, presumptions if we wish to alter the future state and ensure that efficient antimicrobial treatment for illnesses remains available for the foreseeable future (Bartlett et al., 2013; Spellberg et al., 2013).

The very first aim is to methodically compile and immediately report on national statistics on the prevalence of antibiotic resistance amongst various diseases. Reducing needless agricultural antibiotic use is the next aim. In the United States, an astounding 15 million kg (17,000 tons) of antibiotics are used annually, with agriculture accounting for 80% (Bartlett et al., 2013). Resistance will unavoidably persist due to the intolerable amount of environmental pollution. The evidence in Europe disproves any claims of financial catastrophes or severe animal damage. For instance, when growth-promoting antibiotics for cattle were outlawed in Denmark 15 years ago, the country not only avoided a catastrophe but also saw a roughly 15-fold rise in hog output as a result (Bartlett et al., 2013).

Modernizing stewardship methods for antimicrobials is the third goal. Sir Alexander Fleming was the first to flash the red flag that doctors and patients were abusing antibiotics back in 1945 (Fleming, 1945). The misuse of antibiotics is an illustration of the Tragedy of the Commons, which arises from the conflict between what people believe will be to their personal short-term interest and a little overall longer-term detriment to the community (Spellberg, 2014). Furthermore, more research on short-course therapy for many diseases will considerably enhance the science of antibiotic use. Shorter treatment courses are as successful as slightly longer, according to clinical research (Bartlett et al., 2013). New evidence suggests that treatment should be tailored to the normalization of biomarkers for innate immune stimulation rather than being administered for the random and typically more conservative periods

recommended by guidelines. Rice (Rice, 2008) made the final observation that the incorrect belief that "shorter is better" should take the place of the incorrect assumption that patients should keep taking antibiotics until they have finished their complete treatment even after they feel better.

Modernizing techniques for infection control practices is the fourth goal. It is imperative that attempts to boost handwashing percentages continue. By using mechanization and sanitation technologies, we must also ease the burden of hand washing so that catastrophe won't result when people forget to wash their hands regularly. The fifth and sixth jobs are to, correspondingly, revive the research and creation of antimicrobials and to bolster antimicrobials with fresh, infection-treating strategies that do not involve eradicating the bacterium. Bacteria can be prevented from proliferating in the host by gradually depriving them of nutrients (such as iron), actively modifying the host's reaction to pathogens without targeting their targets inside the microbes, and safeguarding the microbiome using probiotics (Spellberg et al., 2013).

Economic, regulatory, and scientific constraints all had a role in modern-day antibiotic financial distress. These obstacles and strategies for overcoming them have been extensively covered elsewhere (America, 2011; Bartlett et al., 2013; Spellberg et al., 2013; Spellberg et al., 2010). The final goal is to create a systematic and planned national strategy to address antibiotic resistance. Antibiotic resistance has been acknowledged as an issue for 15 years, according to Dr. Bartlett. We require integrated guidance from many government entities, academic institutions, professionals, and businesses. To make such collaboration possible, new reporting mechanisms for the government are likely needed (Spellberg & Gilbert, 2014).

It is crucial to raise awareness of AMR and offer access to education to both health personnel and the general population in order to properly execute interventions against AMR (Gu et al., 2021). It has been demonstrated that exposure to details on antibiotics and AMR from trustworthy sources, such as experts or public organization websites, increases behavioral change inspiration and behavioral awareness (Tsuzuki et al., 2020), demonstrating the necessity for medical professionals to actively participate in knowledge dissemination to the public at large.

The correct use of antimicrobial drugs has been promoted by pharmaceutical corporations through public awareness campaigns, although these efforts have been rather small-scale and may not have been particularly successful. To combat AMR, it is crucial to monitor both the usage of antibiotics and the occurrence of organisms that are resistant to them. Both kinds of monitoring have been carried out in Europe and are utilized as markers for AMR management. It was already recognized that the percentage of drug-resistant bacteria differed depending on the kind of bacteria relative to other nations (Kajihara et al., 2021), drug-resistant bacteria monitoring has been carried out in Japan.

A large majority of the antibiotics under research, according to the World Health Organization (Dutescu & Hillier, 2021), provide only marginal therapeutic advantages over already available antibiotics. Actually, 82% of the newly authorized antibiotics are variations on classes of currently used antibiotics that are documented to have developed drug resistance. It is only normal to assume that drug resistance to them will quickly arise. To combat MDR Gram-negative bacteria in hospitals and community-acquired pathogens such as those that cause gonorrhea, TB, and urinary

tract infections, there is obviously an increasing demand for creating novel antimicrobials, notably antibiotics (Delhi, 2001).

6. Treatment and Control

The necessity for ethical deliberation is determined by the ethical ramifications of a choice to act or not to act. This includes AMR prevention and control (Esposito et al., 2011). Uncurable illness is the evident risk from AMR, and this concern is well known. There is now no one answer that can be found to the ongoing ethical challenge of AMR control, leaving only new problems to be raised. Every degree of moral perception may be applied to the many threads of medical practice and authority, prescription writing, medication administration, and financial standing vs rising opposition. There has to be a lot of discussion on how global measures should be put into action to continue the fight against resistance (Esposito et al., 2011).

It is important to think about the options society would have in the absence of antimicrobials (Dancer, 2013). These are focused on preventing and controlling infections, which raises further ethical questions. At the moment of sale, germs may be present in raw meat, vegetables, and other items (Fox et al., 2017). Food that hasn't been thoroughly cleaned or cooked might include organisms that colonize and/or make people sick later on, leading to difficulties. Due to their potential to harbor AMRs that the customer may keep for years, even non-infectious bacteria offer a concern from compromised meals.

Antimicrobial management strives to curtail the overuse of medications in the medical industry (Dellit et al., 2007). Consumers are also shielded from negative medication interactions or adverse outcomes by prudent management. For most institutions, this is handled as a primarily scientific issue, and recommendations for antibiotic options are weighed against factors including diagnosis, concurrent therapy, toxicity, allergies, and length of time. By enhancing the present scope and availability of disease screening methods, management would be effectively supported (Daulaire et al., 2015; Perez et al., 2014).

These enable the faster and more precise identification of the pathogen and infectious features. The importance of diagnostic microbiology in giving the details needed for effective prescription can be attributed to the fact that recognizing the identification of the pathogen assists not only the patient who is ill but also future patients. For private consumers, doctors are more inclined to provide prescriptions for medications because they feel obligated to (Duane et al., 2016). While admitting the conflict between both patients' rights and governance responsibilities, doctors may have a moral need to be questioned about how they handle private vs. public patients. Animals and agriculture account for 50% of antibiotic usage worldwide. Between 2010 and 2030, there will be an anticipated two-thirds rise in livestock utilization (Van Boeckel et al., 2015).

Animals receiving antibiotic doses for improving growth, treatment, or prevention promote AMR globally (Smith et al., 2002; Van den Bogaard et al., 2001). Farmers' families are also susceptible to contracting resistant pathogens from the animals they are caring for (Levy et al., 1976; Van den Bogaard et al., 2001). Then, through slaughterhouses, multi-resistant bacteria go up the food supply chain to customers, a process aptly known as "field to table"(Warren et al., 2008). Consequently, food security is exchanged for AMR control treatments. The safety of antibiotics for human usage shouldn't be compromised by giving them to pets or cattle (Angulo et al., 2009).

Similar to forbidding growth promotion, dedication to transformation can only happen through enforceable regulations, oversight, and monitoring in veterinary care and farming, like the use of foreign cattle and food.

For primarily financial purposes, pharmaceutical corporations are hesitant to explore anti-infective research (Bai et al., 2013). The process of creating new pharmaceuticals is time-consuming and expensive, and there is no guarantee that the findings will be useful (Smolinski et al., 2003). Unlike medications used to treat chronic diseases, antibiotics are typically taken for a short period. Medical institutions could save a novel medication for last-resort use even after it receives approval in the event that resistance arises. Although regulatory obstacles to antibiotic development are typically held responsible for antimicrobials' stagnation, really new antibiotics are not commonly put on hold as a result of these obstacles (So & Shah, 2014). Given the significance of AMR, universities, hospitals, and colleges should devote more time to learning sanitation, disease prevention, microbiology, and prescriptions, preferably with the help of school-based hygiene practices (Lecky et al., 2011).

More questions have been raised regarding the usage of microbiocidal agents in medicine as a result of correlations between antibacterial agents and resistance being established (Sattar, 2010; Vickers, 2017). Strong antimicrobials affect the surface microbiome in a similar way as antibiotics affect the good gut flora, allowing naturally resistant or tolerant microflora to persist and build up stores of ever-more-resistant microorganisms (Dancer, 2013). Antibiotics are medicines that can save lives; they should be administered wisely and efficiently (Control et al., 2017). Follow the whole course of any prescription antibiotic to ensure full efficacy and prevent the development of resistance (Women's & Health, 2012). Healthcare practitioners must take the appropriate safeguards in order to reduce the over- and inappropriate prescription of antibiotics (Fleming-Dutra et al., 2018).

Future Strategies

Rationally using existing antibiotics (Ganguly et al., 2011; Ghafur et al., 2013). Recognizing the causes of the development and spread of antibiotic resistance (Berger-Bächi & Rohrer, 2002; Kotwani et al., 2010; Novo & Manaia, 2010). Lowering the demands on antibiotic adoption by suitable control methods (Tenover & McGowan Jr, 1996). Putting in place a well-organized, national antibiotics program with clear-cut, interconnected roles and duties for each program arm (Bell et al., 2007; Huttner & Harbarth, 2009; Leatherman & McCarthy, 2004; Opintan et al., 2015). Diseases and invasive disorders may be diagnosed quickly and accurately (Bbosa et al., 2014; Holmes et al., 2016; Jindal et al., 2015). Encouragement of the development of new, more potent antibiotics based on the resistance mechanisms already understood. Give many antibiotics to have a stronger impact at a reduced dose.

A variety of different anti-infectives, both traditional and contemporary, are available. These include "traditional" treatments like fresh air, homeopathy, plants, cranberry juice, honey, maggots, salt, heavy metals like silver and copper, sunlight, herbs, and spices, as well as more modern examples like electrolyzed water, UV irradiation, bacteriocins, phage treatments, immune-regulatory techniques, and nanotechnology (Dancer, 2013). The moral discussion around the effectiveness, toxicity, and expense of these alternate remedies is likewise dictated by them. Few have undergone blinded randomized trials hence they continue to be debatable. In spite of caring for patients with TB outside only a few decades ago, putting up a double-blind randomized clinical trial on contact with fresh air, for instance, would present

significant challenges. By adhering to standardized recommendations for non-conventional prescriptions, physicians can avoid using innovative medications in ways that are forbidden (Montanaro et al., 2017).

Some of the cures already discussed include meals, natural items, or irrigation fluids rather than medications. There are no formal guidelines for use, but it is widely accepted in the medical community that 'empathetic' usage of an unregistered product is acceptable as long as the physician and client have taken the available information into account, debated all of their choices, and decided on a course of treatment. Such prescriptions must always be done in an open manner with the proper records.

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

References

A. A. Salyers and C. F. Amabile-Cuevas, "Why are antibiotic resistance genes so resistant to elimination?," Antimicrobial agents and chemotherapy, vol. 41, no. 11, pp. 2321-2325, 1997.

A. Adesiyun, "JS Kaminjolo," Revue Elev. Med. Vet. Pays Trop, vol. 45, no. 3-4, pp. 260-262, 1992.

A. Brantner, "Influence of various parameters on the evaluation of antibacterial compounds by the bioautographic TLC assay," Pharmaceutical and Pharmacological Letters, vol. 7, no. 4, pp. 152-154, 1997.

Á. Castellanos-Ortega et al., "Impact of the Surviving Sepsis Campaign protocols on hospital length of stay and mortality in septic shock patients: results of a three-year follow-up quasi-experimental study," Critical care medicine, vol. 38, no. 4, pp. 1036-1043, 2010.

A. Chokshi, Z. Sifri, D. Cennimo, and H. Horng, "Global contributors to antibiotic resistance," Journal of global infectious diseases, vol. 11, no. 1, p. 36, 2019.

A. Csendes, M. Becerra, P. Burdiles, I. Demian, K. Bancalari, and P. Csendes, "Bacteriological studies of bile from the gallbladder in patients with carcinoma of the gallbladder, cholelithiasis, common bile duct stones and no gallstones disease," The European journal of surgery= Acta chirurgica, vol. 160, no. 6-7, pp. 363-367, 1994.

A. D. So and T. A. Shah, "New business models for antibiotic innovation," Upsala journal of medical sciences, vol. 119, no. 2, pp. 176-180, 2014.

A. Dixit, N. Kumar, S. Kumar, and V. Trigun, "Antimicrobial resistance: progress in the decade since emergence of New Delhi metallo-β-lactamase in India," Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine, vol. 44, no. 1, p. 4, 2019.

A. Fleming, "Penicillin's finder assays its future," New York Times, vol. 26, p. 21, 1945.

A. Fox et al., "Detection and molecular characterization of Livestock-Associated MRSA in raw meat on retail sale in North West England," Letters in Applied Microbiology, vol. 64, no. 3, pp. 239-245, 2017.

A. Ghafur et al., "The Chennai Declaration: a roadmap to tackle the challenge of antimicrobial resistance," Indian Journal of Cancer, vol. 50, no. 1, p. 71, 2013.

A. H. Holmes et al., "Understanding the mechanisms and drivers of antimicrobial resistance," The Lancet, vol. 387, no. 10014, pp. 176-187, 2016.

A. Jiménez-Esquilín and T. Roane, "Antifungal activities of actinomycete strains associated with high-altitude sagebrush rhizosphere," Journal of Industrial Microbiology and Biotechnology, vol. 32, no. 8, pp. 378-381, 2005.

A. Jindal, K. Pandya, and I. Khan, "Antimicrobial resistance: A public health challenge," Medical journal armed forces India, vol. 71, no. 2, pp. 178-181, 2015.

A. Johnston, "Use of antimicrobial drugs in veterinary practice," Bmj, vol. 317, no. 7159, pp. 665-667, 1998.

A. Kotwani, C. Wattal, S. Katewa, P. Joshi, and K. Holloway, "Factors influencing primary care physicians to prescribe antibiotics in Delhi India," Family practice, vol. 27, no. 6, pp. 684-690, 2010.

A. M. Dechet et al., "Outbreak of multidrug-resistant Salmonella enterica serotype Typhimurium definitive type 104 infection linked to commercial ground beef, northeastern United States, 2003–2004," Clinical infectious diseases, vol. 42, no. 6, pp. 747-752, 2006.

A. Marston, "Thin-layer chromatography with biological detection in phytochemistry," Journal of Chromatography A, vol. 1218, no. 19, pp. 2676-2683, 2011.

A. Novo and C. M. Manaia, "Factors influencing antibiotic resistance burden in municipal wastewater treatment plants," Applied Microbiology and Biotechnology, vol. 87, no. 3, pp. 1157-1166, 2010.

A. Van den Bogaard, N. London, C. Driessen, and E. Stobberingh, "Antibiotic resistance of faecal Escherichia coli in poultry, poultry farmers and poultry slaughterers," Journal of Antimicrobial chemotherapy, vol. 47, no. 6, pp. 763-771, 2001.

A. van Hoek, D. Mevius, B. Guerra, P. Mullany, A. Roberts, and H. Aarts, "Acquired Antibiotic Resistance Genes: An Overview," (in English), Frontiers in Microbiology, Review vol. 2, 2011-September-28 2011, doi: 10.3389/fmicb.2011.00203.

B. Berger-Bächi and S. Rohrer, "Factors influencing methicillin resistance in staphylococci," Archives of microbiology, vol. 178, no. 3, pp. 165-171, 2002.

B. Claesson, D. Holmlund, and T. Mätzsch, "Biliary microflora in acute cholecystitis and the clinical implications," Acta chirurgica scandinavica, vol. 150, no. 3, pp. 229-237, 1984.

B. Claesson, D. Holmlund, and T. W. Mätzsch, "Microflora of the gallbladder related to duration of acute cholecystitis," Surgery, gynecology & obstetrics, vol. 162, no. 6, pp. 531-535, 1986.

B. G. Spratt, "Resistance to Antibiotics Mediated by Target Alterations," Science, vol. 264, no. 5157, pp. 388-393, 1994, doi: doi:10.1126/science.8153626.

B. Huttner and S. Harbarth, "The French national campaign to cut antibiotic overuse," PLoS Med, vol. 6, no. 6, p. e1000080, 2009.

B. S. Speer, N. B. Shoemaker, and A. A. Salyers, "Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance," Clinical Microbiology Reviews, vol. 5, no. 4, pp. 387-399, 1992, doi: doi:10.1128/CMR.5.4.387.

B. Spellberg and D. N. Gilbert, "The future of antibiotics and resistance: a tribute to a career of leadership by John Bartlett," Clinical infectious diseases, vol. 59, no. suppl_2, pp. S71-S75, 2014.

B. Spellberg, "Antibiotic judo: working gently with prescriber psychology to overcome inappropriate use," JAMA internal medicine, vol. 174, no. 3, pp. 432-433, 2014.

B. Spellberg, E. Choffnes, D. Relman, and A. Mack, "The antibacterial pipeline: why is it drying up, and what must be done about it," in Antibiotic resistance: implications for global health and novel intervention strategies: workshop summary, 2010: Institute of Medicine, Washington, pp. 299-332.

B. Spellberg, J. G. Bartlett, and D. N. Gilbert, "The future of antibiotics and resistance," New England Journal of Medicine, vol. 368, no. 4, pp. 299-302, 2013.

C. f. D. Control and Prevention, "Antibiotic resistance threats in the United States, 2013 Available at: https://www. cdc. gov/drugresistance/pdf/ar-threats-2013–508. pdf," Accessed January, vol. 10, 2017.

C. f. D. Control, Prevention, and B. A. Aware, "Smart Use, Best Care. CDC website," ed, 2017.

C. Glasner et al., "Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013," Eurosurveillance, vol. 18, no. 28, p. 20525, 2013.

C. Llor and L. Bjerrum, "Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Ther Adv drug Saf," ed: SAGE Publications, 2014.

C. N. Baker, S. A. Stocker, D. H. Culver, and C. Thornsberry, "Comparison of the E Test to agar dilution, broth microdilution, and agar diffusion susceptibility testing techniques by using a special challenge set of bacteria," Journal of clinical microbiology, vol. 29, no. 3, pp. 533-538, 1991.

C. Valgas, S. M. d. Souza, E. F. Smânia, and A. Smânia Jr, "Screening methods to determine antibacterial activity of natural products," Brazilian journal of microbiology, vol. 38, pp. 369-380, 2007.

D. Fuks, C. Cossé, and J.-M. Régimbeau, "Antibiotic therapy in acute calculous cholecystitis," Journal of visceral surgery, vol. 150, no. 1, pp. 3-8, 2013.

D. G. Tsevat, "BA; Harold C. Wiesenfeld, MD, CM; Caitlin Parks, MD; Jeffrey F. Peipert, MD, PhD," Sexually transmitted diseases and infertility. American Journal of Obstetrics & Gynecology, 2017.

D. Gülmez, A. Cakar, B. Şener, G. Hasçelik, and J. Karakaya, "Comparison of different antimicrobial susceptibility testing methods for Stenotrophomonas maltophilia and results of synergy testing," Journal of infection and chemotherapy, vol. 16, no. 5, pp. 322-328, 2010.

D. K. Runyoro, M. I. Matee, O. D. Ngassapa, C. C. Joseph, and Z. H. Mbwambo, "Screening of Tanzanian medicinal plants for anti-Candida activity," BMC complementary and alternative medicine, vol. 6, no. 1, pp. 1-10, 2006.

D. L. Smith, A. D. Harris, J. A. Johnson, E. K. Silbergeld, and J. G. Morris Jr, "Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria," Proceedings of the National Academy of Sciences, vol. 99, no. 9, pp. 6434-6439, 2002.

D. M. Lecky et al., "Development of an educational resource on microbes, hygiene and prudent antibiotic use for junior and senior school children," Journal of antimicrobial chemotherapy, vol. 66, no. suppl_5, pp. v23-v31, 2011.

D. M. Livermore, "Bacterial resistance: origins, epidemiology, and impact," Clinical infectious diseases, vol. 36, no. Supplement_1, pp. S11-S23, 2003.

É. Denes and N. Hidri, "Synergie et antagonisme en antibiothérapie," Antibiotiques, vol. 11, no. 2, pp. 106-115, 2009.

E. M. Grzelak, B. Majer-Dziedzic, and I. M. Choma, "Development of a novel direct bioautography–thin-layer chromatography test: Optimization of growth conditions for Gram-negative bacteria, Escherichia coli," Journal of AOAC International, vol. 94, no. 5, pp. 1567-1572, 2011.

E. Y. Furuya and F. D. Lowy, "Antimicrobial-resistant bacteria in the community setting," Nature Reviews Microbiology, vol. 4, no. 1, pp. 36-45, 2006.

F. C. Tenover and J. E. McGowan Jr, "Reasons for the emergence of antibiotic resistance," The American journal of the medical sciences, vol. 311, no. 1, pp. 9-16, 1996.

F. J. Angulo, P. Collignon, J. H. Powers, T. M. Chiller, A. Aidara-Kane, and F. M. Aarestrup, "World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies for the use of antimicrobials in food production animals," Clinical infectious diseases, vol. 49, no. 1, pp. 132-141, 2009.

F. Koedijk, J. Van Bergen, N. Dukers-Muijrers, A. Van Leeuwen, C. Hoebe, and M. Van der Sande, "The value of testing multiple anatomic sites for gonorrhoea and chlamydia in sexually transmitted infection centres in the Netherlands, 2006–2010," International journal of STD & AIDS, vol. 23, no. 9, pp. 626-631, 2012.

F. Menichetti and G. Sganga, "Definition and classification of intra-abdominal infections," Journal of Chemotherapy, vol. 21, no. sup1, pp. 3-4, 2009.

F. Pieracci and P. Barie, "Management of severe sepsis of abdominal origin," Scandinavian journal of surgery, vol. 96, no. 3, pp. 184-196, 2007.

F. Shahkarami, A. Rashki, and Z. R. Ghalehnoo, "Microbial susceptibility and plasmid profiles of methicillin-resistant Staphylococcus aureus and methicillin-susceptible S. aureus," Jundishapur journal of microbiology, vol. 7, no. 7, 2014.

F. Y. Ramírez-Castillo, A. C. Moreno-Flores, F. J. Avelar-González, F. Márquez-Díaz, J. Harel, and A. L. Guerrero-Barrera, "An evaluation of multidrug-resistant Escherichia coli isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study," Annals of clinical microbiology and antimicrobials, vol. 17, no. 1, pp. 1-13, 2018.

G. A. van Liere, C. J. Hoebe, and N. H. Dukers-Muijrers, "Evaluation of the anatomical site distribution of chlamydia and gonorrhoea in men who have sex with men and in high-risk women by routine testing: cross-sectional study revealing missed opportunities for treatment strategies," Sexually transmitted infections, vol. 90, no. 1, pp. 58-60, 2014.

G. L. Maxwell and W. J. Watson, "Preterm premature rupture of membranes: results of expectant management in patients with cervical cultures positive for group B streptococcus or Neisseria gonorrhoeae," American journal of obstetrics and gynecology, vol. 166, no. 3, pp. 945-949, 1992.

G. Morris-Stiff, P. O'Donohue, S. Ogunbiyi, and W. Sheridan, "Microbiological assessment of bile during cholecystectomy: is all bile infected?," HPB, vol. 9, no. 3, pp. 225-228, 2007.

G. S. Bbosa, N. Mwebaza, J. Odda, D. B. Kyegombe, and M. Ntale, "Antibiotics/antibacterial drug use, their marketing and promotion during the post-antibiotic golden age and their role in emergence of bacterial resistance," Health, vol. 2014, 2014.

H. Harbottle, S. Thakur, S. Zhao, and D. G. White, "Genetics of antimicrobial resistance," (in eng), Anim Biotechnol, vol. 17, no. 2, pp. 111-24, 2006, doi: 10.1080/10495390600957092.

H. Kruse and H. Sørum, "Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments," Applied and environmental microbiology, vol. 60, no. 11, pp. 4015-4021, 1994.

H. Lishman et al., "Exploring the relationship between primary care antibiotic prescribing for urinary tract infections, Escherichia coli bacteraemia incidence and antimicrobial resistance: an ecological study," International journal of antimicrobial agents, vol. 52, no. 6, pp. 790-798, 2018.

H. S. Gold and R. C. Moellering Jr, "Antimicrobial-drug resistance," New England journal of medicine, vol. 335, no. 19, pp. 1445-1453, 1996.

H. Truedson, T. Elmros, and S. Holm, "The incidence of bacteria in gallbladder bile at acute and elective cholecystectomy," Acta Chirurgica Scandinavica, vol. 149, no. 3, pp. 307-313, 1983.

H. Tschäpe, E. Tietze, R. Prager, W. Voigt, E. Wolter, and G. Seltmann, "Plasmid-borne streptothricin resistance in gram-negative bacteria," Plasmid, vol. 12, no. 3, pp. 189-196, 1984/11/01/ 1984, doi: https://doi.org/10.1016/0147-619X(84)90043-X.

H. H. Handsfield, T. O. Lipman, J. P. Harnisch, E. Tronca, and K. K. Holmes, "Asymptomatic gonorrhea in men: diagnosis, natural course, prevalence and significance," New England Journal of Medicine, vol. 290, no. 3, pp. 117-123, 1974.

I. A. Dutescu and S. A. Hillier, "Encouraging the development of new antibiotics: are financial incentives the right way forward? A systematic review and case study," Infection and Drug Resistance, vol. 14, p. 415, 2021.

I. D. S. o. America, "Combating antimicrobial resistance: policy recommendations to save lives," Clinical Infectious Diseases, vol. 52, no. suppl_5, pp. S397-S428, 2011.

I. H. N. Bassolé and H. R. Juliani, "Essential oils in combination and their antimicrobial properties," Molecules, vol. 17, no. 4, pp. 3989-4006, 2012.

I. M. Choma and E. M. Grzelak, "Bioautography detection in thin-layer chromatography," Journal of Chromatography A, vol. 1218, no. 19, pp. 2684-2691, 2011.

J. A. Opintan, M. J. Newman, R. E. Arhin, E. S. Donkor, M. Gyansa-Lutterodt, and W. Mills-Pappoe, "Laboratory-based nationwide surveillance of antimicrobial resistance in Ghana," Infection and drug resistance, pp. 379-389, 2015.

J. Carter, "Deaths involving MRSA: England and Wales, 2008," Health Statistics Quarterly, no. 43, pp. 38-42, 2009.

J. E. Bowling et al., "Detection of inducible clindamycin resistance in beta-hemolytic streptococci by using the CLSI broth microdilution test and erythromycin-

clindamycin combinations," Journal of clinical microbiology, vol. 48, no. 6, pp. 2275-2277, 2010.

J. G. Bartlett, D. N. Gilbert, and B. Spellberg, "Seven ways to preserve the miracle of antibiotics," Clinical Infectious Diseases, vol. 56, no. 10, pp. 1445-1450, 2013.

J. H. Jorgensen et al., "Collaborative evaluation of an erythromycin-clindamycin combination well for detection of inducible clindamycin resistance in beta-hemolytic streptococci by use of the CLSI broth microdilution method," Journal of clinical microbiology, vol. 49, no. 8, pp. 2884-2886, 2011.

J. Pogliano, N. Pogliano, and J. A. Silverman, "Daptomycin-mediated reorganization of membrane architecture causes mislocalization of essential cell division proteins," Journal of bacteriology, vol. 194, no. 17, pp. 4494-4504, 2012.

J. S. Bell, M. Väänänen, H. Ovaskainen, U. Närhi, and M. S. Airaksinen, "Providing patient care in community pharmacies: practice and research in Finland," Annals of Pharmacotherapy, vol. 41, no. 6, pp. 1039-1046, 2007.

K. E. Fleming-Dutra, J. A. Linder, D. Hyun, J. K. Iskander, P. Thorpe, and S. Laird, "Be antibiotics aware: smart use, best care," 2018.

K. K. Perez et al., "Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic-resistant Gram-negative bacteremia," Journal of Infection, vol. 69, no. 3, pp. 216-225, 2014.

L. B. Reller, M. Weinstein, J. H. Jorgensen, and M. J. Ferraro, "Antimicrobial susceptibility testing: a review of general principles and contemporary practices," Clinical infectious diseases, vol. 49, no. 11, pp. 1749-1755, 2009.

L. B. Rice, "The Maxwell Finland Lecture: for the duration—rational antibiotic administration in an era of antimicrobial resistance and Clostridium difficile," Clinical infectious diseases, vol. 46, no. 4, pp. 491-496, 2008.

L. Bai, L. C. Morton, and Q. Liu, "Climate change and mosquito-borne diseases in China: a review," Globalization and health, vol. 9, no. 1, pp. 1-22, 2013.

L. Elleuch et al., "Bioactive secondary metabolites from a new terrestrial Streptomyces sp. TN262," Applied biochemistry and biotechnology, vol. 162, no. 2, pp. 579-593, 2010.

L. J. Berghaus, S. Giguère, K. Guldbech, E. Warner, U. Ugorji, and R. D. Berghaus, "Comparison of Etest, disk diffusion, and broth macrodilution for in vitro susceptibility testing of Rhodococcus equi," Journal of clinical microbiology, vol. 53, no. 1, pp. 314-318, 2015.

L. Karthik, G. Kumar, T. Keswani, A. Bhattacharyya, S. S. Chandar, and K. Bhaskara Rao, "Protease inhibitors from marine actinobacteria as a potential source for antimalarial compound," PloS one, vol. 9, no. 3, p. e90972, 2014.

L. M. Baddour et al., "Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association," Circulation, vol. 132, no. 15, pp. 1435-1486, 2015.

L. Piddock, "Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy?," The Journal of antimicrobial chemotherapy, vol. 38, no. 1, pp. 1-3, 1996.

L. S. Roope et al., "The challenge of antimicrobial resistance: what economics can contribute," Science, vol. 364, no. 6435, p. eaau4679, 2019.

L. W. Martin et al., "Expression of Pseudomonas aeruginosa Antibiotic Resistance Genes Varies Greatly during Infections in Cystic Fibrosis Patients," Antimicrobial Agents and Chemotherapy, vol. 62, no. 11, pp. e01789-18, 2018, doi: doi:10.1128/AAC.01789-18.

M. A. Puskarich et al., "Association between timing of antibiotic administration and mortality from septic shock in patients treated with a quantitative resuscitation protocol," Critical care medicine, vol. 39, no. 9, p. 2066, 2011.

M. A. Wikler, "Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard," Clsi (Nccls), vol. 26, pp. M7-A7, 2006.

M. Ali-Shtayeh and S. I. Abu Ghdeib, "Antifungal activity of plant extracts against dermatophytes," mycoses, vol. 42, no. 11-12, pp. 665-672, 1999.

M. Balouiri, S. Bouhdid, E. Harki, M. Sadiki, W. Ouedrhiri, and S. K. Ibnsouda, "Antifungal activity of Bacillus spp. isolated from Calotropis procera AIT. Rhizosphere against Candida albicans," Asian J. Pham. Clin. Res, vol. 8, pp. 213-217, 2015.

M. Bassetti, M. Peghin, A. Vena, and D. R. Giacobbe, "Treatment of infections due to MDR Gram-negative bacteria," Frontiers in medicine, vol. 6, p. 74, 2019.

M. C. J. Maiden, "Horizontal Genetic Exchange, Evolution, and Spread of Antibiotic Resistance in Bacteria," Clinical Infectious Diseases, vol. 27, no. Supplement_1, pp. S12-S20, 1998, doi: 10.1086/514917.

M. Haque, "Antibiotic Resistance: A Global Threat."

M. K. Glynn, C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar, and F. J. Angulo, "Emergence of Multidrug-Resistant Salmonella enterica SerotypeTyphimurium DT104 Infections in the United States," New England Journal of Medicine, vol. 338, no. 19, pp. 1333-1339, 1998.

M. Lertcanawanichakul and S. Sawangnop, "A comparison of two methods used for measuring the antagonistic activity of Bacillus species," Walailak Journal of Science and Technology (WJST), vol. 5, no. 2, pp. 161-171, 2008.

M. Mehrabani, A. Kazemi, S. A. A. Mousavi, M. Rezaifar, H. Alikhah, and A. Nosky, "Evaluation of antifungal activities of Myrtus communis L. by bioautography method," Jundishapur Journal of Microbiology, vol. 6, no. 8, 2013.

M. S. Smolinski, M. A. Hamburg, and J. Lederberg, "Microbial threats to health," Emergence, detection and response, 2003.

M. Sartelli et al., "Complicated intra-abdominal infections worldwide: the definitive data of the CIAOW Study," World Journal of Emergency Surgery, vol. 9, no. 1, pp. 1-10, 2014.

M. T. Silva, S. M. Simas, T. G. Batista, P. Cardarelli, and T. C. Tomassini, "Studies on antimicrobial activity, in vitro, of Physalis angulata L.(Solanaceae) fraction and physalin B bringing out the importance of assay determination," Memorias do Instituto Oswaldo Cruz, vol. 100, pp. 779-782, 2005.

M.-L. Lambert et al., "Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study," The Lancet infectious diseases, vol. 11, no. 1, pp. 30-38, 2011.

N. C. C. f. Women's and C. s. Health, "Antibiotics for early-onset neonatal infection: antibiotics for the prevention and treatment of early-onset neonatal infection," 2012.

N. Datta and V. M. Hughes, "Plasmids of the same Inc groups in Enterobacteria before and after the medical use of antibiotics," Nature, vol. 306, no. 5943, pp. 616-617, 1983.

N. Daulaire, A. Bang, G. Tomson, J. N. Kalyango, and O. Cars, "Universal access to effective antibiotics is essential for tackling antibiotic resistance," Journal of Law, Medicine & Ethics, vol. 43, no. S3, pp. 17-21, 2015.

N. Delhi, "Ministry of Health and family welfare," Government of India, pp. 7-30, 2001.

N. Ganguly, N. Arora, S. Chandy, M. Fairoze, J. Gill, and U. Gupta, "Global Antibiotic Resistance Partnership (GARP) India Working Group," Rationalizing antibiotic use to limit antibiotic resistance in India. Indian J Med Res, vol. 134, pp. 281-94, 2011.

N. J. Vickers, "Animal communication: when i'm calling you, will you answer too?," Current biology, vol. 27, no. 14, pp. R713-R715, 2017.

N. Montanaro, M. Melis, S. Proni, G. Chiabrando, and D. Motola, "Six-year activity on approval of compassionate use of medicines by the Ethics Committee of the University Hospital of Bologna (Italy): time to update rules and recommendations," European journal of clinical pharmacology, vol. 73, no. 4, pp. 479-485, 2017.

Ohnishi, Golparian et al. 2011, Cámara, Serra et al. 2012, Fifer, Natarajan et al. 2016).

O. Galili, S. Eldar, I. Matter, H. Madi, A. Brodsky, and I. Galis, "The effect of bactibilia on the course and outcome of laparoscopic cholecystectomy," European Journal of Clinical Microbiology & Infectious Diseases, vol. 27, no. 9, pp. 797-803, 2008.

P. Arora, N. Nagelkerke, and S. Sgaier, "Erratum: HIV, HSV-2 and syphilis among married couples in India: Patterns of discordance and concordance (Sexually Transmitted Infections (2013) 89 (678," Sexually Transmitted Infections, vol. 89, no. 8, p. 678, 2013.

P. Boerlin et al., "Antimicrobial Resistance and Virulence Genes of Escherichia coli Isolates from Swine in Ontario," Applied and Environmental Microbiology, vol. 71, no. 11, pp. 6753-6761, 2005, doi: doi:10.1128/AEM.71.11.6753-6761.2005.

P. G. Engelkirk and J. L. Duben-Engelkirk, Laboratory diagnosis of infectious diseases: essentials of diagnostic microbiology. Lippincott Williams & Wilkins, 2008.

P. Gupta, V. Khare, D. Kumar, A. Ahmad, G. Banerjee, and M. Singh, "Comparative evaluation of disc diffusion and E-test with broth micro-dilution in susceptibility testing of amphotericin B, voriconazole and caspofungin against clinical Aspergillus isolates," Journal of clinical and diagnostic research: JCDR, vol. 9, no. 1, p. DC04, 2015.

P. K. Mukherjee and K. Raghu, "Effect of temperature on antagonistic and biocontrol potential of shape Trichoderma sp. on Sclerotium rolfsii," Mycopathologia, vol. 139, no. 3, pp. 151-155, 1997.

P. S. Morley et al., "Antimicrobial drug use in veterinary medicine," Journal of veterinary internal medicine, vol. 19, no. 4, pp. 617-629, 2005.

R. E. Warren et al., "Imported chicken meat as a potential source of quinolone-resistant Escherichia coli producing extended-spectrum β-lactamases in the UK," Journal of Antimicrobial Chemotherapy, vol. 61, no. 3, pp. 504-508, 2008.

R. Ferrer et al., "Effectiveness of treatments for severe sepsis: a prospective, multicenter, observational study," American journal of respiratory and critical care medicine, vol. 180, no. 9, pp. 861-866, 2009.

R. L. White, D. S. Burgess, M. Manduru, and J. A. Bosso, "Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test," Antimicrobial agents and chemotherapy, vol. 40, no. 8, pp. 1914-1918, 1996.

R. Smith and J. Coast, "The true cost of antimicrobial resistance," Bmj, vol. 346, 2013.

S. A. Sattar, "Promises and pitfalls of recent advances in chemical means of preventing the spread of nosocomial infections by environmental surfaces," American journal of infection control, vol. 38, no. 5, pp. S34-S40, 2010.

S. B. Levy et al., "Nomenclature for New Tetracycline Resistance Determinants," Antimicrobial Agents and Chemotherapy, vol. 43, no. 6, pp. 1523-1524, 1999, doi: doi:10.1128/AAC.43.6.1523.

S. B. Levy, "Balancing the drug-resistance equation," Trends Microbiol., vol. 2, pp. 341-342, 1994.

S. B. Levy, G. B. FitzGerald, and A. B. Macone, "Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm," New England Journal of Medicine, vol. 295, no. 11, pp. 583-588, 1976.

S. Dewanjee, M. Gangopadhyay, N. Bhattacharya, R. Khanra, and T. K. Dua, "Bioautography and its scope in the field of natural product chemistry," Journal of pharmaceutical analysis, vol. 5, no. 2, pp. 75-84, 2015.

S. Duane et al., "Using qualitative insights to change practice: exploring the culture of antibiotic prescribing and consumption for urinary tract infections," BMJ open, vol. 6, no. 1, p. e008894, 2016.

S. Esposito, C. Tagliabue, S. Bosis, and N. Principi, "Levofloxacin for the treatment of Mycoplasma pneumoniae-associated meningoencephalitis in childhood," International journal of antimicrobial agents, vol. 37, no. 5, pp. 472-475, 2011.

S. G. Pereira, A. C. Rosa, and O. Cardoso, "Virulence factors as predictive tools for drug resistance in Pseudomonas aeruginosa," Virulence, vol. 6, no. 7, pp. 679-683, 2015.

S. J. Dancer, "Infection control in the post-antibiotic era," Healthcare infection, vol. 18, no. 2, pp. 51-60, 2013.

S. L. S. Pavelquesi, A. C. A. de Oliveira Ferreira, A. R. M. Rodrigues, C. M. de Souza Silva, D. C. Orsi, and I. C. R. da Silva, "Presence of Tetracycline and Sulfonamide Resistance Genes in Salmonella spp.: Literature Review," Antibiotics, vol. 10, no. 11, p. 1314, 2021. [Online]. Available: https://www.mdpi.com/2079-6382/10/11/1314.

S. Magaldi et al., "Well diffusion for antifungal susceptibility testing," International journal of infectious diseases, vol. 8, no. 1, pp. 39-45, 2004.

S. N. Kumar, B. Nambisan, A. Sundaresan, C. Mohandas, and R. J. Anto, "Isolation and identification of antimicrobial secondary metabolites from Bacillus cereus associated with a rhabditid entomopathogenic nematode," Annals of microbiology, vol. 64, no. 1, pp. 209-218, 2014.

S. Stefani, "Emergence of multi-drug resistance gram-positive bacteria and new active antibiotics," Current Medicinal Chemistry-Anti-Infective Agents, vol. 4, no. 3, pp. 235-257, 2005.

S. T. Leatherman and D. McCarthy, Quality of health care for children and adolescents: a chartbook. Commonwealth Fund New York, NY, 2004.

S. Tsuzuki et al., "Factors associated with sufficient knowledge of antibiotics and antimicrobial resistance in the Japanese general population," Scientific reports, vol. 10, no. 1, pp. 1-9, 2020.

T. H. Dellit et al., "Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship," Clinical infectious diseases, vol. 44, no. 2, pp. 159-177, 2007.

T. Kajihara, K. Yahara, A. Hirabayashi, K. Shibayama, and M. Sugai, "Japan nosocomial infections surveillance (JANIS): current status, international collaboration, and future directions for a comprehensive antimicrobial resistance surveillance system," Japanese Journal of Infectious Diseases, vol. 74, no. 2, pp. 87-96, 2021.

T. M. Khan, Y. L. Kok, A. Bukhsh, L.-H. Lee, K.-G. Chan, and B.-H. Goh, "Incidence of methicillin resistant Staphylococcus aureus (MRSA) in burn intensive care unit: a systematic review," Germs, vol. 8, no. 3, p. 113, 2018.

T. P. Van Boeckel et al., "Global trends in antimicrobial use in food animals," Proceedings of the National Academy of Sciences, vol. 112, no. 18, pp. 5649-5654, 2015.

T. Saga and K. Yamaguchi, "History of antimicrobial agents and resistant bacteria," Jmaj, vol. 52, no. 2, pp. 103-108, 2009.

V. M. D'Costa, K. M. McGrann, D. W. Hughes, and G. D. Wright, "Sampling the antibiotic resistome," Science, vol. 311, no. 5759, pp. 374-377, 2006.

V. M. Hughes and N. Datta, "Conjugative plasmids in bacteria of the 'pre-antibiotic'era," Nature, vol. 302, no. 5910, pp. 725-726, 1983.

V. V. Nguyen, H. T. Dong, S. Senapin, N. Pirarat, and C. Rodkhum, "Francisella noatunensis subsp. orientalis, an emerging bacterial pathogen affecting cultured red tilapia (Oreochromis sp.) in Thailand," Aquaculture Research, vol. 47, no. 11, pp. 3697-3702, 2016.

W. Ashford, R. Golash, and V. Hemming, "Penicilunase-producing Neisseria gonorrhoeae," The Lancet, vol. 308, no. 7987, pp. 657-658, 1976.

W. H. Organization, "Global action plan on antimicrobial resistance. 2015," ed, 2019.

W. H. Organization, Global incidence and prevalence of selected curable sexually transmitted infections-2008. World Health Organization, 2012.

W. Witte, "Medical consequences of antibiotic use in agriculture," vol. 279, ed: American Association for the Advancement of Science, 1998, pp. 996-997.

WHO, "Global action plan on antimicrobial resistance," WHO Report, 2015.

Y. Achermann, E. J. Goldstein, T. Coenye, and M. E. Shirtliff, "Propionibacterium acnes: from commensal to opportunistic biofilm-associated implant pathogen," Clinical microbiology reviews, vol. 27, no. 3, pp. 419-440, 2014.

Y. Gu, Y. Fujitomo, and N. Ohmagari, "Outcomes and future prospect of Japan's national action plan on antimicrobial resistance (2016–2020)," Antibiotics, vol. 10, no. 11, p. 1293, 2021.

About The Authors

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkiye. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 68 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.
E-mail: msafdar@cuvas.edu.pk , ORCID: 0000 0002 3720 2090

Prof. Dr. Mehmet OZASLAN received his PhD in 1995 at the Institute of Natural Sciences at Cukurova University, Turkiye. He is a professor of molecular biology and genetics. His research interests include cancer genetics, molecular virology, molecular genetics, microbiology, genetic mutations, etc. He has published more than 200 research articles in national and international well-reputed journals. He has also written many book chapters as well as edited books. He is an editor and editor-in-chief of many well-reputed national and international journals.
E-mail: ozaslanmd@gantep.edu.tr, ORCID: 0000 0001 9380 4902

Shafeeq ur Rehman graduated from the Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur. He continues to work on FMD and PPR viruses as his area of interest. He is still working as a Research Assistant in FMDV and PPRV project in Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan.
Email: shafeequrrehmanbhutta@gmail.com, ORCID: 0000-0003-3571-8226

Minal Hussain graduated from Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur.
Email: 2019-cu-bios-023@cuvas.edu.pk, ORCID: 0009-0004-0143-5644

Furqan Shafqat graduated from Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in medical biotechnology from University of Wollongong Australia.
Email: furqanshafqat007@gmail.com ORCID: 0000-0002-2563-913X

Salman Nazeer graduated from Cholistan University of Veterinary and Animal Sciences Bahawalpur. He has a bachelor's degree in Applied Microbiology. He continues to work on antimicrobial resistance, virology, and immunology as his area of interest.
E-mail: 2019-cu-micro-091@cuvas.edu.pk, ORCID: 0009-0003-8606-3482

Fazal e Subhani graduated from Cholistan University of Veterinary and Animal Sciences Bahawalpur. He has a bachelor's degree in Applied Microbiology. He continues to work on antimicrobial resistance, virology, and immunology as his area of interest.
E-mail: 2019-cu-micro-076@cuvas.edu.pk, ORCID: 0009-0002-7413-612X

Muhammad Naveed Nawaz graduated from Cholistan University of Veterinary and Animal Sciences Bahawalpur. He has a bachelor's degree in Applied Microbiology. He continues to work on antimicrobial resistance, virology, cancer, bacteriophages, and immunology as his area of interest.
E-mail: 2019-cu-micro-058@cuvas.edu.pk, ORCID: 0000-0002-2479-6547

Muhammad Abdullah Mueen graduated from Cholistan University of Veterinary and Animal Sciences Bahawalpur. He has a bachelor's degree in applied microbiology. He continues to work on antimicrobial resistance, virology, cancer, bacteriophages, and immunology as his area of interest.
E-mail: abdullahblouch1@gmail.com, ORCID: 0009-0001-3383-754X

To Cite This Chapter:
Safdar, M., et al. (2023). **Antimicrobial resistance (amr): a global challenge in disease prevention and control**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 2-29). ISRES Publishing

RECENT ADVANCES IN THE APPLICATION OF CRISPR-CAS9

Shafeeq ur REHMAN
Furqan SHAFQAT
Minal HUSSAIN
Mehmet OZASLAN
Muhammad SAFDAR

Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) were initially discovered as an adaptive immune system effector in prokaryotes (Lander, 2016). CRISPR is a group of short DNA sequences present in the genomes of prokaryotes that have been acquired by bacteriophage attack (Lander, 2016). It provides prokaryotes with a defensive mechanism from reinfection by identical bacteriophages. Genetic manipulation for human disorders is now possible because of the development of this technique into a gene-modification tool in eukaryotic cells (Mali et al., 2013). The Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR-Cas9) system is made up of the Cas9 endonuclease and the selective-target single guide RNA (sgRNA). A selective-target sgRNA, which is composed of a CRISPR RNA (crRNA) and a transactivating crRNA (tracrRNA), creates a double-strand break (DSB) by driving the Cas9 protein to cleave at a specific location. CRISPR-Cas9 gives the benefits of simply designing the genomic sites and multiplexing over transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs) since target identification is dependent on RNA–DNA interactions. Unlike ZFNs and TALENs, which need time-consuming protein engineering procedures for every single modifying site, the Cas9 nuclease needs just a single selective-target sgRNA for every modifying site. The protospacer adjacent motifs (PAMs) and spacer sequence of crRNAs (20 nucleotides) affect Cas9's target selectivity (Doudna & Charpentier, 2014). For accurate targeting, the seed sequence at the 30 ends of the spacer sequence is essential (Doudna & Charpentier, 2014). When the seed site and the desired DNA have enough similarity, Cas9 will cleave. Off-target cleavage, on the other hand, happens when DNA sequences include a small mismatch yet have some similarity with the sgRNA seed domain (Doudna & Charpentier, 2014). The purpose of this study is to limit CRISPR-Cas9's off-target consequences. For instance, reduced gRNAs (less than 20 nucleotides) have been shown to minimize off-target impacts while maintaining on-target genetic modifications (Fu et al., 2014). Furthermore, using a Cas9 nickase and two sgRNAs, each cleaving at a different location of the target, significantly reduces off-target impacts (Ran et al., 2013). When Cas9 ribonucleoproteins (RNPs) are delivered into cells, they have less off-target impacts than DNA plasmids containing Cas9 and sgRNA (Kim et al., 2014). Because the Cas9 protein is temporary and capable of rapidly cleaving the desired DNA, Cas9 RNP administration reduces the risk of Cas9-caused off-target impacts (Yip, 2020).

Cancer is a hereditary disease characterized by epigenetic and genetic aberrations. Existing cancer treatments are restricted owing to the complication of cancer's progression, highlighting the need for new treatment strategies. For example, the CRISPR-Cas9-based genetic manipulation technique allows for accurate modification of almost any genome, allowing for functional clarification of genes implicated in tumorigenesis as well as the repair of tumor-inducing alterations. As a result, this technique might be used to cure illnesses such as cancer (Barrangou & Doudna, 2016; Sánchez-Rivera & Jacks, 2015). Despite its advantages and capabilities, delivering

CRISPR-Cas9 to specific cells *in vivo* and avoiding or decreasing undesirable off-target effects remain significant challenges that must be overcome before therapeutic applications may be considered (M. Chen et al., 2019).

The global population is growing at an exponential rate, yet the quantity of food accessible is increasing arithmetically (Adhikari & Poudel, 2020). The population is forecast to exceed 10 billion by 2050 (Clarke & Zhang, 2013). Food security challenges may arise as a result of the growing human population and the detrimental effect of climate conditions. A drop in fertile lands and a reduction in production may worsen the situation. According to current reports from the International Rice Research Institute (IRRI), one hectare of productive land is wasted every 7.7 seconds, and the impact may be more significant if the world temperature increasing speed continues (Stamm et al., 2011). Crop production ability must almost double to ensure food security, and extremely resistant varieties against diverse stresses must be engineered to attain this target (Jones et al., 2014). The main challenge for world food security is a rise in agricultural crop yield employing the newest breeding methods (Z. Khan, S. H. Khan, et al., 2021). Traditional breeding approaches, on the other hand, are unable to assure food safety and maximize food supply (Rasheed et al., 2020). Conventional breeding approaches, like hybridization, have been used to optimize varietals and crop growth, which has increased crop productivity to a certain degree (Rasheed, Wassan, et al., 2021). This includes the use of biological and physical alterations, as well as the detection of biological contrivances, to increase agricultural yields. A 100 % success rate is impossible to obtain due to many factors (Samanta et al., 2016). Furthermore, the characteristics of transgene insertion into the plant host genome cause public concern about consumable crops and are not always understood due to a deficiency of information about the procedures used or the advantages that result. As a result, using biotechnological methods for crop development is critical to overcome these constraints (Jaganathan et al., 2018; Rasheed, Gill, et al., 2021).

Delivery Strategies for CRISPR-Cas9

Viral Vectors

During induction, the Adeno-associated virus (AAV) remain effectively treated in the nucleus and are gradually dispersed by cell growth. As a consequence, AAV-mediated plasmids delivery of transgenic plants provides a safe technique to create genes in the short term (Daya & Berns, 2008). AAVs may be used to deliver CRISPR-Cas9 in two ways: AAVs may be used to convert cells and insert Cas9, sgRNAs, and/or donor motifs, for beginning (Y. Yang et al., 2016). Furthermore, AAVs are valuable for *in vitro* uses as well as *in vivo* CRISPR-Cas9 genetic modification, particularly where genome incorporation is a problem and electrodeposition are not an optional alternative for the kind of cell of relevance. AAV vectors, on the other hand, are hampered by their poor replication capability (4.7 kb). To produce a gene-editing event in mice, researchers employed two different AAV carriers, one producing Cas9 and the rest of the genes releasing a donor DNA and a sgRNA sequence (Y. Yang et al., 2016). By its huge size (4.2 kb), The commonly used Streptococcus pyogenes Cas9 is challenging to administer using AAV. A shorter Staphylococcus aureus Cas9 strain (SaCas9; 3.15 kb) is a more practical choice (Ran et al., 2015). Nevertheless, the provision of appropriate PAM sequences for addressing SaCas9 is a limitation (Ma et al., 2019). Second, through the homology-directed repair (HDR) route, AAV particles can be employed as a gene knocking donor pattern (Eyquem et al., 2017). AAV donating patterns have a better knocking efficacy than nonviral aiming approaches (Bak & Porteus, 2017). Likewise, transgenic AAVs' restricted replication

capability can be overcome by separating big transgenic organisms into two distinct AAV vectors, allowing for successive Recombination that is homologous (Bak & Porteus, 2017). An additional drawback of AAVs is their limited gene binding effectiveness. Under ideal circumstances, particular homologous crossover happens in only 0.1 % to 1% of the entire cell line (Deyle & Russell, 2009). Presently, ZFNs are exclusively used in AAV-based gene altering experiments published on ClinicalTrials.gov to introduce a corrected replication of the gene into the genetics of severe Hemophilia patients (George, 2017) or chromatin structure in mucopolysaccharidosis genotypes I and II (Sawamoto et al., 2018). Because AAV-based administration is likely to become more widespread, commercial studies utilizing AAV-mediated CRISPR-Cas9 genetic manipulation may be in the future. Some other infectious vector utilized for CRISPR-Cas9 distribution is lentiviruses (LVs). LV vectors offer a larger replication capability (8 kb) than AAV vectors, allowing Cas9 and sgRNA to be cloned into a single LV vector. LVs are also easier to make than AAVs. In both proliferating and pervading all nature and humanity cells, the LV transmission mechanism is very effective (Kotterman et al., 2015). These benefits suggest that LV matrices are a kind of vector perfect platform for *ex vivo* and *in vitro* administration (Kotterman et al., 2015). Nevertheless, the most difficult aspect of LV technologies is stochastic incorporation entering the genome of host cells. The presence of LVs near gene mutations may result in their stimulation, leading to cancer (Popescu et al., 1990). This eliminates the possibility of using LV-mediated CRISPR-Cas9 for *in vivo* gene manipulation in clinical studies (Rothe et al., 2013). Furthermore, insertion/deletion alterations produced by bacteriophages have caused some therapeutic studies to fail (Check, 2005). implying that employing LVs in patients might be dangerous. The creation of integration-defective lentiviruses with mutation integrase-expressing plasmids might enhance the reliability of LV transmission (K.-C. Liu et al., 2014). Nonetheless, backdrop assimilation happens to vary degrees and seems to be inevitable (Saeed et al., 2014). When CRISPR-Cas9 is delivered efficiently using in the case of bacteriophages, this leads in a greater proportion of cutting than when alternative approaches are used. Even though this is generally favorable, in some disease circumstances, such as spinal cord injuries and ocular diseases, a little amount of preparation or conversion in a percentage of the cells may provide therapeutic benefits (Cai et al., 2018). As a result, excessive trimming may pose a risk in certain situations. Each medical condition should be taken into account when determining the efficacy of genetic manipulation.

Nonviral Physical Methods

Microinjection is a method that involves using a microscope and a syringe to inject sgRNAs and Cas9 actively into cells. The molecular mass of Cas9, which is normally a hindrance in viral administration because of its restricted multiplying capabilities, is not an issue in inoculation since the cannula protrudes through the cell surface to carry the contents directly into the nucleus. Furthermore, manually injecting payloads into cells allows for precise payload dosage. Microinjection, on contrary, takes a long time and is technically difficult, resulting in a low throughput method. Moreover, since infusion requires a microscope, this approach is not suitable for *in vivo* patient work. The majority of microinjection techniques are utilized to generate transgenic animal studies in animal zygotes (Horii et al., 2014; Y. Ma et al., 2014). Transfection is a common method of physical distribution. It uses electrical current pulses to promote the brief release of apertures in cellular membranes, allowing the payload to be delivered into cells. Because it successfully transports payloads into a broad range of cell types, functionalization is extensively utilized *in vitro* and *ex vivo* genetic

manipulation. This is preferable to traditional translation procedures, which are sometimes hindered in tricky cell types like primary cells. The improvement of stem cell treatments, particularly for the treatment of malignant cancers, has been aided by *ex vivo* gene editing using transfection (Dever et al., 2016). *ex vivo* modified donor hematological spinal cord and brain cells are implanted and reintroduced into patients for therapy (Romero et al., 2018). Even though *in vivo* electroporators are now accessible and have been shown to effectively alter genes in animals (Melo & Blackshaw, 2018). The use of laser ablation in individuals for *in vivo* genetic manipulation is still not widely accepted. Furthermore, the cost of cell lysis genetic manipulation is generally considerable since it requires significant adjusting the Cas9-to-sgRNA ratio and electrodeposition parameters for each cell in the body. Significantly, the high electric charge induced by electrokinetic causes a significant proportion of mortality, implying that this approach may not be appropriate for strain cell types (Melo & Blackshaw, 2018).

Nonviral Chemical Methods

For nucleotide distribution, liposome nanoparticle (LNPs) is extensively utilized (Pensado et al., 2014). Vesicles are round entities made up of phospholipid bilayers that develop in water. Since plasmid DNA and cell membranes are both polarized, they repel each other, preventing nucleic acids from entering cells. Opposite charges nucleic acids are encapsulated in positively charged exosomes, allowing the combinations to fuse through cell membranes and enter cells (Pensado et al., 2014). DNA ("all-in-one" plasmid), mRNA (Cas9 and sgRNA), or protein may all be used to administer the CRISPR-Cas9. The Lipofectamine solution is now the most widely used method for LNP production. Through Lipofectamine production, the CRISPR-Cas9 system has been effectively administered *in vitro* and *in vivo* for gene editing (Zuris et al., 2015). Polyethylene and poly-L-lysine are non-lipid polymeric chemicals that are often employed to create nanostructures for CRISPR-Cas9 delivery (Zhen et al., 2015). Likewise, polyamide chemicals promote cellular uptake into cells by encapsulating CRISPR-Cas9 payloads in positive electrode compounds (Longo et al., 2013). Since nanomaterials distribution does not include viruses, it is a safer option. Furthermore, LNPs does not damage cells as much as transfection does. As a result, the U.S. Food and Drug Administration has authorized this delivery device for medication administration (Allen & Cullis, 2013). In medical tests for the therapy of diverse disorders, both polymer-based and lipid chemicals were employed to administer CRISPR-Cas9 (F. Chen et al., 2020). However, as contrasted to viral transmission and electrodeposition, the effectiveness, which depends only on the endocytosis route, is poor. Chemical transfected approaches, for example, in the human embryonic stem, fewer than 10% of the cells produced enhanced green fluorescent protein. After every cell division, transgenic translation likewise diminishes over time (Moore et al., 2005). Cell-penetrating peptides (CPPs) are small peptides having the capacity to penetrate cell membranes on their own. They've been used to make it easier to transfer a range of payloads into cells (Lim et al., 2016). Alternatively, CPPs may be coupled to Cas9 and sgRNAs (Suresh et al., 2017). alternatively, in the majority of instances, only coupled to Cas9 and then complexed with sgRNAs to generate RNPs during distribution (Ngwa et al., 2017). Since stochastic incorporation and indel mutations are not issues, CPPs seem to be a safer bet for Cas9 RNP distribution. Nevertheless, as contrasted to antiviral and teaching, CPP-based distribution is ineffective, due to a low proportion of gene manipulation (Lino et al., 2018). While CPP-based delivery is effective in *ex vivo* and *in vitro*, they are not effective in humans (Henriques et al., 2005). Cas9 RNPs may be delivered

effectively using gold nanoparticles (AuNPs). AuNPs do not cause an immune reaction after injection because they are unreactive (Zhang, 2015). As a consequence, their actual safety increases. AuNPs (15 nm) are initially linked to a 5' thiol changed mono nucleotide pattern hybridized to single-stranded donor DNA in Lee et al. CRISPR-Gold techniques (K. Lee et al., 2017). Following that, Cas9 RNPs are loaded onto donor DNA before being coated with entire silicate nanoparticles and the copolymer PAsp(DET) (K. Lee et al., 2017). These findings suggest that CRISPR-Gold is more able to induce HDR *in vitro* than Lipofectamine transfected or nucleofection. furthermore, mice were given CRISPR-Gold intramuscularly, prompting HDR to correct a missense mutation in the gene product *in vivo* with a 5 % success rate (K. Lee et al., 2017).

Extracellular Vesicles Deliver CRISPR-Cas9 Gene Editing

Although virus vector-based transportation is exceedingly effective, there is a danger of genetic modifications with LVs, and AAVs have a restricted ability for payload replication. Physiochemical approaches, on the other hand, are seldom successful *in vivo*, limiting their use in clinical studies. Multiple investigations have recently shown that extracellular vesicles (EVs) can efficiently transport Cas9 RNPs *in vivo* and *in vitro*, implying that this technology might be used in therapeutic settings (Choi et al., 2016; Mangeot et al., 2019).

The transcription and identity of the viral membrane and/or viral structural proteins are required for the production of EVs **(Figure 1)** (Montagna et al., 2018). If both the phages and viral matrix proteins are formed into EVs, the name virus-like nanoparticles (VLPs) are employed. The target sequence is coupled to the Gag fusion protein so that they may both be integrated into a molecule at the same time. The trypsin from Pol causes breakage along with the Gag protein complex during the viral developmental phase, exposing the specific protein for distribution (Bell & Lever, 2013). In compartments, there are no or few viral special proteins. The Gag protein chain and proteolytic degradation are not required for particle production. When virion components are highly conveyed, vesicles develop from the breaking of the cell membrane (Bell & Lever, 2013). EVs, on the other hand, do not include a viral vector, except for LVs **(Figure 1)**. As a result, EVs are incapable of integrating into their hosts' chromosomes or reproducing (Fuenmayor et al., 2017). Because of their higher safety characteristic, EVs are a safe method of viral transmission. Furthermore, brief Cas9 activation in cells via EVs considerably minimizes the risk of off-target impacts caused by long-term Cas9 transcription (X. Wu et al., 2014). Just since normal plasmid transfected into packing cells is needed, the manufacturing of EVs is likewise easy and cost-effective. VLPs have been used frequently in the creation of vaccines (Fuenmayor et al., 2017). Vaccination predicated on VLPs transmits epitope peptides to hosts, triggering an immune reaction, and is much safer than using weakened viruses (Mohsen et al., 2017). Additional molecules, such as fluorochromes, Cre directly correspond, and mammalian protease 8, have also been delivered using VLPs (Kaczmarczyk et al., 2011). As a result, the present study will focus on Cas9 peptide transport through EVs. Choi et al. (Choi et al., 2016) showed that the Cas9 protein may be wrapped into VLPs for temporary Cas9 engagement in cells, preventing CRISPR-Cas9 off-target impacts. In a nonviral Gag/Pol production system, the Cas9 genome was linked to the Gag gene. VSV-G and wild-type Gag/Pol co-transfection cassettes resulted in VLPs. The fused plasmid's protease breakage site between Cas9 and Gag enabled the Cas9 protein to be released after viral development (Choi et al., 2016). Furthermore, relative to LVs releasing Cas9 after incorporation into genomes,

Cas9-preloaded VLPs reduced off-target effects implying that transitory Cas9 treatment with VLPs is beneficial. This is the first study to show Cas9 packing into VLPs for genetic manipulation, even if it did not show the maximum usage of sgRNAs to Cas9 to form RNPs before distribution (Choi et al., 2016). The retroviral leukemia virus Gag-Cas9 expression template was co-transfected with the baboon membrane, VSV-G, sgRNA, and wild-type Gag-Pol plasmids to create nanoblades (Mangeot et al., 2019). *In vitro* in primary cells and induced pluripotent stem cells (iPSCs), and also *in vivo* in mice, nanoblade-based gene modification was reported (Mangeot et al., 2019). The findings of this work have paved the way for *in vitro* and *in vivo* Submits gene manipulation. VLP creation, unlike vesicle synthesis, needs the wild-type Gag-Pol plasmid supplying protease to release Cas9. However, during VLP packing, competing between Gag-Cas9 and wild-type Gag proteins limits the amount of Cas9 protein that may be used (Briggs et al., 2004).

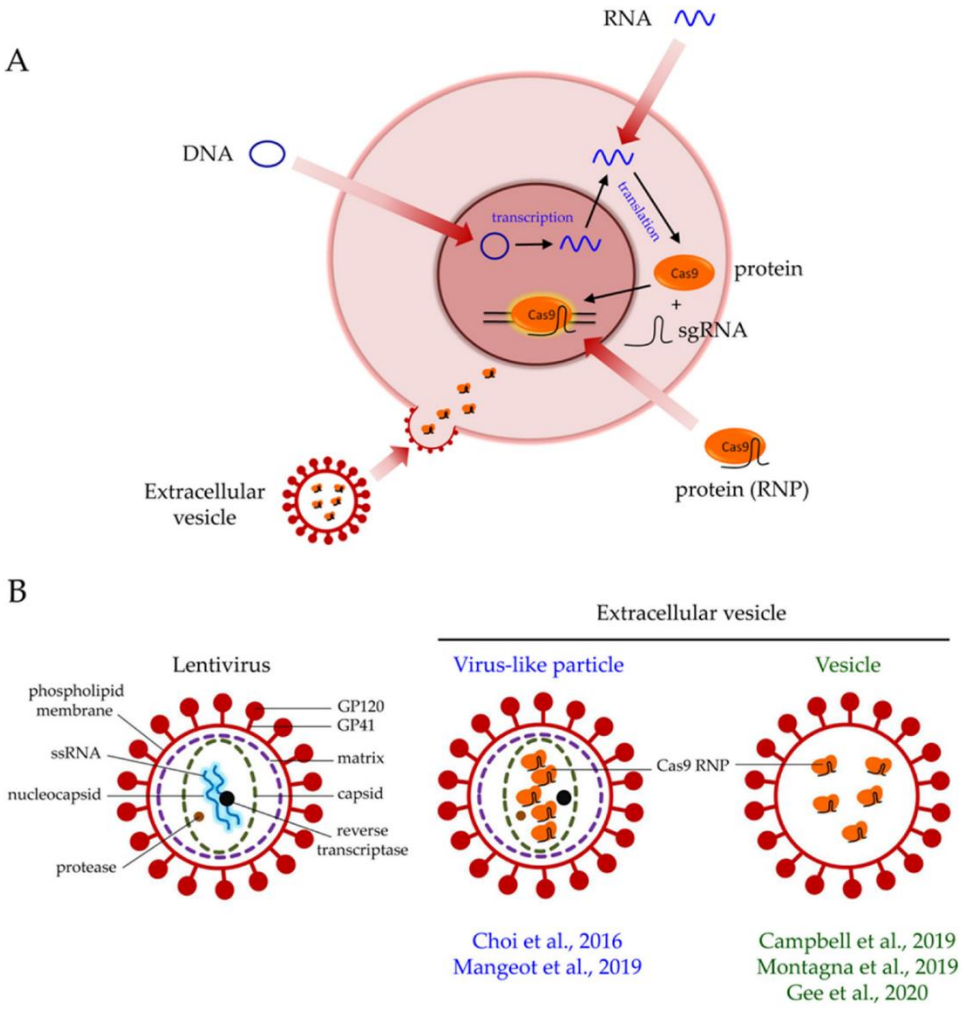


Figure 1. Cas9 RNPs are delivered by EV. (A) Cas9 may be supplied in DNA, mRNA, or protein form. When Cas9 is in the nucleus, the protein structure allows it to operate immediately. EVs are transduced into cells, releasing pre-loaded Cas9 RNPs for effective gene manipulation. (B) Differences in structure between lentiviruses, virus-like particles, and vesicles (Yip, 2020).

CRISPR-Cas9 Mechanism and Function

According to Makarova et al. (Makarova et al., 2011), the CRISPR-Cas9 system aids three stages of the immune response: adaptation, expression, and interference. Small

plasmids are introduced into the CRISPR-Cas9 series as novel spacers during the adaptation stage, while crRNA transcription and maturation occur during the expression stage. Finally, during the interference phase, the crRNAs cause Cas proteins to match the splitters. Makarova et al. (Makarova et al., 2011) proposed a hierarchical classification of CRISPR/Cas into three major divisions, including I, II, and III, in research on the genesis of CRISPR-Cas9 and Cas proteins. Cas9 is a big multipurpose sole sign protein in the Type II system that is responsible for both crRNA synthesis and target DNA slicing (Makarova et al., 2011). Several Cas proteins are used as sign proteins in Type I and III systems. The Type II system, in comparison to the other systems, has a simpler design and can be developed more quickly as a genome-modifying tool. Genome evaluation, sgRNA cloning, plant conversion, genomic DNA harvesting, and sequence evaluation to validate the findings are all part of the CRISPR-Cas9 genetic modification process **(Figure 2)**. *Streptococcus pyogenes* was found to yield a short trans-noncoding RNA that was shown to direct crRNA synthesis via RNase III and Cas9 by matching bases with duplicated sites of pre-crRNA transcripts. Jinek et al. (Jinek et al., 2012) discovered evidence for a Type II system and used tracrRNA and crRNA to create a sgRNA. Any sequence may be transformed from the 50 end 20-bp sgRNA sequence, which is a target associating DNA sequence. According to the results, a sgRNA may be used to program three pairs of PAM bases to cause DSB near the Cas9 nuclease. In various CRISPR/Cas systems, PAMs are sequenced and located in different ways. A typical SpCas9 PAM is 50-NGG-30, and a PAM was used to complement a 20-bp target DNA sequence. RuvC and HNH have two domains in common with Cas9 nuclease. The RuvC and HNH domains, according to Jinek et al. (Jinek et al., 2012), may complement DNA but not RNA. As a result, this system is both efficient and customizable, and it functions similar to a conventional CRISPR-Cas9 technique, that is often employed to edit genes, with non-homologous end joining (NHEJ) or HDR facilitating the modifications. Several RNAs have a complex and well-studied function in CRISPR-Cas9. The guided RNA (mgRNA) is a unique RNA sequence that recognizes certain DNA sites and directs the Cas9 nuclease to induce genetic changes. As a result, the DNA is unfolded, and the gene is aligned with the Cas9 protein, causing double-stranded breakage. CrRNA is the directed DNA of the Cas9 protein, while tracrRNA is a scaffold that links the crRNA molecule to the Cas9 protein and helps in sorting pre-crRNAs from mature crRNAs produced by CRISPR-Cas9 (Rasheed, Gill, et al., 2021).

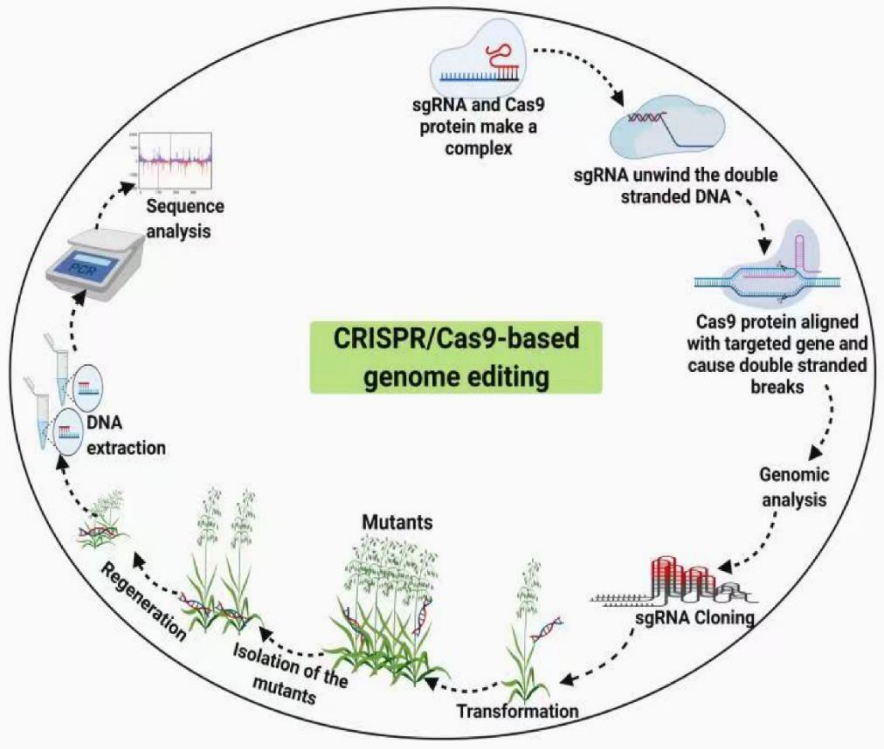


Figure 2. Cas9-mediated genome manipulation process. The Cas9 genome manipulation system includes a combination of Cas9 protein and sgRNA, as well as DNA unwinding by sgRNA, gene cutting by Cas9, analytical tools, cloning, and transformation. For manipulation, there is no requirement for a third-party component (Rasheed, Gill, et al., 2021).

CRISPR-Cas9 applications in cancer treatment

Cancer cells undergo (epi)genome editing for therapeutic purposes

Knowing that cancer is a genetic illness caused by the accumulation of genetic/epigenetic abnormalities, employing CRISPR-Cas9 to repair oncogenic genome/epigenome abnormalities might be a potential cancer therapeutic approach **(Figure 3 a)**.

A urothelium-specialized human uroplakin II (hUP II) promoter and a cancer-specialized human telomerase reverse transcriptase (hTERT) promoter, for instance, regulated sgRNA and Cas9 and production in a cellular model of bladder cancer (Y. Liu et al., 2014b). hBax, E-cad, and p21 were stimulated by sgRNA Cas9 solely in bladder cancer cells, resulting in cancer cell-specific pro-apoptotic and anti-proliferative actions. Aubrey et al. (Aubrey et al., 2015) found that knocking down the myeloid-cell leukemia 1 (MCL-1) gene in human Burkitt's lymphoma (BL) cells using the lentiviral CRISPR-Cas9 approach increased the rate of BL cell apoptosis, which resulted in cancer progression limitation in a human BL xenograft model through the recurrent expression of sgRNA. Protein kinase C (PKC), a tumor inhibitor, was also shown to be deficient in colon cancer cell lines derived from a patient. In a xenograft model, CRISPR technology was used to repair a PKC mutation, which decreased tumor development (Antal et al., 2015). These findings show that knocking down genes essential in cancer cell proliferation and survival significantly lowers the growth of cancer cells and induces apoptosis, limiting tumor development. Conversely, cancer cells may be killed by knocking in a therapeutic transgene at a specific site using CRISPR-Cas9-mediated homologous recombination (Wan et al., 2017).

Furthermore, this approach may be used to modify the epigenome of cancer cells, indicating its potential role as a novel epigenetic treatment. Because cancer is so heterogeneous, genomic abnormality profiles differ not just across patients' tumors, but also between tumors at different phases or from various parts of the patient, making (epi)genome modification in cancer very difficult. Furthermore, since untreated cells have a growth advantage over edited cells, this approach needs a great editing efficiency. The former proliferates faster than the latter, invalidating the therapeutic impact. It's also essential to choose the right delivery methods, particularly *in vivo* (M. Chen et al., 2019).

Combating carcinogenic virus infection

Epstein-Barr virus (EBV) infection in nasopharyngeal carcinoma, Human papillomavirus (HPV) infection in cervical cancer, BL and Hodgkin's lymphoma (HL), infection in nasopharyngeal carcinoma, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection in liver cancer is all examples of carcinogenic virus infection. The CRISPR-Cas9 system has intrinsic potential for the protection and elimination of viral infection because of its antiviral activity as bacterial adaptive immunity. The viral genome-specialized Cas9-sgRNA might be used to specifically target and destroy viral oncogenes, and also the genes essential for viral replication and survival, resulting in viral genome mutations, decreased viral oncogene production, and eventually tumor cell mortality **(Figure 3 b)**. As a result, fighting infection caused by a virus and removing these microbes may be able to prevent or even revert tumorigenesis, bringing hope to those who are suffering from cancer caused by viruses (M. Chen et al., 2019).

Cervical cancer is most often caused by the HPV. When E6 and E7 oncoproteins of HPV are expressed in normal cells, they cause cancer to develop and then persist (Clifford et al., 2019; Hoppe-Seyler et al., 2018). CRISPR-Cas9 targeting genes and promoters of the E6 and E7 resulted in the suppression of cervical cancer development and reversion of the malignant phenotype, according to several studies (Zhen et al., 2016). Finding a distinct desired sequence may be difficult because of the small length of the E6 and E7 genes. As a result, more specific targeting of these oncogenes might be a strong therapeutic strategy for cervical cancer. Chronic HBV infection of hepatocytes is directly associated with the development of hepatocellular carcinoma (HCC). In the cell nucleus, the dsDNA of HBV virion is converted to double-stranded covalently closed circular (ccc)DNA, which is used to make viral protein-coding mRNAs and pre-genomic RNA. HBV DNA modification and elimination using CRISPR-Cas9 have been shown to effectively suppress viral replication in both mouse models and cells in earlier research (Dong et al., 2015; Liu et al., 2015; Seeger & Sohn, 2016). Such models do not accurately represent HBV infection in individuals who have HBV cccDNA primarily in their hepatocytes. To do so, the scientists used a plasmid encoding HBV to create a huge quantity of HBV cccDNA in mice, which is a much better way of mimicking chronic HBV infection *in vivo* (Dong et al., 2015). HBV cccDNA in the liver was significantly reduced when anti-HBV CRISPR was given hydrodynamically into the tail vein. Moreover, using CRISPR-Cas9 to target sites that encode the antigen of HBV suppressed HBV growth and served as an antiviral (Zhen et al., 2015). As previously stated, the present therapy only partially scavenges the virus. The cccDNA molecule is required for HBV removal from infected hepatocytes, suggesting that more research is needed. HCV, like HBV, aids in the formation of HCC.

Price et al. (Price et al., 2015) developed Francisella novicida Cas9 (FnCas9) to specifically attack the HCV viral RNA genome, resulting in reduced viral protein synthesis and suppression of HCV infection, using RNA-guided RNA detection. FnCas9 might be utilized to combat several viruses at once, such as HBV and HCV, since it can attack both DNA and RNA.

BL ,HL, and Nasopharyngeal carcinoma have all been associated with EBV (Ok et al., 2015; Taylor et al., 2015). EBV infection mostly affects epithelial cells and B cells. The ability of CRISPR-Cas9 to target the EBV genome has been shown (van Diemen et al., 2016; Wang & Quake, 2014). To target the EBV genes required for episome stability, CRISPR-Cas9 was also inserted via a lentiviral transfection approach. In EBV-positive lymphoma cells, most of the EBV genome was lost, up to 95 %. CRISPR-Cas9 can prevent and cure cancer caused by a viral infection, to these solid evidences. Nevertheless, the broad range of viral targets is a challenge for current treatment methods, and simultaneously targeting many critical loci in the viral genome may be essential (M. Chen et al., 2019).

Stromal-targeting therapies

Stromal cells and Cancer cells interact and modify one another throughout carcinogenesis, forming a symbiotic association that promotes cancer growth and therapeutic resilience. CRISPR-Cas9 might be used to reprogram the stroma of tumors to make them antitumor **(Figure 3 c)**.

Yang et al. (L. Yang et al., 2016) discovered that genes implicated in glutamine production were upregulated in cancer-associated fibroblasts (CAFs) in a study comparing fibroblasts from healthy ovarian tissue with fibroblasts with developed ovarian cancer. Inactivation of glutamine synthetase (GS), a glutamine-producing enzyme, reduces cancer cell development but does not affect CAFs' ability to promote cancer cell growth (L. Yang et al., 2016). As a result, researchers believe that CRISPR-induced matrix GS knockdown might be an economical way to suppress cancer growth and provide therapeutic advantages. Likewise, the stroma is prominent in pancreatic cancer, which has been thought to act as a physical obstacle preventing chemotherapy drugs from reaching cancer cells (Zambirinis & Miller, 2017). According to Sherman et al. (Sherman et al., 2014), the vitamin D receptor (VDR) is a major regulator of pancreatic stellate cells. VDR activation resulted in the remodeling of reactive stroma and the reduction of fibrosis-related inflammatory markers in a cancer model, resulting in increased drug availability (gemcitabine) and cancer prevention (Sherman et al., 2014). CRISPR technology may improve drug penetration, stroma reprogramming, and VDR activation to provide anticancer benefits. Stromal cells are simpler to genetically alter than cancer cells, which have a lot of (epi)genetic heterogeneity and dynamics. Furthermore, stromal cells that have been genetically modified may not have the same fitness disadvantage as cancer cells that have been therapeutically modified (M. Chen et al., 2019).

Anticancer drug development

The discovery of novel tumor-targeted therapies is an important component of cancer treatment. Drug target validation, detection of drug-resilient genes, and the discovery of novel drug genomic loci are currently major challenges in the production of anticancer drugs **(Figure 3 d)**.

CRISPR-Cas9 technology is used to confirm drug targets and identify resistance genes. In cancer cells, the cysteine 528 residue of exportin-1 was shown to be the main

target of Selinexor (Neggers et al., 2015). According to transcriptome sequencing and computational analysis, the kinesin-5 A133P mutation might be the cause of ispinesib resistance (Kasap et al., 2014). Kasap et al. (Kasap et al., 2014) used to induce the A133P mutation in HeLa cells using CRISPR-Cas9, resulting in better ispinesib resistance in mutant cancer cells. Ispinesib was also shown to be a primary target of kinesin-5 in cancer cells. Furthermore, by targeting the exons that encode a protein, the CRISPR-Cas9 technology has been widely employed to find novel therapeutic genomic regions in cancer (J. Shi et al., 2015). In melanoma cells, researchers used a CRISPR-Cas9 knockout library with 64751 unique sgRNAs to find novel and more potential genes whose removal resulted in resistance to the BRAF protein kinase inhibitor vemurafenib (Shalem et al., 2014). In the genomes of acute myeloid leukemia (AML), the CRISPR-Cas9 technique was also used to identify therapeutic targets and check for genetic susceptibility (Tzelepis et al., 2016). KAT2A has been suggested as a promising target for further research, and suppression of KAT2A has been proposed as a treatment for AML. These outcomes are exciting. Because of its high effectiveness and relative ease, the CRISPR-Cas9 technique has a lot of potential for confirming drug targets, detecting drug resistance genes, and discovering novel therapeutic targets. This will make it easier to develop anticancer drugs (M. Chen et al., 2019).

Cancer immunotherapy

Cancer immunotherapy is a quickly growing discipline currently, because of the clinical improvements it has gained in a variety of cancers. Cancer immunotherapy, instead of directly targeting tumors, employs a variety of techniques to combat tumors via innate or adaptive immunity **(Figure 3 e)**.

Unleashing T lymphocytes against cancers by suppressing immunological checkpoints like programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte antigen 4 (CTLA-4), and programmed cell death 1 ligand 1 (PD-L1) is an approach that has been effectively adapted to the clinic (Hoos, 2016; Zhou et al., 2017). As a result, using CRISPR-Cas9 technology to knock down these genes, such as the PD-1 and CTLA-4 genes, might be critical for improving cancer immunotherapy efficacy. CRISPR-Cas9 was just used to modify the genome of human T cells, and CRISPR-mediated indel mutations were shown to decrease PD-1 expression. Su et al. (Su et al., 2016) recently showed that plasmid-encoded Cas9 and sgRNA electroporation into human T lymphocytes reduced PD-1 expression while increasing T cell immunological reactions to cancer cells and their ability to efficiently destroy tumor cells. Cell therapy is another technique that has shown potential. It fights cancer by genetically altering immune cells *in vitro* and then delivering them to patients. Anti-CD19 chimeric antigen receptor T cells (CAR-T) had already been shown to be effective in the treatment of leukemia (Maude et al., 2014). Due to their high efficacy in hematological tumors, several scientists are focusing on the production of CAR-T cells for solid tumors (Johnson et al., 2015). CRISPR-Cas9 technology, which is based on the simplicity of use and adaptability, can impair many genomic sites at once, resulting in universal CAR-T cells that lack PD-1, human leukocyte antigen-I (HLA-I), and endogenous T cell receptor (TCR) (M. Chen et al., 2019; Ren et al., 2017). As a result of the integrated gene alteration that includes inhibitory receptors, histocompatibility, and endogenous TCR, researchers believe that CAR-T cell treatment will become more trustworthy, efficient, and secure in the future (M. Chen et al., 2019).

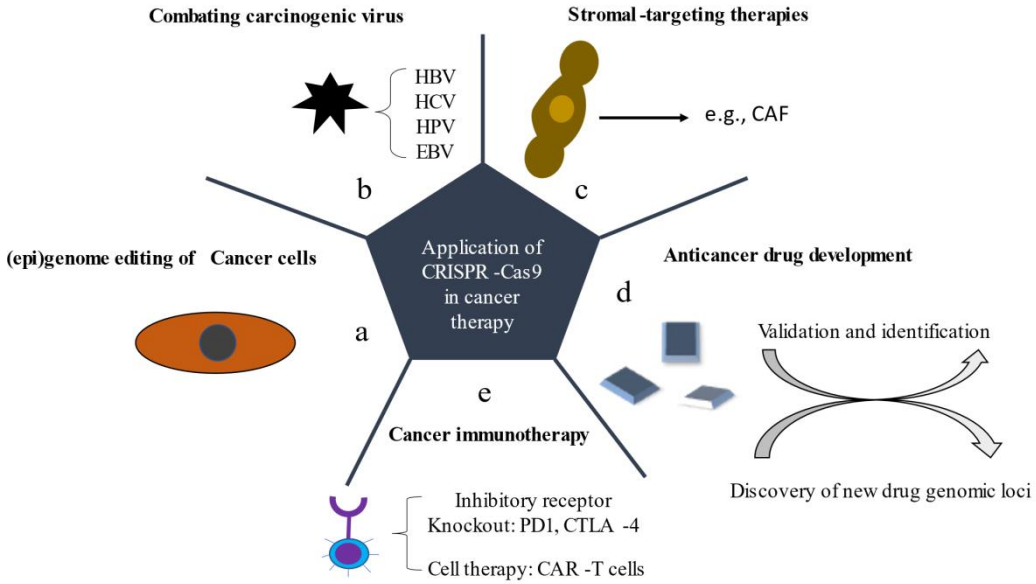


Figure 3. CRISPR-Cas9 has the capacity to be used in cancer treatment. a. Cancer cell (epi)genome modification for therapeutic purposes. Knocking down genes associated with cancer cell growth and longevity inhibits tumor development by reducing cancer cell development and promoting apoptosis. b. Combating carcinogenic virus infection. Viral oncogenes, and also the genes essential for viral survival and replication, may be specifically targeted and destroyed using the viral genome-specialized Cas9-sgRNA, which all lead to viral genome changes, reduce viral oncogene production, and eventually cause tumor apoptosis. c. Stromal-targeting therapies. To attain anticancer actions, CRISPR-Cas9 may be employed to reprogram tumor stroma. d. Anticancer drug development. Particular drug targets are verified, resistance genes are found, and novel drug genomic loci in cancer are identified using CRISPR-Cas9 technique. e. Cancer immunotherapy. CRISPR-Cas9 technique might be vital for improving the effectiveness of cancer immunotherapy dependent on T cells by knocking out inhibitory receptors like CTLA-4 and PD1. Cell therapy is a promising option as well (e.g., CAR-T cell therapy) (M. Chen et al., 2019).

Crop Improvement Using CRISPR-Cas9

The CRISPR-Cas9 approach is the most widely utilized, and it has a lot of benefits, including efficiency, cheap editing, extreme versatility, and the capacity to spontaneously direct several gene reproductions (Braatz et al., 2017). This is particularly useful for reproducing polyploid crop species that are difficult to progress utilizing traditional breeding methodologies, as it allows the grouping of a variety of biallelic mutants and monoallelic, as well as a phenotypic allelic sequence, in the first generation, which is typically not possible using traditional breeding strategies. This approach to genome editing is more effective and diverse (Hsu et al., 2014).

Because of its beneficial characteristics, the CRISPR-Cas9 method has been employed for various species of plant and its use gives the right answer to various plant breeding challenges (Gao, 2018). CRISPR-Cas9 is used to enhance monocot and dicot crop productivity, immunity to disease, quality, and climate variability tolerance (Ma & Liu, 2016). Cereal crops, vegetables, and fruits have all had their genomic

information altered using CRISPR-Cas9. The most prevalent use is the induction of indels that cause frameshift mutations to knock out particular genes (Ricroch et al., 2017).

Improve Quality and Yield using CRISPR-Cas9

Crop species undergo radical changes as a result of CRISPR-Cas9 (T. Wang et al., 2021). Because of its potential, several laboratories worldwide have been developed to utilize this powerful approach. Here are a few of the applications that are being explored to improve agricultural quality and production. CRISPR-Cas9 is utilized to generate crops with superior nutritional qualities and defensive traits and to achieve the greatest landmark of genome modification in modern technology. The crop seeds of Camelina sativa, which is frequently grown due to its greater proportion of fatty acids, were used to make a commercial oil. The Brassinosteroid Insensitive1 (BRI1) gene and JAZI, a jasmonate-zim-domain protein, were also modified, with a mutation rate ranging from 26% to 84 % (Jaganathan et al., 2018). Cas9 was utilized to alter a flowering locus, squamosal promotor binding protein, in a related species, and the plants had a 90% mutation frequency in late flowering (Hyun et al., 2015). A multiple CRISPR-Cas9 system was developed and employed in the first generation to manipulate six changed ABO receptors PYL genes with a mutation rate of 13 % to 93 % (Zhengjing Zhang et al., 2016). RNA-facilitated endonucleases were used to change the Nicotiana benthamiana's green fluorescent gene (Nekrasov et al., 2013). Later, the tobacco rattle virus was employed to alter plant gene directions, allowing them to generate and control transcriptional characteristics. Cas9 was also used to study the function of GS3, two QTLs, and Gn1a in rice grain size and quantity (Shen et al., 2018). Usman et al. (Usman, Nawaz, Zhao, Liao, et al., 2020) looked into Cas9-based changes to the OsSPL16 gene in rice and discovered an increase in rice grain production. Cas9 mutagenesis of CLBG1 in watermelon improved seed germination and reduced seed size (Y. Wang et al., 2021). Cas9 has the potential to enhance every characteristic of crops, perhaps leading to an agricultural revolution in the future. Rice cooking quality was enhanced by using Cas9 to alter the WAXY allele of granule-bound starch synthase (Mahmuda et al., 2021). The interruption of MIR396f and MIR396e, which increases rice production under reduced nitrogen circumstances, was examined by Zhang et al. (J. Zhang et al., 2020). Rice seed dormancy is regulated by the grain production modulator miR156, according to research (Miao et al., 2019). CRISPR-Cas9 was used to modify three homologs in rice to produce novel rice mutants with higher production and odor (Usman, Nawaz, Zhao, Liu, et al., 2020). The quality of rice cooking is often lowered when there is a significant concentration of protein. selective mutation of amino acid carrier genes might result in lots of mutants with several diverse characteristics. In rice high-production varieties, Cas9 produced two mutants, OsAAP6 and OsAAP10, which demonstrated considerable improvements in rice production and taste (S. Wang et al., 2020). Based on the findings, we believe that subsequent alteration of amino acid genes might contribute to the development of elevated production and high-quality rice varieties. Using Cas9 to alter the GS3 gene, many rice mutants with excellent yielding characteristics were produced. Rice complex characteristics might be enhanced using CRISPR-Cas9, according to this research (Zeng et al., 2020). The use of CRISPR-Cas9 to selectively mutate TaSBEIIa resulted in increased amylose wheat with much higher tolerated starch concentrations (J. Li et al., 2021). The effects of altering TaSBEIIa on the content, structure, and characteristics of starch in spring wheat were studied using CRISPR-Cas9 (J. Li et al., 2021). CRISPR-Cas9 has been used to produce high-quality oils from Brassica napus, such as oleic acid (Okuzaki et

al., 2018). In Camelina sativa, CRISPR is employed to enhance pod-shattering tolerance by altering the activity of the ALCATRAZ INDEHSCENT and JAGGED genes (Ellison et al., 2020; Morineau et al., 2017). Another research reveals that the ALCATRAZ and INDEHSCENT genes, which are required for fruit dehiscence in Brassica species, are impacted. Similarly, Zaman et al. (Zaman et al., 2019) used Cas9 to modify the genome of the JAGGED gene in Brassica napus, discovering that this gene is important in the creation of pod shattering tolerance. ALCATRAZ's function in Brassica napus pod shattering tolerance was also investigated (Braatz et al., 2017). CRISPR-Cas9 has been extensively used in plants, particularly in tomatoes, due to its commercial relevance and simplicity of transformation. Tomatoes with targeted modifications to the synthetic gene SP5G had early blooming and many brushes, indicating a timely cultivation (Soyk et al., 2017). Liang et al. (Liang et al., 2014) revealed the targeted deletion of genes implicated in the production of phytic acids in maize, such as ZmIPK1A, ZmIPK, and ZmMRP4. The needle structure trait in tomatoes was discovered using a CRISPR-Cas9-mediated disruption of the SISG07 gene (Brooks et al., 2014). The apple protoplast's taste and quality were also improved using CRISPR-Cas9 (Malnoy et al., 2016). CRISPR-Cas9 was employed to develop steroidal glycoalkaloids in some potato varieties to influence St16Dox. Two SGA-free potato lines with a knockout in the St16Dox gene were developed as a result of the research (Toda et al., 2019).

Using CRISPR-Cas9 to Develop Disease-Resistant Varieties

Plant diseases have a significant impact on agricultural quality and yield (Singh, 2021); the primary pathogens include bacteria, nematodes, fungi, insects, and viruses all of which result in significant crop output losses. Disease-tolerant crops are now being generated using CRISPR-Cas9 technology (Z. Khan, T. Saboor, et al., 2021). The emergence of fatal insect epidemics, as well as other biotic stresses, has become a serious concern (Al-Sadi et al., 2012). Knowing the links or interactions between plants and pathogens is critical for plant defense against these attacks (Kettles & Kanyuka, 2016). In the wheat crop, there is an increasing demand for gluten-free wheat products. Wheat that is coeliac-safe reduces the chance of chronic diseases. Conventional breeding approaches will not be able to increase these characteristics. CRISPR-Cas9 technology has recently been used to alter glutenin genes to develop gluten-free crops. These approaches result in children with decreased or no gliadins, potentially reducing individuals' exposure to coeliac disorder epitopes (Jouanin et al., 2020). Verma et al. (Verma et al., 2021) explored the role of CRISPR-Cas9 in the production of gluten-free wheat to reduce the incidence of the coeliac disorder. Researchers concluded that the wheat genome has been effectively altered using CRISPR-Cas9. In the future, this approach might be used to make wheat rice that is safe from coeliac disease. The Elf4g gene was altered in rice to improve tolerance to the tungro spherical virus (Macovei et al., 2018). Bacterial leaf blight is a serious disease that reduces rice yields dramatically. This gene, OsSWEET14, was targeted by Cas9 to produce pathogen resistance (Sam et al., 2021). This demonstrates that selective mutagenesis of any sensitive gene may result in considerable variance in plant disease resistance. For example, Cas9 was used to knock out the OsERF922 gene, leading to enhance resistance to blast generated by Magnaporthe oryzae (F. Wang et al., 2016). Likewise, Cas9 was used to conduct targeted mutations in SWEET1E to develop blight-resistant plants (Zhou et al., 2015). As a consequence, genetic modification has been effectively used to study plant-pathogen interactions, and CRISPR-Cas9 is a promising way of generating disease-tolerant crops by altering disease-causing genes. Cas9 was used, for example, to cause changes in the CsLOBI

gene, which causes citrus canker, resulting in increased citrus canker resistance. The mutation frequencies were greater in two mutant lines, DLoB9 and DLoB10. CsLOBI frameshift mutations and alterations in activity led to an enhanced Xanthomonas citri resistance (Jia et al., 2017). Cas9 alteration of the effector attaching element in the CsLOBI promoter region was used to enhance citrus resistance to Xanthomonas (A. Peng et al., 2017). In wheat protoplasts, the Cas9 method was used to alter the gene TaMLO (Zhang et al., 2014), leading to the production of increased powdery mildew resistance (Wang et al., 2014). Likewise, by targeting EDRI homologs, Cas9 was utilized to provide resistance to powdery mildew in wheat (Zhang et al., 2017). In tomatoes, MLO gene variants were produced that improved powdery mildew resistance (Nekrasov et al., 2017). Viruses are thought to be responsible for 50% of all plant disorders, resulting in significant losses in agricultural production and quality (Zaidi et al., 2016). The genome's targeting efficacy was greatly improved by DNA virus amplicons. Geminivirus replicons were used for Cas9 transient expression to combat the dwarf virus in hexaploid wheat, leading to a 12-fold rise in gene expression (Gil-Humanes et al., 2017). CRISPR-Cas9 is used to target the geminivirus genome and block viral development (Ji et al., 2018). Instead of treating viral infections, Cas9 may be used to alter viral DNA (Zaidi et al., 2016). Cas9-based viral genome modification might be increased by employing virus promoters to regulate sgRNA cassata production (Ji et al., 2018). Francisella novicida has currently been discovered to have a unique Cas9 ortholog, which has been applied to modify the genome of RNA-based viruses. TMV and cucumber mosaic virus replication was slowed by the FnCas9 gene, which conferred resistance to both. Cas9 is therefore a strong tool for improving crop genomic composition and increasing tolerance to viruses and other sporadic pathogens (T. Zhang et al., 2018).

Using CRISPR-Cas9 to develop climate-smart crops

In important crops like cotton, potatoes, maize, rice, and wheat, Cas9 has been used to combat abiotic stresses. The plant breeding system has been revolutionized by the development of abiotic stress-tolerant cultivars or climate-smart. CRISPR-Cas9 has been used to alter any gene in plants and introduce any trait. Many genes associated with abiotic stress resistance have been found and manipulated into crops by molecular breeders (Razzaq et al., 2021). Slmapk3 protein gene mutants produced by CRISPR-Cas9 increased tomato defensive response to drying stress (L. Wang et al., 2017). Cas9 was used to study two genes in wheat protoplasts, TaDREB2 and TaDREB3, that are associated with abiotic stress tolerance. Alterations in gene expression were detected in roughly 70% of implanted wheat protoplasts using the T7 endonuclease assay (Joshi et al., 2020). Cas9 was used to enhance drought stress tolerance in plants in a detailed study. The Cas9 technique was used to alter two mitogen-activated genes in rice, OsMKP2, and betaine aldehyde dehydrogenase OsBADH2. These genes were transferred into the host genome via particle bombardment and the protoplast transformation method, conferring resistance to a variety of stresses (Shan et al., 2013). The OsAnn3 gene in rice was modified to protect it from cold stress, and its function in genome-modified plants was investigated (Shen et al., 2017). To investigate the stress mechanism in rice, the gene SAPK2 was modified. Previous research has shown that this gene improves rice salinity and drought resistance (Lou et al., 2017). Novel variations of the ARGOS8 gene, which is particularly important in maize, have been used to establish drought resistance (Shi et al., 2017). Drought resistance was conferred in rice by Cas9-mediated mutation of Leaf1,2, which affected ROS scavenging and protein expression profiles (Liao et al., 2019). Zhang et al. (A. Zhang et al., 2019) used directed

mutagenesis of the OsRR22 gene to improve rice salinity resistance. Drought stress has a significant impact on chickpea, a major legume crop. Only one research has been done so far to use CRISPR to cause mutations in genes. Cas9 was used to target two genes, RVE7 and 4CL in chickpea to enhance drought stress resistance. Cas9 gene knockout is a unique technique that might lead to the production of drought-resistant Chickpea cultivars in the long run (Badhan et al., 2021). CRISPR-activated AREB-1 Arabidopsis plants have better drought resistance than wild-type plants, according to De Melo et al. (de Melo et al., 2020). Herbicide resistance may also be introduced into crops using CRISPR. Kuang et al. (Kuang et al., 2020) examined how base alteration aided in the OsALS1's artificial evolution in plants, resulting in novel herbicide-resistant rice germplasm. Likewise, Cas-based mutations were used to investigate two genetic factors, Drb2b and Drb2a, which were found to regulate salinity and drought endurance in soybeans (Curtin et al., 2018). Drought stress is countered by the mitogen-activated protein kinase gene, which protects its membrane from oxidative stress and controls gene transcription. In tomatoes, the role of SIMPAK3 in drought stress was investigated using Cas9 technology to create mutant variants in the SIMPAK3 gene for drought resistance (L. Wang et al., 2017). Crop productivity and stress tolerance are controlled by a large number of genes. These findings suggest that the CRISPR-Cas9 approach has a lot of promise for generating climate-adaptive crops. Cas9 might be used to modify genes to improve resistance to several abiotic stresses, such as heavy metals, nutritional shortages, extreme heat, and drought, according to a recent study (Anwar & Kim, 2020).

Crop Domestication Using CRISPR-Cas9

Crop domestication and crop selection have resulted in the production of high-yielding cultivars that are well-suited to their natural environments. Despite this, the expanding world's population suffers a variety of agricultural issues, such as changing climate, biotic and abiotic stress fluctuations, and agricultural land degradation, as well as the need for more sustainable and specific farming techniques. Relatives of current cultivated and orphan crops are thought to be a rich source of new variability. Their limited productivity and unattractive appearance, however, inhibit commercial production (Lyzenga et al., 2021). De novo subjugation by gene modification has recently been proposed as a strategy for quickly domesticating orphan and wild crops, using recalled genetic differences and domesticated plant characteristics (Zsögön et al., 2018). Meanwhile, since they are well-defined, contain a basic genomic structure, and are monogenetic in origin, many traditional domesticated genes are well-suited for Cas base manipulation (Li et al., 2018). Ground cherry has some undesirable traits that cause fruit to drop, such as strong stems and little fruit. The SP5G gene was mutated using the CRISPR base gene-editing technology, which resulted in a huge quantity of fruits (Lemmon et al., 2018). These results indicate that Cas-based genetic manipulation might accelerate the process of domestication while also adding value and utility to orphan crops. Wild plants are domesticated using CRISPR-Cas9 to meet human needs. All major crops, like rice, wheat, and maize, were domesticated by ancient farmers. Our ancestors, on the other hand, relied on a small number of originator species and easily selected crops with superior qualities such as higher yield and breeding convenience, resulting in a decrease in plant natural variety. Domestication of wild plants, with genetic variation as a major consideration in the picking procedure, might help in the conservation of this variety. Wild tomatoes have been domesticated using the CRISPR-Cas9 technology, which is stress-resistant but has a lot of flaws in their fruit yield (Zsögön et al., 2018). Six important yield QTLs were altered in one research, and all lines exhibited increased fruit quality and quantity

(Zsögön et al., 2018). Other crops, like bananas, quinoa and potatoes are critical in the region since they are nutritious and adapt well to the environment. Despite these characteristics, they are unsuitable for large-scale production due to their fruit loss and low production. CRISPR-Cas9 is a very effective approach for genetically modifying crops and achieving desired crop characteristics. This method was currently employed to increase the quantity and size of ground cherry flowers (Lemmon et al., 2018). Researchers are hopeful that by identifying genes that control the domestication mechanism, they will be able to modify the genome and enhance the world food supply. Here are several unique strategies that may help us enhance the effectiveness of genetic modification using CRISPR-Cas9. The first step is to conduct a thorough screening of the characteristics that have to be modified. Genome modification, whether polygenic or monogenetic, requires the understanding of genetic data regarding desired features. The choice of an effective tool is vital to acquire excellent outcomes for editing. Off-target impacts should be observed, and this may be accomplished by developing sequences with a strong affinity for one another. The difficulties of developing sequences that have a strong affinity with one another. The agriculture sector's issues are presenting a major danger to crop yield. Using knockout approaches to bring about desired alterations in agricultural crops, there are several sources of genetic diversity that may be used and investigated. The greater the efficiency of CRISPR-Cas9, the greater the possibilities for effective modification and the more appealing the results (Rasheed, Gill, et al., 2021).

Summary

For *in vitro* applications, CRISPR-Cas9 gene-editing technology has been well-developed. Most delivery systems are adequate for quick editing if patients' safety is not an issue. The most difficult issues for *in vitro* research are off-target impacts caused by longer Cas9 exposure. CRISPR-Cas9 delivery in *ex vivo* contexts are also advancing quickly. Hematological disorders may be treated by harvesting hematopoietic stem cells of individuals for *ex vivo* alteration before the autologous transformation of the altered cells again into individuals. *ex vivo* cell treatment, on the other hand, is negated by the expense and effort required to collect stem cells from each individual for autologous transplants. As a strong modifying tool, CRISPR-cas9 offers significant therapeutic promise for improving our anticancer strategy, although with certain limitations. We believe that cancer treatment based on genetic engineering will bring a revolution. Modern cancer study should also allow chemists, physicists, and engineers to collaborate with biologists and doctors to get a comprehensive knowledge of cancer's various features, including biochemical and genetic aspects. We anticipate that ongoing improvements and developments in CRISPR-Cas9 technology will improve the efficacy and safety of treatment in the future, giving hope to cancer patients. Using diverse approaches, the objective of developing healthy and cheap crops to fulfill the rising worldwide need for food may encounter problems. The employment of modern methods to increase crop variety will be a key element. When compared to conventional breeding strategies, modern breeding technologies enable researchers to modify the genome and introduce the desired gene into the genome more efficiently. As a result, using this technology to improve crop output, quality, and tolerance to diseases may become a key subject of study in the future.

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

Acknowledgments

The authors concede the support of the Cholistan University of Veterinary & Animal Sciences-Bahawalpur, Pakistan, during the write-up.

Conflict of Interest

The authors declare no conflict of interest.

References

Adhikari, P., & Poudel, M. (2020). CRISPR-Cas9 in agriculture: Approaches, applications, future perspectives, and associated challenges. *Malaysian Journal of Halal Research*, *3*(1), 6-16.

Al-Sadi, A., Al-Moqbali, H., Al-Yahyai, R., & Al-Said, F. (2012). AFLP data suggest a potential role for the low genetic diversity of acid lime (Citrus aurantifolia Swingle) in Oman in the outbreak of witches' broom disease of lime. *Euphytica*, *188*(2), 285-297.

Allen, T. M., & Cullis, P. R. (2013). Liposomal drug delivery systems: from concept to clinical applications. *Advanced drug delivery reviews*, *65*(1), 36-48.

Antal, C. E., Hudson, A. M., Kang, E., Zanca, C., Wirth, C., Stephenson, N. L., Trotter, E. W., Gallegos, L. L., Miller, C. J., & Furnari, F. B. (2015). Cancer-associated protein kinase C mutations reveal kinase's role as tumor suppressor. *Cell*, *160*(3), 489-502.

Anwar, A., & Kim, J.-K. (2020). Transgenic breeding approaches for improving abiotic stress tolerance: recent progress and future perspectives. *International journal of molecular sciences*, *21*(8), 2695.

Aubrey, B. J., Kelly, G. L., Kueh, A. J., Brennan, M. S., O'Connor, L., Milla, L., Wilcox, S., Tai, L., Strasser, A., & Herold, M. J. (2015). An inducible lentiviral guide RNA platform enables the identification of tumor-essential genes and tumor-promoting mutations in vivo. *Cell reports*, *10*(8), 1422-1432.

Badhan, S., Ball, A. S., & Mantri, N. (2021). First report of CRISPR/Cas9 mediated DNA-free editing of 4CL and RVE7 genes in chickpea protoplasts. *International journal of molecular sciences*, *22*(1), 396.

Bak, R. O., & Porteus, M. H. (2017). CRISPR-mediated integration of large gene cassettes using AAV donor vectors. *Cell reports*, *20*(3), 750-756.

Barrangou, R., & Doudna, J. A. (2016). Applications of CRISPR technologies in research and beyond. *Nature biotechnology*, *34*(9), 933-941.

Bell, N. M., & Lever, A. M. (2013). HIV Gag polyprotein: processing and early viral particle assembly. *Trends in microbiology*, *21*(3), 136-144.

Braatz, J., Harloff, H.-J., Mascher, M., Stein, N., Himmelbach, A., & Jung, C. (2017). CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoeologous gene copies in polyploid oilseed rape (Brassica napus). *Plant Physiology*, *174*(2), 935-942.

Briggs, J. A., Simon, M. N., Gross, I., Kräusslich, H.-G., Fuller, S. D., Vogt, V. M., & Johnson, M. C. (2004). The stoichiometry of Gag protein in HIV-1. *Nature structural & molecular biology*, *11*(7), 672-675.

Brooks, C., Nekrasov, V., Lippman, Z. B., & Van Eck, J. (2014). Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. *Plant Physiology*, *166*(3), 1292-1297.

Cai, B., Sun, S., Li, Z., Zhang, X., Ke, Y., Yang, J., & Li, X. (2018). Application of CRISPR/Cas9 technologies combined with iPSCs in the study and treatment of retinal degenerative diseases. *Human genetics*, *137*(9), 679-688.

Check, E. (2005). Gene therapy put on hold as third child develops cancer. *Nature*, *433*(7026), 561-562.

Chen, F., Alphonse, M., & Liu, Q. (2020). Strategies for nonviral nanoparticle-based delivery of CRISPR/Cas9 therapeutics. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *12*(3), e1609.

Chen, M., Mao, A., Xu, M., Weng, Q., Mao, J., & Ji, J. (2019). CRISPR-Cas9 for cancer therapy: Opportunities and challenges. *Cancer letters*, *447*, 48-55.

Choi, J., Dang, Y., Abraham, S., Ma, H., Zhang, J., Guo, H., Cai, Y., Mikkelsen, J., Wu, H., & Shankar, P. (2016). Lentivirus pre-packed with Cas9 protein for safer gene editing. *Gene therapy*, *23*(7), 627-633.

Clarke, J. L., & Zhang, P. (2013). Plant biotechnology for food security and bioeconomy. *Plant Molecular Biology*, *83*(1), 1-3.

Clifford, G. M., Tenet, V., Georges, D., Alemany, L., Pavón, M. A., Chen, Z., Yeager, M., Cullen, M., Boland, J. F., & Bass, S. (2019). Human papillomavirus 16 sub-lineage dispersal and cervical cancer risk worldwide: Whole viral genome sequences from 7116 HPV16-positive women. *Papillomavirus Research*, *7*, 67-74.

Curtin, S. J., Xiong, Y., Michno, J. M., Campbell, B. W., Stec, A. O., Čermák, T., Starker, C., Voytas, D. F., Eamens, A. L., & Stupar, R. M. (2018). Crispr/cas9 and talen s generate heritable mutations for genes involved in small rna processing of glycine max and medicago truncatula. *Plant Biotechnology Journal*, *16*(6), 1125-1137.

Daya, S., & Berns, K. I. (2008). Gene therapy using adeno-associated virus vectors. *Clinical microbiology reviews*, *21*(4), 583-593.

de Melo, B. P., Lourenço-Tessutti, I. T., Paixão, J. F. R., Noriega, D. D., Silva, M. C. M., de Almeida-Engler, J., Fontes, E. P. B., & Grossi-de-Sa, M. F. (2020). Transcriptional modulation of AREB-1 by CRISPRa improves plant physiological performance under severe water deficit. *Scientific reports*, *10*(1), 1-10.

Dever, D. P., Bak, R. O., Reinisch, A., Camarena, J., Washington, G., Nicolas, C. E., Pavel-Dinu, M., Saxena, N., Wilkens, A. B., & Mantri, S. (2016). CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. *Nature*, *539*(7629), 384-389.

Deyle, D. R., & Russell, D. W. (2009). Adeno-associated virus vector integration. *Current opinion in molecular therapeutics*, *11*(4), 442.

Dong, C., Qu, L., Wang, H., Wei, L., Dong, Y., & Xiong, S. (2015). Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. *Antiviral research*, *118*, 110-117.

Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*(6213), 1258096.

Ellison, E. E., Nagalakshmi, U., Gamo, M. E., Huang, P.-j., Dinesh-Kumar, S., & Voytas, D. F. (2020). Multiplexed heritable gene editing using RNA viruses and mobile single guide RNAs. *Nature plants*, *6*(6), 620-624.

Eyquem, J., Mansilla-Soto, J., Giavridis, T., van der Stegen, S. J., Hamieh, M., Cunanan, K. M., Odak, A., Gönen, M., & Sadelain, M. (2017). Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature*, *543*(7643), 113-117.

Fu, Y., Sander, J. D., Reyon, D., Cascio, V. M., & Joung, J. K. (2014). Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. *Nature biotechnology*, *32*(3), 279-284.

Fuenmayor, J., Gòdia, F., & Cervera, L. (2017). Production of virus-like particles for vaccines. *New biotechnology*, *39*, 174-180.

Gao, C. (2018). The future of CRISPR technologies in agriculture. *Nature Reviews Molecular Cell Biology*, *19*(5), 275-276.

George, L. A. (2017). Hemophilia gene therapy comes of age. *Blood Advances*, *1*(26), 2591-2599.

Gil-Humanes, J., Wang, Y., Liang, Z., Shan, Q., Ozuna, C. V., Sánchez-León, S., Baltes, N. J., Starker, C., Barro, F., & Gao, C. (2017). High-efficiency gene targeting in hexaploid wheat using DNA replicons and CRISPR/Cas9. *The Plant Journal*, *89*(6), 1251-1262.

Henriques, S. T., Costa, J., & Castanho, M. A. (2005). Translocation of β-galactosidase mediated by the cell-penetrating peptide pep-1 into lipid vesicles and human HeLa cells is driven by membrane electrostatic potential. *Biochemistry*, *44*(30), 10189-10198.

Hoos, A. (2016). Development of immuno-oncology drugs—from CTLA4 to PD1 to the next generations. *Nature reviews Drug discovery*, *15*(4), 235-247.

Hoppe-Seyler, K., Bossler, F., Braun, J. A., Herrmann, A. L., & Hoppe-Seyler, F. (2018). The HPV E6/E7 oncogenes: key factors for viral carcinogenesis and therapeutic targets. *Trends in microbiology*, *26*(2), 158-168.

Horii, T., Arai, Y., Yamazaki, M., Morita, S., Kimura, M., Itoh, M., Abe, Y., & Hatada, I. (2014). Validation of microinjection methods for generating knockout mice by CRISPR/Cas-mediated genome engineering. *Scientific reports*, *4*(1), 1-6.

Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, *157*(6), 1262-1278.

Hyun, Y., Kim, J., Cho, S. W., Choi, Y., Kim, J.-S., & Coupland, G. (2015). Site-directed mutagenesis in Arabidopsis thaliana using dividing tissue-targeted RGEN of the CRISPR/Cas system to generate heritable null alleles. *Planta*, *241*(1), 271-284.

Jaganathan, D., Ramasamy, K., Sellamuthu, G., Jayabalan, S., & Venkataraman, G. (2018). CRISPR for crop improvement: an update review. *Frontiers in plant science*, *9*, 985.

Ji, X., Si, X., Zhang, Y., Zhang, H., Zhang, F., & Gao, C. (2018). Conferring DNA virus resistance with high specificity in plants using virus-inducible genome-editing system. *Genome biology*, *19*(1), 1-7.

Jia, H., Zhang, Y., Orbović, V., Xu, J., White, F. F., Jones, J. B., & Wang, N. (2017). Genome editing of the disease susceptibility gene Cs LOB 1 in citrus confers resistance to citrus canker. *Plant Biotechnology Journal*, *15*(7), 817-823.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*, *337*(6096), 816-821.

Johnson, L. A., Scholler, J., Ohkuri, T., Kosaka, A., Patel, P. R., McGettigan, S. E., Nace, A. K., Dentchev, T., Thekkat, P., & Loew, A. (2015). Rational development and characterization of humanized anti–EGFR variant III chimeric antigen receptor T cells for glioblastoma. *Science translational medicine*, *7*(275), 275ra222-275ra222.

Jones, J. D., Witek, K., Verweij, W., Jupe, F., Cooke, D., Dorling, S., Tomlinson, L., Smoker, M., Perkins, S., & Foster, S. (2014). Elevating crop disease resistance

with cloned genes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1639), 20130087.

Joshi, R. K., Bharat, S. S., & Mishra, R. (2020). Engineering drought tolerance in plants through CRISPR/Cas genome editing. *3 Biotech*, *10*(9), 1-14.

Jouanin, A., Gilissen, L. J., Schaart, J. G., Leigh, F. J., Cockram, J., Wallington, E. J., Boyd, L. A., van den Broeck, H. C., van der Meer, I. M., & America, A. (2020). CRISPR/Cas9 gene editing of gluten in wheat to reduce gluten content and exposure—reviewing methods to screen for coeliac safety. *Frontiers in Nutrition*, *7*, 51.

Kaczmarczyk, S. J., Sitaraman, K., Young, H. A., Hughes, S. H., & Chatterjee, D. K. (2011). Protein delivery using engineered virus-like particles. *Proceedings of the National Academy of Sciences*, *108*(41), 16998-17003.

Kasap, C., Elemento, O., & Kapoor, T. M. (2014). DrugTargetSeqR: a genomics-and CRISPR-Cas9–based method to analyze drug targets. *Nature chemical biology*, *10*(8), 626-628.

Kettles, G. J., & Kanyuka, K. (2016). Dissecting the molecular interactions between wheat and the fungal pathogen Zymoseptoria tritici. *Frontiers in plant science*, *7*, 508.

Khan, Z., Khan, S. H., & Ahmad, A. (2021). Challenges and future perspective of CRISPR/Cas technology for crop improvement. In *CRISPR crops* (pp. 289-306). Springer.

Khan, Z., Saboor, T., Ashfaq, M., Saddique, A., & Khanum, P. (2021). Disease resistance in crops through CRISPR/Cas. In *CRISPR Crops* (pp. 151-175). Springer.

Kim, S., Kim, D., Cho, S. W., Kim, J., & Kim, J.-S. (2014). Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. *Genome research*, *24*(6), 1012-1019.

Kotterman, M. A., Chalberg, T. W., & Schaffer, D. V. (2015). Viral vectors for gene therapy: translational and clinical outlook. *Annual review of biomedical engineering*, *17*, 63-89.

Kuang, Y., Li, S., Ren, B., Yan, F., Spetz, C., Li, X., Zhou, X., & Zhou, H. (2020). Base-editing-mediated artificial evolution of OsALS1 in planta to develop novel herbicide-tolerant rice germplasms. *Molecular plant*, *13*(4), 565-572.

Lander, E. S. (2016). The heroes of CRISPR. *Cell*, *164*(1-2), 18-28.

Lee, K., Conboy, M., Park, H. M., Jiang, F., Kim, H. J., Dewitt, M. A., Mackley, V. A., Chang, K., Rao, A., & Skinner, C. (2017). Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair. *Nature biomedical engineering*, *1*(11), 889-901.

Lemmon, Z. H., Reem, N. T., Dalrymple, J., Soyk, S., Swartwood, K. E., Rodriguez-Leal, D., Van Eck, J., & Lippman, Z. B. (2018). Rapid improvement of domestication traits in an orphan crop by genome editing. *Nature plants*, *4*(10), 766-770.

Li, J., Jiao, G., Sun, Y., Chen, J., Zhong, Y., Yan, L., Jiang, D., Ma, Y., & Xia, L. (2021). Modification of starch composition, structure and properties through editing of TaSBEIIa in both winter and spring wheat varieties by CRISPR/Cas9. *Plant Biotechnology Journal*, *19*(5), 937-951.

Li, J., Zhang, X., Sun, Y., Zhang, J., Du, W., Guo, X., Li, S., Zhao, Y., & Xia, L. (2018). Efficient allelic replacement in rice by gene editing: a case study of the NRT1. 1B gene. *Journal of Integrative Plant Biology*, *60*(7), 536-540.

Liang, Z., Zhang, K., Chen, K., & Gao, C. (2014). Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. *Journal of Genetics and Genomics*, *41*(2), 63-68.

Liao, S., Qin, X., Luo, L., Han, Y., Wang, X., Usman, B., Nawaz, G., Zhao, N., Liu, Y., & Li, R. (2019). CRISPR/Cas9-induced mutagenesis of semi-rolled leaf1, 2 confers curled leaf phenotype and drought tolerance by influencing protein expression patterns and ROS scavenging in rice (Oryza sativa L.). *Agronomy*, *9*(11), 728.

Lim, S., Koo, J.-H., & Choi, J.-M. (2016). Use of cell-penetrating peptides in dendritic cell-based vaccination. *Immune network*, *16*(1), 33-43.

Lino, C. A., Harper, J. C., Carney, J. P., & Timlin, J. A. (2018). Delivering CRISPR: a review of the challenges and approaches. *Drug delivery*, *25*(1), 1234-1257.

Liu, K.-C., Lin, B.-S., Gao, A.-D., Ma, H.-Y., Zhao, M., Zhang, R., Yan, H.-H., Yi, X.-F., Lin, S.-J., & Que, J.-W. (2014). Integrase-deficient lentivirus: opportunities and challenges for human gene therapy. *Current gene therapy*, *14*(5), 352-364.

Liu, X., Hao, R., Chen, S., Guo, D., & Chen, Y. (2015). Inhibition of hepatitis B virus by the CRISPR/Cas9 system via targeting the conserved regions of the viral genome. *Journal of General Virology*, *96*(8), 2252-2261.

Liu, Y., Zeng, Y., Liu, L., Zhuang, C., Fu, X., Huang, W., & Cai, Z. (2014). Synthesizing AND gate genetic circuits based on CRISPR-Cas9 for identification of bladder cancer cells. *Nature communications*, *5*(1), 1-7.

Longo, P. A., Kavran, J. M., Kim, M.-S., & Leahy, D. J. (2013). Transient mammalian cell transfection with polyethylenimine (PEI). In *Methods in enzymology* (Vol. 529, pp. 227-240). Elsevier.

Lou, D., Wang, H., Liang, G., & Yu, D. (2017). OsSAPK2 confers abscisic acid sensitivity and tolerance to drought stress in rice. *Frontiers in plant science*, *8*, 993.

Lyzenga, W. J., Pozniak, C. J., & Kagale, S. (2021). Advanced domestication: harnessing the precision of gene editing in crop breeding. *Plant Biotechnology Journal*, *19*(4), 660-670.

Ma, D., Xu, Z., Zhang, Z., Chen, X., Zeng, X., Zhang, Y., Deng, T., Ren, M., Sun, Z., & Jiang, R. (2019). Engineer chimeric Cas9 to expand PAM recognition based on evolutionary information. *Nature communications*, *10*(1), 1-9.

Ma, X., & Liu, Y. G. (2016). CRISPR/Cas9-based multiplex genome editing in monocot and dicot plants. *Current protocols in molecular biology*, *115*(1), 31.36. 31-31.36. 21.

Ma, Y., Shen, B., Zhang, X., Lu, Y., Chen, W., Ma, J., Huang, X., & Zhang, L. (2014). Heritable multiplex genetic engineering in rats using CRISPR/Cas9. *PloS one*, *9*(3), e89413.

Macovei, A., Sevilla, N. R., Cantos, C., Jonson, G. B., Slamet-Loedin, I., Čermák, T., Voytas, D. F., Choi, I. R., & Chadha-Mohanty, P. (2018). Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice tungro spherical virus. *Plant Biotechnology Journal*, *16*(11), 1918-1927.

Mahmuda, B. M., Cao, N., Wei, X., Xie, L., Jiao, G., Tang, S., Nese, S., Shao, G., & Hu, P. (2021). Improved Eating and Cooking Quality of indica Rice Cultivar YK17 via Adenine Base Editing of Wxa Allele of Granule-Bound Starch Synthase I (GBSS I). *Rice Science*, *28*(5), 427.

Makarova, K. S., Haft, D. H., Barrangou, R., Brouns, S. J., Charpentier, E., Horvath, P., Moineau, S., Mojica, F. J., Wolf, Y. I., & Yakunin, A. F. (2011). Evolution

and classification of the CRISPR–Cas systems. *Nature Reviews Microbiology*, *9*(6), 467-477.

Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., & Church, G. M. (2013). RNA-guided human genome engineering via Cas9. *Science*, *339*(6121), 823-826.

Malnoy, M., Viola, R., Jung, M.-H., Koo, O.-J., Kim, S., Kim, J.-S., Velasco, R., & Nagamangala Kanchiswamy, C. (2016). DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. *Frontiers in plant science*, *7*, 1904.

Mangeot, P. E., Risson, V., Fusil, F., Marnef, A., Laurent, E., Blin, J., Mournetas, V., Massouridès, E., Sohier, T. J., & Corbin, A. (2019). Genome editing in primary cells and in vivo using viral-derived Nanoblades loaded with Cas9-sgRNA ribonucleoproteins. *Nature communications*, *10*(1), 1-15.

Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N. J., Chew, A., Gonzalez, V. E., Zheng, Z., & Lacey, S. F. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. *New England Journal of Medicine*, *371*(16), 1507-1517.

Melo, J. d., & Blackshaw, S. (2018). In vivo electroporation of developing mouse retina. In *Retinal Gene Therapy* (pp. 101-111). Springer.

Miao, C., Wang, Z., Zhang, L., Yao, J., Hua, K., Liu, X., Shi, H., & Zhu, J.-K. (2019). The grain yield modulator miR156 regulates seed dormancy through the gibberellin pathway in rice. *Nature communications*, *10*(1), 1-12.

Mohsen, M. O., Zha, L., Cabral-Miranda, G., & Bachmann, M. F. (2017). Major findings and recent advances in virus–like particle (VLP)-based vaccines. Seminars in immunology,

Montagna, C., Petris, G., Casini, A., Maule, G., Franceschini, G. M., Zanella, I., Conti, L., Arnoldi, F., Burrone, O. R., & Zentilin, L. (2018). VSV-G-enveloped vesicles for traceless delivery of CRISPR-Cas9. *Molecular Therapy-Nucleic Acids*, *12*, 453-462.

Moore, J. C., Van Laake, L. W., Braam, S. R., Xue, T., Tsang, S.-Y., Ward, D., Passier, R., Tertoolen, L. L., Li, R. A., & Mummery, C. L. (2005). Human embryonic stem cells: genetic manipulation on the way to cardiac cell therapies. *Reproductive toxicology*, *20*(3), 377-391.

Morineau, C., Bellec, Y., Tellier, F., Gissot, L., Kelemen, Z., Nogué, F., & Faure, J. D. (2017). Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid Camelina sativa. *Plant Biotechnology Journal*, *15*(6), 729-739.

Neggers, J. E., Vercruysse, T., Jacquemyn, M., Vanstreels, E., Baloglu, E., Shacham, S., Crochiere, M., Landesman, Y., & Daelemans, D. (2015). Identifying drug-target selectivity of small-molecule CRM1/XPO1 inhibitors by CRISPR/Cas9 genome editing. *Chemistry & biology*, *22*(1), 107-116.

Nekrasov, V., Staskawicz, B., Weigel, D., Jones, J. D., & Kamoun, S. (2013). Targeted mutagenesis in the model plant Nicotiana benthamiana using Cas9 RNA-guided endonuclease. *Nature biotechnology*, *31*(8), 691-693.

Nekrasov, V., Wang, C., Win, J., Lanz, C., Weigel, D., & Kamoun, S. (2017). Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Scientific reports*, *7*(1), 1-6.

Ngwa, V. M., Axford, D. S., Healey, A. N., Nowak, S. J., Chrestensen, C. A., & McMurry, J. L. (2017). A versatile cell-penetrating peptide-adaptor system for efficient delivery of molecular cargos to subcellular destinations. *PloS one*, *12*(5), e0178648.

Ok, C. Y., Li, L., & Young, K. H. (2015). EBV-driven B-cell lymphoproliferative disorders: from biology, classification and differential diagnosis to clinical management. *Experimental & Molecular Medicine*, *47*(1), e132-e132.

Okuzaki, A., Ogawa, T., Koizuka, C., Kaneko, K., Inaba, M., Imamura, J., & Koizuka, N. (2018). CRISPR/Cas9-mediated genome editing of the fatty acid desaturase 2 gene in Brassica napus. *Plant Physiology and Biochemistry*, *131*, 63-69.

Peng, A., Chen, S., Lei, T., Xu, L., He, Y., Wu, L., Yao, L., & Zou, X. (2017). Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene Cs LOB 1 promoter in citrus. *Plant Biotechnology Journal*, *15*(12), 1509-1519.

Pensado, A., Seijo, B., & Sanchez, A. (2014). Current strategies for DNA therapy based on lipid nanocarriers. *Expert Opinion on Drug Delivery*, *11*(11), 1721-1731.

Popescu, N. C., Zimonjic, D., & DiPaolo, J. A. (1990). Viral integration, fragile sites, and proto-oncogenes in human neoplasia. *Human genetics*, *84*(5), 383-386.

Price, A. A., Sampson, T. R., Ratner, H. K., Grakoui, A., & Weiss, D. S. (2015). Cas9-mediated targeting of viral RNA in eukaryotic cells. *Proceedings of the National Academy of Sciences*, *112*(19), 6164-6169.

Ran, F., Cong, L., Yan, W. X., Scott, D. A., Gootenberg, J. S., Kriz, A. J., Zetsche, B., Shalem, O., Wu, X., & Makarova, K. S. (2015). In vivo genome editing using Staphylococcus aureus Cas9. *Nature*, *520*(7546), 186-191.

Ran, F. A., Hsu, P. D., Lin, C.-Y., Gootenberg, J. S., Konermann, S., Trevino, A. E., Scott, D. A., Inoue, A., Matoba, S., & Zhang, Y. (2013). Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell*, *154*(6), 1380-1389.

Rasheed, A., Fahad, S., Aamer, M., Hassan, M., Tahir, M., & Wu, Z. (2020). Role of genetic factors in regulating cadmium uptake, transport and accumulation mechanisms and quantitative trait loci mapping in rice. a review. *Applied Ecology and Environmental Research*, *18*(3), 4005-4023.

Rasheed, A., Gill, R. A., Hassan, M. U., Mahmood, A., Qari, S., Zaman, Q. U., Ilyas, M., Aamer, M., Batool, M., & Li, H. (2021). A critical review: recent advancements in the use of CRISPR/Cas9 technology to enhance crops and alleviate global food crises. *Current Issues in Molecular Biology*, *43*(3), 1950-1976.

Rasheed, A., Wassan, G. M., Khanzada, H., Solangi, A. M., Aamer, M., Ruicai, H., Jianmin, B., & Ziming, W. (2021). QTL underlying iron toxicity tolerance at seedling stage in backcross recombinant inbred lines (BRILs) population of rice using high density genetic map. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *49*(1), 12158-12158.

Razzaq, M. K., Aleem, M., Mansoor, S., Khan, M. A., Rauf, S., Iqbal, S., & Siddique, K. H. (2021). Omics and CRISPR-Cas9 approaches for molecular insight, functional gene analysis, and stress tolerance development in crops. *International journal of molecular sciences*, *22*(3), 1292.

Ren, J., Liu, X., Fang, C., Jiang, S., June, C. H., & Zhao, Y. (2017). Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. *Clinical cancer research*, *23*(9), 2255-2266.

Ricroch, A., Clairand, P., & Harwood, W. (2017). Use of CRISPR systems in plant genome editing: toward new opportunities in agriculture. *Emerging Topics in Life Sciences*, *1*(2), 169-182.

Romero, Z., DeWitt, M., & Walters, M. C. (2018). Promise of gene therapy to treat sickle cell disease. *Expert Opinion on Biological Therapy*, *18*(11), 1123-1136.

Rothe, M., Modlich, U., & Schambach, A. (2013). Biosafety challenges for use of lentiviral vectors in gene therapy. *Current gene therapy*, *13*(6), 453-468.

Saeed, M. Q., Dufour, N., Bartholmae, C., Sieranska, U., Knopf, M., Thierry, E., Thierry, S., Delelis, O., Grandchamp, N., & Pilet, H. (2014). Comparison Between several integrase-defective lentiviral vectors reveals increased integration of an HIV vector bearing a D167H mutant. *Molecular Therapy-Nucleic Acids*, *3*, e213.

Sam, V. H., Van, P. T., Ha, N. T., Ha, N. T. T., Huong, P. T. T., Hoi, P. X., Phuong, N. D., & Le Quyen, C. (2021). Design and transfer of OsSWEET14-editing T-DNA construct to bac thom 7 rice cultivar. *Academia Journal of Biology*, *43*(1).

Samanta, M. K., Dey, A., & Gayen, S. (2016). CRISPR/Cas9: an advanced tool for editing plant genomes. *Transgenic research*, *25*(5), 561-573.

Sánchez-Rivera, F. J., & Jacks, T. (2015). Applications of the CRISPR–Cas9 system in cancer biology. *Nature reviews cancer*, *15*(7), 387-393.

Sawamoto, K., Chen, H.-H., Alméciga-Díaz, C. J., Mason, R. W., & Tomatsu, S. (2018). Gene therapy for Mucopolysaccharidoses. *Molecular genetics and metabolism*, *123*(2), 59-68.

Seeger, C., & Sohn, J. A. (2016). Complete spectrum of CRISPR/Cas9-induced mutations on HBV cccDNA. *Molecular Therapy*, *24*(7), 1258-1266.

Shalem, O., Sanjana, N. E., Hartenian, E., Shi, X., Scott, D. A., Mikkelsen, T. S., Heckl, D., Ebert, B. L., Root, D. E., & Doench, J. G. (2014). Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*, *343*(6166), 84-87.

Shan, Q., Wang, Y., Li, J., Zhang, Y., Chen, K., Liang, Z., Zhang, K., Liu, J., Xi, J. J., & Qiu, J.-L. (2013). Targeted genome modification of crop plants using a CRISPR-Cas system. *Nature biotechnology*, *31*(8), 686-688.

Shen, C., Que, Z., Xia, Y., Tang, N., Li, D., He, R., & Cao, M. (2017). Knock out of the annexin gene OsAnn3 via CRISPR/Cas9-mediated genome editing decreased cold tolerance in rice. *Journal of Plant Biology*, *60*(6), 539-547.

Shen, L., Wang, C., Fu, Y., Wang, J., Liu, Q., Zhang, X., Yan, C., Qian, Q., & Wang, K. (2018). QTL editing confers opposing yield performance in different rice varieties. *Journal of Integrative Plant Biology*, *60*(2), 89-93.

Sherman, M. H., Ruth, T. Y., Engle, D. D., Ding, N., Atkins, A. R., Tiriac, H., Collisson, E. A., Connor, F., Van Dyke, T., & Kozlov, S. (2014). Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. *Cell*, *159*(1), 80-93.

Shi, J., Gao, H., Wang, H., Lafitte, H. R., Archibald, R. L., Yang, M., Hakimi, S. M., Mo, H., & Habben, J. E. (2017). ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnology Journal*, *15*(2), 207-216.

Shi, J., Wang, E., Milazzo, J. P., Wang, Z., Kinney, J. B., & Vakoc, C. R. (2015). Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains. *Nature biotechnology*, *33*(6), 661-667.

Singh, R. (2021). Genome Editing for Plant Disease Resistance. In *Emerging Trends in Plant Pathology* (pp. 577-590). Springer.

Soyk, S., Müller, N. A., Park, S. J., Schmalenbach, I., Jiang, K., Hayama, R., Zhang, L., Van Eck, J., Jimenez-Gomez, J. M., & Lippman, Z. B. (2017). Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. *Nature Genetics*, *49*(1), 162-168.

Stamm, P., Ramamoorthy, R., & Kumar, P. P. (2011). Feeding the extra billions: strategies to improve crops and enhance future food security. *Plant Biotechnology Reports*, *5*(2), 107-120.

Su, S., Hu, B., Shao, J., Shen, B., Du, J., Du, Y., Zhou, J., Yu, L., Zhang, L., & Chen, F. (2016). CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients. *Scientific reports*, *6*(1), 1-14.

Suresh, B., Ramakrishna, S., & Kim, H. (2017). Cell-penetrating peptide-mediated delivery of Cas9 protein and guide RNA for genome editing. In *Eukaryotic Transcriptional and Post-Transcriptional Gene Expression Regulation* (pp. 81-94). Springer.

Taylor, G. S., Long, H. M., Brooks, J. M., Rickinson, A. B., & Hislop, A. D. (2015). The immunology of Epstein-Barr virus–induced disease. *Annual review of immunology*, *33*, 787-821.

Toda, E., Koiso, N., Takebayashi, A., Ichikawa, M., Kiba, T., Osakabe, K., Osakabe, Y., Sakakibara, H., Kato, N., & Okamoto, T. (2019). An efficient DNA-and selectable-marker-free genome-editing system using zygotes in rice. *Nature plants*, *5*(4), 363-368.

Tzelepis, K., Koike-Yusa, H., De Braekeleer, E., Li, Y., Metzakopian, E., Dovey, O. M., Mupo, A., Grinkevich, V., Li, M., & Mazan, M. (2016). A CRISPR dropout screen identifies genetic vulnerabilities and therapeutic targets in acute myeloid leukemia. *Cell reports*, *17*(4), 1193-1205.

Usman, B., Nawaz, G., Zhao, N., Liao, S., Qin, B., Liu, F., Liu, Y., & Li, R. (2020). Programmed editing of rice (Oryza sativa L.) OsSPL16 gene using CRISPR/Cas9 improves grain yield by modulating the expression of pyruvate enzymes and cell cycle proteins. *International journal of molecular sciences*, *22*(1), 249.

Usman, B., Nawaz, G., Zhao, N., Liu, Y., & Li, R. (2020). Generation of high yielding and fragrant rice (Oryza sativa L.) lines by CRISPR/Cas9 targeted mutagenesis of three homoeologs of cytochrome P450 gene family and OsBADH2 and transcriptome and proteome profiling of revealed changes triggered by mutations. *Plants*, *9*(6), 788.

van Diemen, F. R., Kruse, E. M., Hooykaas, M. J., Bruggeling, C. E., Schürch, A. C., van Ham, P. M., Imhof, S. M., Nijhuis, M., Wiertz, E. J., & Lebbink, R. J. (2016). CRISPR/Cas9-mediated genome editing of herpesviruses limits productive and latent infections. *PLoS pathogens*, *12*(6), e1005701.

Verma, A. K., Mandal, S., Tiwari, A., Monachesi, C., Catassi, G. N., Srivastava, A., Gatti, S., Lionetti, E., & Catassi, C. (2021). Current status and perspectives on the application of CRISPR/Cas9 Gene-editing system to develop a low-gluten, non-transgenic wheat variety. *Foods*, *10*(10), 2351.

Wan, L., Wen, H., Li, Y., Lyu, J., Xi, Y., Hoshii, T., Joseph, J. K., Wang, X., Loh, Y.-H. E., & Erb, M. A. (2017). ENL links histone acetylation to oncogenic gene expression in acute myeloid leukaemia. *Nature*, *543*(7644), 265-269.

Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., Liu, Y.-G., & Zhao, K. (2016). Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. *PloS one*, *11*(4), e0154027.

Wang, J., & Quake, S. R. (2014). RNA-guided endonuclease provides a therapeutic strategy to cure latent herpesviridae infection. *Proceedings of the National Academy of Sciences*, *111*(36), 13157-13162.

Wang, L., Chen, L., Li, R., Zhao, R., Yang, M., Sheng, J., & Shen, L. (2017). Reduced drought tolerance by CRISPR/Cas9-mediated SlMAPK3 mutagenesis in

tomato plants. *Journal of Agricultural and Food Chemistry*, *65*(39), 8674-8682.

Wang, S., Yang, Y., Guo, M., Zhong, C., Yan, C., & Sun, S. (2020). Targeted mutagenesis of amino acid transporter genes for rice quality improvement using the CRISPR/Cas9 system. *The Crop Journal*, *8*(3), 457-464.

Wang, T., Zhang, C., Zhang, H., & Zhu, H. (2021). CRISPR/Cas9-mediated gene editing revolutionizes the improvement of horticulture food crops. *Journal of Agricultural and Food Chemistry*, *69*(45), 13260-13269.

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., & Qiu, J.-L. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature biotechnology*, *32*(9), 947-951.

Wang, Y., Wang, J., Guo, S., Tian, S., Zhang, J., Ren, Y., Li, M., Gong, G., Zhang, H., & Xu, Y. (2021). CRISPR/Cas9-mediated mutagenesis of ClBG1 decreased seed size and promoted seed germination in watermelon. *Horticulture research*, *8*.

Wu, X., Kriz, A. J., & Sharp, P. A. (2014). Target specificity of the CRISPR-Cas9 system. *Quantitative biology*, *2*(2), 59-70.

Yang, L., Achreja, A., Yeung, T.-L., Mangala, L. S., Jiang, D., Han, C., Baddour, J., Marini, J. C., Ni, J., & Nakahara, R. (2016). Targeting stromal glutamine synthetase in tumors disrupts tumor microenvironment-regulated cancer cell growth. *Cell metabolism*, *24*(5), 685-700.

Yang, Y., Wang, L., Bell, P., McMenamin, D., He, Z., White, J., Yu, H., Xu, C., Morizono, H., & Musunuru, K. (2016). A dual AAV system enables the Cas9-mediated correction of a metabolic liver disease in newborn mice. *Nature biotechnology*, *34*(3), 334-338.

Yip, B. H. (2020). Recent advances in CRISPR/Cas9 delivery strategies. *Biomolecules*, *10*(6), 839.

Zaidi, S. S.-e.-A., Tashkandi, M., Mansoor, S., & Mahfouz, M. M. (2016). Engineering plant immunity: using CRISPR/Cas9 to generate virus resistance. *Frontiers in plant science*, *7*, 1673.

Zaman, Q. U., Chu, W., Hao, M., Shi, Y., Sun, M., Sang, S.-F., Mei, D., Cheng, H., Liu, J., & Li, C. (2019). CRISPR/Cas9-mediated multiplex genome editing of JAGGED gene in Brassica napus L. *Biomolecules*, *9*(11), 725.

Zambirinis, C. P., & Miller, G. (2017). Cancer manipulation of host physiology: lessons from pancreatic cancer. *Trends in molecular medicine*, *23*(5), 465-481.

Zeng, Y., Wen, J., Zhao, W., Wang, Q., & Huang, W. (2020). Rational improvement of rice yield and cold tolerance by editing the three genes OsPIN5b, GS3, and OsMYB30 with the CRISPR–Cas9 system. *Frontiers in plant science*, *10*, 1663.

Zhang, A., Liu, Y., Wang, F., Li, T., Chen, Z., Kong, D., Bi, J., Zhang, F., Luo, X., & Wang, J. (2019). Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Molecular breeding*, *39*(3), 1-10.

Zhang, H., Zhang, J., Wei, P., Zhang, B., Gou, F., Feng, Z., Mao, Y., Yang, L., Zhang, H., & Xu, N. (2014). The CRISPR/C as9 system produces specific and homozygous targeted gene editing in rice in one generation. *Plant Biotechnology Journal*, *12*(6), 797-807.

Zhang, J., Zhou, Z., Bai, J., Tao, X., Wang, L., Zhang, H., & Zhu, J.-K. (2020). Disruption of MIR396e and MIR396f improves rice yield under nitrogen-deficient conditions. *National science review*, *7*(1), 102-112.

Zhang, T., Zheng, Q., Yi, X., An, H., Zhao, Y., Ma, S., & Zhou, G. (2018). Establishing RNA virus resistance in plants by harnessing CRISPR immune system. *Plant Biotechnology Journal*, *16*(8), 1415-1423.

Zhang, X. (2015). Gold nanoparticles: recent advances in the biomedical applications. *Cell biochemistry and biophysics*, *72*(3), 771-775.

Zhang, Y., Bai, Y., Wu, G., Zou, S., Chen, Y., Gao, C., & Tang, D. (2017). Simultaneous modification of three homoeologs of Ta EDR 1 by genome editing enhances powdery mildew resistance in wheat. *The Plant Journal*, *91*(4), 714-724.

Zhang, Z., Mao, Y., Ha, S., Liu, W., Botella, J. R., & Zhu, J.-K. (2016). A multiplex CRISPR/Cas9 platform for fast and efficient editing of multiple genes in Arabidopsis. *Plant cell reports*, *35*(7), 1519-1533.

Zhen, S., Hua, L., Liu, Y., Gao, L., Fu, J., Wan, D., Dong, L., Song, H., & Gao, X. (2015). Harnessing the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated Cas9 system to disrupt the hepatitis B virus. *Gene therapy*, *22*(5), 404-412.

Zhen, S., Lu, J.-J., Wang, L.-J., Sun, X.-M., Zhang, J.-Q., Li, X., Luo, W.-J., & Zhao, L. (2016). In vitro and in vivo synergistic therapeutic effect of cisplatin with human papillomavirus16 E6/E7 CRISPR/Cas9 on cervical cancer cell line. *Translational oncology*, *9*(6), 498-504.

Zhou, G., Sprengers, D., Boor, P. P., Doukas, M., Schutz, H., Mancham, S., Pedroza-Gonzalez, A., Polak, W. G., De Jonge, J., & Gaspersz, M. (2017). Antibodies against immune checkpoint molecules restore functions of tumor-infiltrating T cells in hepatocellular carcinomas. *Gastroenterology*, *153*(4), 1107-1119. e1110.

Zhou, J., Peng, Z., Long, J., Sosso, D., Liu, B., Eom, J. S., Huang, S., Liu, S., Vera Cruz, C., & Frommer, W. B. (2015). Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. *The Plant Journal*, *82*(4), 632-643.

Zsögön, A., Čermák, T., Naves, E. R., Notini, M. M., Edel, K. H., Weinl, S., Freschi, L., Voytas, D. F., Kudla, J., & Peres, L. E. P. (2018). De novo domestication of wild tomato using genome editing. *Nature biotechnology*, *36*(12), 1211-1216.

Zuris, J. A., Thompson, D. B., Shu, Y., Guilinger, J. P., Bessen, J. L., Hu, J. H., Maeder, M. L., Joung, J. K., Chen, Z.-Y., & Liu, D. R. (2015). Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. *Nature biotechnology*, *33*(1), 73-80.

About The Authors

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkiye. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 68 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.

E-mail: msafdar@cuvas.edu.pk , ORCID: 0000 0002 3720 2090

Prof. Dr. Mehmet ÖZASLAN received his PhD in 1995 at the Institute of Natural Sciences at Cukurova University, Turkiye. He is a professor of molecular biology and genetics. His research interests include cancer genetics, molecular virology, molecular genetics, microbiology, genetic mutations, etc. He has published more than 200 research articles in national and international well-reputed journals. He has also written many book chapters as well as edited books. He is an editor and editor-in-chief of many well-reputed national and international journals.
E-mail: ozaslanmd@gantep.edu.tr, ORCID: 0000 0001 9380 4902

Shafeeq ur Rehman graduated from the Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur. He continues to work on FMD and PPR viruses as his area of interest. He is still working as a Research Assistant in FMDV and PPRV project in Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan.
Email: shafeequrrehmanbhutta@gmail.com, ORCID: 0000-0003-3571-8226

Minal Hussain graduated from Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur.
Email: 2019-cu-bios-023@cuvas.edu.pk ORCID: 0009-0004-0143-5644

Furqan Shafqat graduated from Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in medical biotechnology from University of Wollongong Australia.
Email: furqanshafqat007@gmail.com, ORCID: 0000-0002-2563-913X

To Cite This Chapter:
Rehman, S et al., (2023). **Recent advances in the application of crispr-CAS9**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 30-58). ISRES Publishing

ANTI CRISPR PROTEINS AS A NEW TOOL FOR SYNTHETIC BIOLOGY

Shafeeq ur REHMAN
Furqan SHAFQAT
Muhammad YOUNUS
Muhammad SAFDAR

Introduction

Bacteria develop by random genetic drift (RGD) and horizontal gene transfer (HGT). HGT is a technique of lateral gene transfer that enables bacteria to receive and incorporate exogenous DNA into their genome (Brito, 2021; Mejdani, 2019; Soucy et al., 2015). Phages (bacteria-specific viruses) are a key contribution to HGT since they can effectively attach bacterial cells and deliver DNA into the cytoplasm. This vector-based technique of HGT comes with the substantial caveat of possible destruction and mortality for the bacterial host (Mejdani, 2019). That guard against phage invasion and other lateral gene transfer components microorganisms have evolved methods to destroy invading DNA sequences. One of these ways is CRISPR-Cas-related genes. However, there is a range of different CRISPR technologies they all involve an RNA-directed protein complex that may anneal to a foreign DNA and/or RNA sequence and via nuclease, capability to induce destruction (Tang et al., 2019). As hypothesized, by this method, CRISPR-Cas presents a considerable danger to phage infection (Mejdani, 2019).

To evade CRISPR-Cas systems bacteriophages code for CRISPR-Cas inhibitors termed Acr (Acrs). These Acrs are tiny phage-encoded proteins that may interact and suppress CRISPR-Cas nuclease activity, hence facilitating lateral gene transfer (Davidson et al., 2020). Whereas CRISPR-Cas mechanisms are a reflection of the co-evolution between bacteria and phages, CRISPR-Cas technologies have also been exploited for biotechnological reasons (Hampton et al., 2020). Through the RNA-directed nuclease functionality of CRISPR-Cas systems, bacterial DNA patterns may be changed, leading to novel bacterial strains that can be exploited for study.

The detection of invading nucleic acids is one of the most prevalent cellular immunity techniques in all living species. The existence of DNA in the cytoplasm can stimulate the cGAS–STING signaling mechanism in eukaryotes, resulting in innate immunity induction via the generation of type I interferons1 (Jia & Patel, 2021). Pathogens have adapted a variety of strategies to resolve such defenses, which is unsurprising (Cheng et al., 2020; Eaglesham & Kranzusch, 2020). To circumvent antiviral mechanisms, phages have developed a variety of counter techniques including alteration of limitation areas or destruction of restriction-modification cofactors (Hampton et al., 2020). The choice for point mutations in the CRISPR–Cas aimed sequence (Deveau et al., 2008) or the establishment of nucleus-like structures (Malone et al., 2020; Mendoza et al., 2020) that protect target identification or the production of Acr proteins that instantly neutralize the CRISPR–Cas effector complex (Bondy-Denomy et al., 2013; Samson et al., 2013). CRISPR–Cas processes are found in around 85 % of archaeal and almost 40 % of bacterial genomes that have been entirely sequenced (Makarova et al., 2020). Prominently, CRISPR–Cas technologies are varied, and they are nowadays divided into two broad categories, each of which contains six different kinds based on phylogenetic analysis, cas gene structure, and method of action (Makarova et al., 2015; Makarova et al., 2020). Multisubunit effector complexes make up class 1 systems (that comprise types I, III, and IV), while single-subunit effector

proteins make up class 2 methods (that contain types II, V, and VI). Despite these variances, all mechanisms follow the same fundamental action principle, which is separated into three phases: adaptation, expression, and interference (Marraffini, 2015; Van Der Oost et al., 2014). Small DNA segments originating from the attacker, termed as spacers, are incorporated into the host CRISPR locus to immunize the host cell during the initial, adaption stage; this inclusion of spacers produces a genetic record of the disease. The CRISPR loci are translated into precursor CRISPR-derived RNAs (crRNAs) and then converted into short mature crRNAs in the second stage of protection. Individual crRNAs join with Cas proteins to create crRNA–effector complexes during the destination interference phase, that are accountable for sequence-particular identification and destruction of invading nucleic acids. By experiencing rapid mutation, phages can avoid CRISPR–Cas immunity by requiring complementarity between crRNAs and phage nucleic acid goals (Andersson & Banfield, 2008; Deveau et al., 2008). Phages have developed protein blockers of CRISPR–Cas networks, termed as Acr proteins, to resist the adaptive nature of CRISPR–Cas monitoring complexes, that were initially found in 2013 (Bondy-Denomy et al., 2013). Acr have been identified for five CRISPR–Cas subtypes, comprising class 2 types II, V, and VI, as well as class 1 types I and III, with none found for type IV yet. Different techniques have been used to uncover distinct Acr that have no sequence characteristics in common. Biochemical and structural analyses have discovered particular pathways of activity for 29 Acr, revealing four main techniques for combating CRISPR–Cas processes: inhibiting CRISPR–Cas complicated assembly, attempting to block target interaction, preventing target breakage, and degrading cyclic oligonucleotide sensing molecules. Cas suppression via dimer synthesis and post-translational alteration of Cas effectors are two further developing Acrmethods (Davidson et al., 2020; Hwang & Maxwell, 2019; Marino et al., 2020). CRISPR–Cas technologies have been reinvented as strong genetic modification techniques for gene and genome editing (Cong et al., 2013; Jinek et al., 2012; Mali et al., 2013), gene regulation (Qi et al., 2013), imaging, and molecular diagnosis (Adli, 2018; Hsu et al., 2014), due to their RNA-guided, sequence-specific recognition capabilities. Gene manipulation has evolved from a basic scientific technique to a technology employed in a variety of medicinal applications due to its ease and resilience.

Discovery of CRISPR–Cas inhibitors

The Ist lively CRISPR–Cas inhibitor was found in an almost similar class to the Pseudomonas species phage. Despite possessing promoter region regions that really should have been recognized by an updated type I F CRISPR–Cas system(Pawluk et al., 2017), those phages were capable of infecting and replicating with in Pseudomonas strains with just as explored I F CRISPR–Cas system8 (Meaden et al., 2021). Sequence analysis identified the chromosomal area responsible for the 'anti-CRISPR' trait along with the particular gene implicated(Pawluk et al., 2018; Touchon & Rocha, 2010). Five different proteins (AcrF1, AcrF2, AcrF3, AcrF4, and AcrF5) have been demonstrated to inhibit the kind I F CRISPR–Cas system(Bondy-Denomy et al., 2015). Due to the particular reason none of these proteins interfered with cas gene expression or crRNA molecule maturation, this was assumed that they prevented CRISPR–Cas interference(Marino et al., 2020). In a further investigation, four other different families of tiny proteins were displayed to suppress *P. aeruginosa* type I E CRISPR–Cas system10 (Borges et al., 2017)

Those proteins were coded by genes found among the same phage family as the class I F Acrgene(Stanley et al., 2019). Unlike the class, I F Acrmolecules, not analogs of both class I E Acr amino acids have been recognized in MGEs from various genera of bacterial species(Liu et al., 2020; Peng et al., 2020). The nine previously discovered Acr protein domains did not share any system is defined features, which might lead to the recognition of supplementary Acr proteins and also peptides among other taxa or active over different CRISPR–Cas types8(e Pacheco, 2019). The proteomic background of the gene producing those proteins, on the other hand, was highly similar(Derrien et al., 2012). Acrphages also produced a suspected transcription regulator known as Acr Associated (Aca1; produced by aca1), which featured a helix–turn–helix pattern directly ahead of something like the Acr genes(Shehreen, 2021), and an Aca regulating proteins appears to check the activity in both Acrand the aca alleles at optimum action even through the phage infection series(Stanley et al., 2019).

A 'guilt-by-association' bioinformatic technique was utilized in subsequent investigations to find the possible Acrgene in MGEs depending on its genomic position downstream of the probable regulator aca1(Yin et al., 2019). Analogous putative Acrgene was discovered upstream of genes producing a helical structure protein of different sequences from a family than Aca1(Pawluk et al., 2018; Stanley et al., 2019). As a result of this novel Aca protein family (Aca2), additional putative Acr genes have been identified(Walker, 2017). This study discovered and functionally verified five novel groups of class I F Acr protein found in MGEs throughout the Proteobacterial phylum (Pawluk et al., 2018; Pawluk, 2016). Following that, the very first inhibitor of a types II CRISPR–Cas systems are found utilizing this guilt-by-association strategy(Davidson et al., 2020). Three small protein families (AcrIIC1, AcrIIC2, and AcrIIC3) have been demonstrated to hamper Neisseria meningitides type II CRISPR–Cas9 activity(Zhu et al., 2019), both in its natural bacterial environment and when utilized like a genome-editing technology in cultured human cells (Porto et al., 2020). To find antagonists of class II A CRISPR–Cas9 systems, researchers utilised a different bioinformatic technique(Bondy-Denomy, 2018). The researchers discovered bacterial strains that had crRNAs (complementary ribosomal RNA) that matched protospacers with their own genome and generate type II-A CRISPR–Cas9 systems(Yaung et al., 2014). Due to self-targeting by both the CRISPR–Cas pathway could be assumed that it results in induction of apoptosis(Reinshagen et al., 2018), that is hypothesised that every single genome containing self-targeting would also have Acr genes in an MGEs present inside the same cell (Pinilla-Redondo et al., 2020; Yin et al., 2019).

The development of such an indigenous Acr protein of the MGEs would render the CRISPR system inactive and the cell is allowed to survive selfs-targeting(Hille & Charpentier, 2016). Using these criteria, 4 novel families of Acr proteins that originate to block the type II-A CRISPR–Cas pathway of Listeria monocytogenes12 (AcrIIA1, AcrIIA2, AcrIIA3 also AcrIIA4)(Osuna et al., 2020). Both of such Acr proteins, AcrIIA2 and AcrIIA4, have been found to remain efficacious in contrast to the Streptococcus pyogenes type II-A CRISPR–Cas9 protein(Zhang & Marchisio, 2021). Acr protein is coded with genes originating in siphophages, myophages, and presumptive recombinant essentials besides pathogenicity landmasses(Leon, 2021). Except for the periodic existence of the aca genes just down-streams of that Acrgene, there are no shared genomic traits(Pawluk et al., 2018). Acrgene is discovered in phages at the head and the tail morphogenetic gene, as well as at the very end of their genome(Stanley et al., 2019). Many MGEs contain 2 or 3 Acr genes in a single operon,

also the significant variety in Acrgene arrangements and pairings implies that they may have been traded across MGEs (Birkholz et al., 2019; Yi et al., 2020). Acr protein is categorized established on that CRISPR–Cas system it suppresses (Marino et al., 2020). The accepted naming standard comprises the kind of system that is suppressed, a number value relating to the proteins family, also the origin of a specific Acr protein(Forsberg et al., 2021). AcrIIC1Nme, for instance, is functional at odds with the type C CRISPR–Cas systems(Thavalingam, 2019); that is the first Acrreported for that scheme, and that is expressed as an N. meningitides genome as an integrated MGE(Pawluk, Amrani, et al., 2016).

Why inhibit CRISPR-Cas?

Among the eagerness and successes in Crs investigation and medical advancement, it might not be instantly understandable why Is it successful to suppress the Cas proteins. Suppression, on the opposing hand, is essential for CRISPR to work properly. CRISPR is already having an impact on several seemingly unrelated and perhaps surprising features of our daily life, such as our environments (Webber et al., 2015), our foods (Scheben et al., 2017), and our heath (Babačić et al., 2019; C.-H. Huang et al., 2018; Karimian et al., 2019; Lino et al., 2018). As a result, kill switch blockers that completely stop CRISPR-Cas systems may be required in a variety of conditions. The development of a conveniently given inhibitor to stop activity could be crucial for therapeutic research and, eventually, FDA agreement of some CRISPR-based drugs. Many approved drugs, such as vitamins K and prothrombin complicated concentrated for anticoagulants, for example, anticoagulant (Yasaka et al., 2002) & heparin protamine sulphate (CR, 1948), include an antiserum that can be donated to the incident of unintended abuse or to reduce the reaction. Importantly, these medications have just a short-term impact on the body, but CRISPR has a long-term effect, forming the accessibility of an emergency- stop further critical. The creation of gene drives to propagate a characteristic across a population's inhabitants or cause wild populations of species to collied totally (Hammond et al., 2016) is one of the suggested uses of CRISPR. The discovery of universally applied CRISPR inhibitors to fight unintended or intentional gene drive misuse, as well as the deploy of Crs contrary to human populations, could become a major worldwide security concern. Off-target effects (Barboni et al., 2017; Naeem et al., 2020; F. Zhang et al., 2019) are another practical reason for suppressing CRISPR. In recent work using CRISPR in the human fetus, researchers showed that disrepair division by-products may survive beyond cellular division, evolving in particular gene loss of whole chromosomes (Zuccaro et al., 2020), Inhibitors may work in two ways to reduce off-target effects. The first is timed inhibition, which prevents considerable off-target cleavage. This technique is based on the idea that on-target cleavage happens quickly because of complete guide-target complementarity, but off-target activity takes longer and folds predominantly after the on-target focus has been cut and altered. This approach was advanced by including an L7Ae: K-tum suppression mechanism to reduce Cas9 transcription and translation at the same time (Shen et al., 2019). Similar results were obtained with a timed distribution of the Acr protein AcrllA4 (Shin et al., 2017). Sufficient enzyme inhibition is the second route through which inhibitors may reduce off-target editing. Although this is comparable to timed suppression, it usually includes the effector and inhibitor being delivered at the same time. "Off-tissue" editing is a similar notion. It is an on-the-move location: when editing is not desired in a tissue or organ, rather than an inaccurate genetic locus or target, that is altered in this situation. Unrestricted CRISPR-mediated editing exposes a variety of organs and cell types to potentially harmful off-target alterations, such as loss of large genomic

sequences or chromosomal rearrangements. that may not be illness relevance (Kosicki et al., 2018). If at everything is possible, off-tissue modification should be prohibited.

Acr proteins

Acr proteins are expressed by plages to aid phages to escape bacteria and archaea Crs methods. Acrs were first discovered in *P. aeruginosa* phages as a collection of five genes 157), and they are also expressed by genetic elements in a few bacteria (Pawluk et al., 2014). They were able to infect *P. aeruginosa* cultures by inhibiting the bacterium's type IF CRISPR-Cas defensive mechanism. Experiments using translationally inept copies of the genes demonstrated that suppression was translation-based, suggesting a protein-based mechanism. Soon later, the same group discovered that few of these phages included blockers of CRISPR *P. aeruginosa* type 1-E mechanisms (Pawluk et al., 2014). Overall of the Anti CRISPR proteins known till 2017 targeted class I systems. Cas9 and Cas12 enzymes, on the other hand, are developed from class 2 systems and have been widely employed in biotechnology. 3 Acrs that suppress the type 2-C systems of Neisseria meningitidis and Brackiella oedipodis were the first described instances of a class 2 Acr (Pawluk, Amrani, et al., 2016). Listeria monocytogenes phages have Acr-encoding genes that block type II-A system is a system, such as the well-known SpCas9. Despite the fact that LmoCas9 and SpCas9 have only 53% sequence similarity, acrIIA2 and acrllA 4 generated proteins that could block target binding in SpCas9, proving that AcrllA2 and Acrl1A4 are broad-spectrum inhibitors of type II-A Cas9 orthologs (Rauch et al., 2017). AcrllA2 and AcrllA4 e shown to inhibit SpCas9 in human cells in the same investigation. A fifth type II-A inhibitor was found. this time from *Streptococcus thermophilus* phages, using a strategy that focused on naturally existing Acr activity in phage strains to find possible Acrs. Later, AcrilA5 was discovered to block all types II Cas9s, including all Cas9 homologs utilized in genome engineering. While no inhibition of type 2-B Francisella novicida (Fn)Cas9 was seen in plaque experiments (Garcia et al., 2019). AcrllAS-dependent suppression of FnCas9 was reported in vitro (Song et al., 2019; Watters et al., 2020). Using a guilt-by-association technique, three novel proteins. AcrllA13-15, was recently discovered as exclusively inhibiting S. aureus (Sa) Cas9 but not SpCas9 in living cells (Watters et al., 2018; Watters et al., 2020). SaCas9 inhibitors are a useful addition to the CRISPR-Cas9 toolkit because of SaCas9's modest size and relative simplicity of administration. Another extensively utilized Cas enzyme, type V Cas 12a, has also been shown to contain Acr inhibitors, Acrs, also known as AcrVALI-5, was discovered to impede self-targeting by Cas12a systems in two separate investigations (Watters et al., 2018).

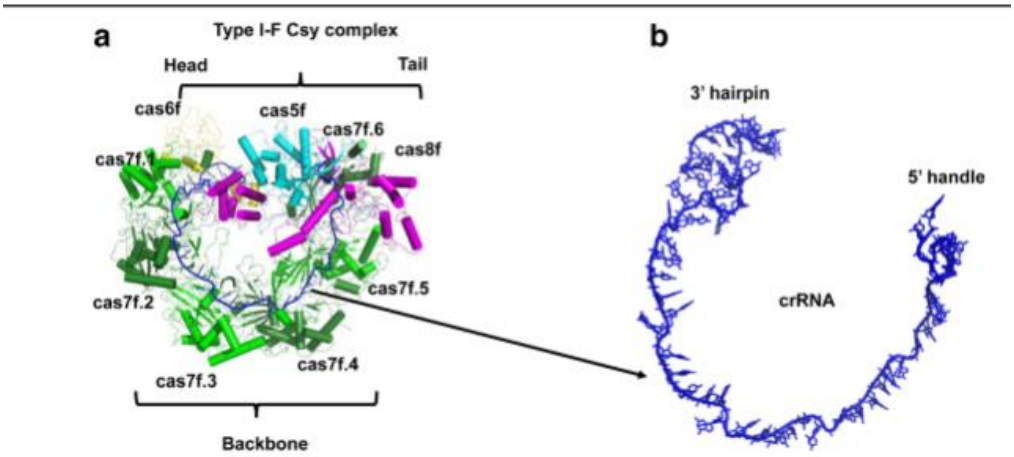
Structure of Acr proteins

Framework biologist's research on Acr proteins has solved the geometries of Acr proteins or different fields of activity such as interaction with target CRISPR-Cas effectors. The structures of 7 Acr proteins have been revealed: AcrF1, 2, 3, 10, AcrIIA1,4, and AcrIIC1. Though, there is still a need for a systematic and comprehensive theoretical design of antiCRISPR amino acids.

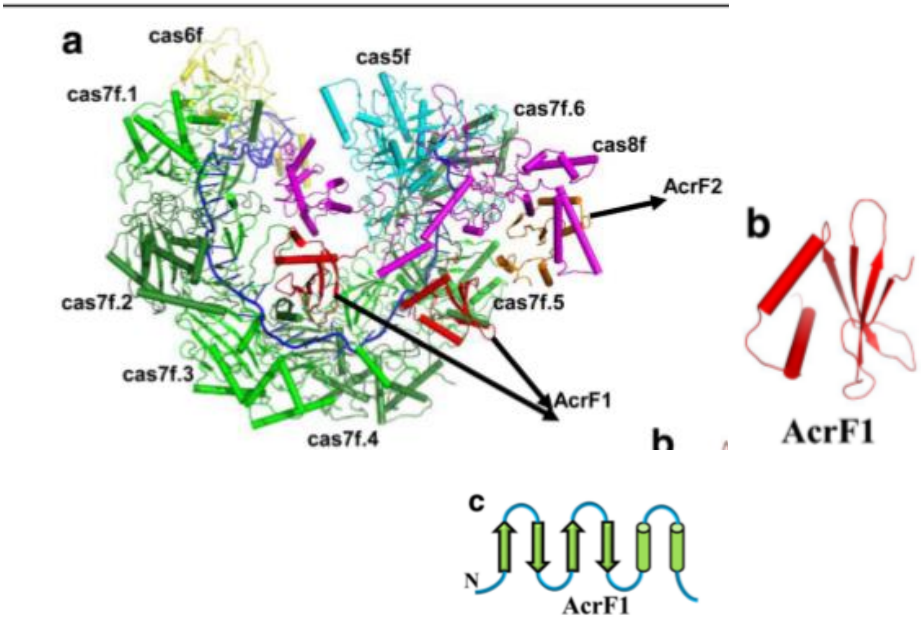
Structure of AcrF1

Choudhury and colleagues first discovered that the electron microscopy structure of a subtype I-F Csy molecule related to two unique Acr proteins, AcrF1 or AcrF2, with just an average resolving of 3.4 Å was linked to two distinct Acr proteins, AcrF1 or

AcrF2 (Chowdhury et al., 2017). The Cas6f head, Cas7f backbone, and Cas8f-Cas5f tail make up the overall structure of the type I-F Csy complex, which has a nearly closed ring topology. The 60-nucleotide crRNA, which resembles a string and tethers the complex's protein subunits together, plays an important structural role in compound formation. Gene 35 of the Pseudomonas aeruginosa phage JBD30 encodes the 78-amino-acid amino acid AcrF1. The general structure of AcrF1 is fairly simple, divided into 4 anti-parallel strands and two anti-helices (β1↑-β2↓-β3↑-β4↓-α1-α2). These 4 anti-parallel -strands create a -sheet that binds to the 2 anti -helices at the C-terminus to produce a hydrophilic core (Maxwell et al., 2016).



Structural view of Type I-F Csy complex (a)Complex of type IF Csy structure The type IF Csy complex's cassf, cassif, cast, and cassf subunits are colored cyan, yellow, green, and magenta, respectively. The crRNA is blue in colour. (b)An expanded image of the aRNA structure, which looks like a string.



Structural view of type I-F Csy complex bound to AcrF1/2 .(a)The type I-F structure Two Acr proteins, AcrF1 and Acr F2, were bound by the Csy complex. The type I-F Cay complex's cas5f, cas6f cas7f, , and cas8f subunits are coloured as shown in Fig.

1a. The structures AcrF1 and AcrF2 are coloured red and aronge, respectively.(b)Also, topolocical perspective. (c)3D structure of AcrF1

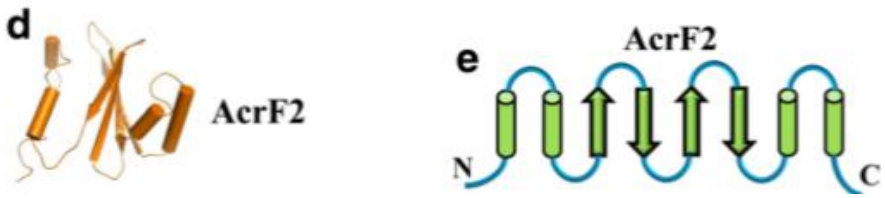
Table 1. Anti –CRISPR protein and their mechanism of action

Anti CRISPR (sources)	Size (amino acids)	CRISPR inhibited	Inhibition mechanism	Struture (PDB code)	References
AcrE1 (*P.aeruginosa*)	100	Type I-E	–	–	(Pawluk et al., 2014)
AcrE2 (*P.aeruginosa*)	84	Type I-E	–	–	(Pawluk et al., 2014)
AcrE3 (*P.aeruginosa*)	68	Type I-E	–	–	(Pawluk et al., 2014)
AcrE4 (*P.aeruginosa*)	52	Type I-E	–	–	(Pawluk et al., 2014)
AcrF1 (*P.aeruginosa*)	78	Type I-F	Inhibits DNA binding	2LW5,5UZ9, 6ANV,6B46, 5UZ9,6B47	(Bondy-Denomy et al., 2013; Chowdhury et al., 2017; Guo et al., 2017)
AcrF2 (*P.aeruginosa*)	90	Type I-F	The binding site of dsDNA is partially overlapped.	5GNF,5GQH, 5B71	(Bondy-Denomy et al., 2013; Chowdhury et al., 2017; Guo et al., 2017)
AcrF3 (*P.aeruginosa*)	139	Type I-F	The entrance to the DNA binding tunnel is blocked, preventing fresh sequence acquisition.		(Bondy-Denomy et al., 2013; X. Wang et al., 2016)
AcrF4 (*P.aeruginosa*)	100	Type I-F	___		(Bondy-Denomy et al., 2013)
AcrF5 (*P.aeruginosa*)	79	Type I-E/F	___		(Bondy-Denomy et al., 2013)
AcrF6 (*P.aeruginosa*)	100	Type I-F	____		(Pawluk, Amrani, et al., 2016)
AcrF7 (*P.aeruginosa*)	67	Type I-F	___		(Pawluk, Amrani, et al., 2016)
AcrF8 (*P.atrosepticum*)	92	Type I-F	____		(Pawluk, Amrani, et al., 2016)

AcrF9 (*V.parahaemolyticus*	68	Type I-F	——		(Pawluk, Amrani, et al., 2016)
AcrF10 (*S.xiamenensis*)	97	Type I-F	DNA mimic blocks DNA binding	6ANW,6B48	(Guo et al., 2017; Pawluk, Amrani, et al., 2016)
AcrIiA1 (*L.monocytogenes*	149	Type II-A	——	5Y6A	(Ka et al., 2018; Rauch et al., 2017)
AcrIIA2 (*L.monocytogenes)*	123	Type II-A	Inhibits DNA binding	——	(Rauch et al., 2017)
AcrIiA3 (*L.monocytogenes)*	125	Type II-A	——	——	(Rauch et al., 2017)
AcrIiA4 (*L.monocytogenes)*	87	Type II-A	PAM mimic inhibits DNA binding by interacting with the active site inside the RuvC domain and preventing the HNH domain from changing conformation.	5XBL, SVW1,5VZL	(Dong et al., 2017; Rauch et al., 2017; Shin et al., 2017)
AcrllA5 (*S.thermophilus*)	140	Type II-A	——	——	(Hynes et al., 2017)
AcrIiC1 (*N.meningitidis*)	85	Type II-C	Protects the catalytic core by binding the HNH domain.	5VGB	(Harrington et al., 2017; Pawluk, Amrani, et al., 2016)
AcrIiC2 (*N.meningitidis*	123	Type II-C	——	—	(Pawluk, Amrani, et al., 2016)
AcrIiC3 (*N.meningitidis*)	116	Type II-C	Cas9 dimerization is induced, which hinders DNA binding.	——	(Harrington et al., 2017; Pawluk, Amrani, et al., 2016)

Structure of AcrF2

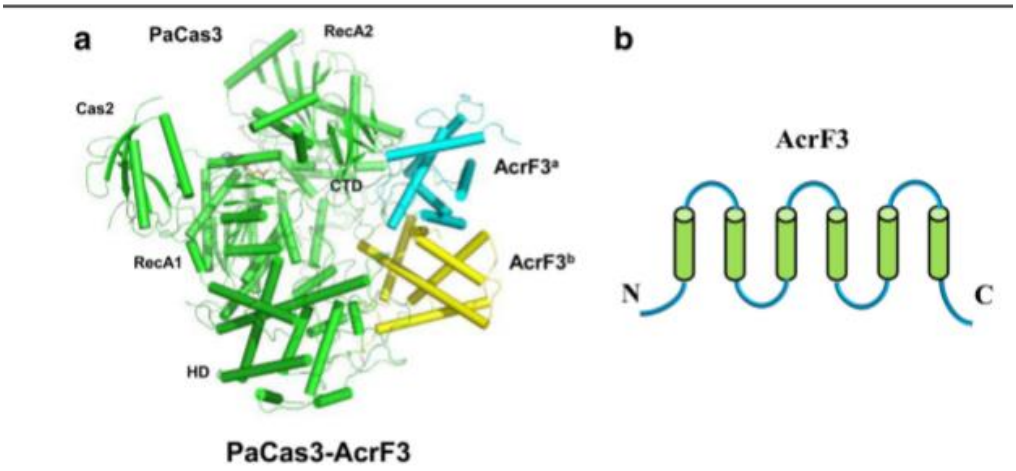
AcrF2, a small alkaline protein, is a 90-amino acid protein produced by *P. aeruginosa* phage D3112 via nucleotide Thirty. The geometry of AcrF2 coupled to the kind I-F Csy molecule was identified by using a single-particle cryo-electron microscope. AcrF2's logical narrative is sandwiched, with 4 antiparallel -strands flanked on each side by two -helices(α1,2 -β1,4↑-β2,3↓-α3,4) except for 2-helices at the N-terminus, AcrF2 has a topological structure that is quite similar to AcrF1(Chowdhury et al., 2017; Guo et al., 2017).



(d) AcrF2 topological view (e) AcrF2 helices and strands are shown by green cylinders and arrows, respectively.

Structure of AcrF3

AcrF3 is a bigger protein than AcrF1 or AcrF2 and is coded by gene -35 from the *P. aeruginosa* phage JBD. It is greater than AcrF1,2 and has 139 proteins (Table 1). Zhu's group discovered the crystalline structure of AcrF3 in combination with PaCas3 in 2016, with clarity of 2.6 Å (Mallon & Bailey, 2016; X. Wang et al., 2016). Wang and colleagues then utilized a cryo-EM single-molecule technique to solve a 4.2-residue PaCas3 (residue 106–1076)–AcrF3 complex using the molecular structure of AcrF3 with a resolution of 1.5Å. The all framework of AcrF3 consists of 6 -helices. The gel filtration chromatographic findings indicate that AcrF3 has a dimeric structure. The HD domain range is RecA1, RecA2, and PaCas3's CTD form a groove around the AcrF3 dimer. PaCas3 and AcrF3 form a dense complex with many H-bonds and a significant hydrophobic relationship (J. Wang et al., 2016).



PaCas3-AcrF3

Structure of PaCas3 in complex with AcrF3 (a)PaCas3 is coloured green in this structure of the PaCas3-AcrF3 complex. Respectively, the AcrF3 dimer is coloured cyon and yellow. Cas2, RecA1/2, HD, and CTD domains of PaCas3 have been tagged. (b)AcrF3 topology.

Structure of AcrIIA4

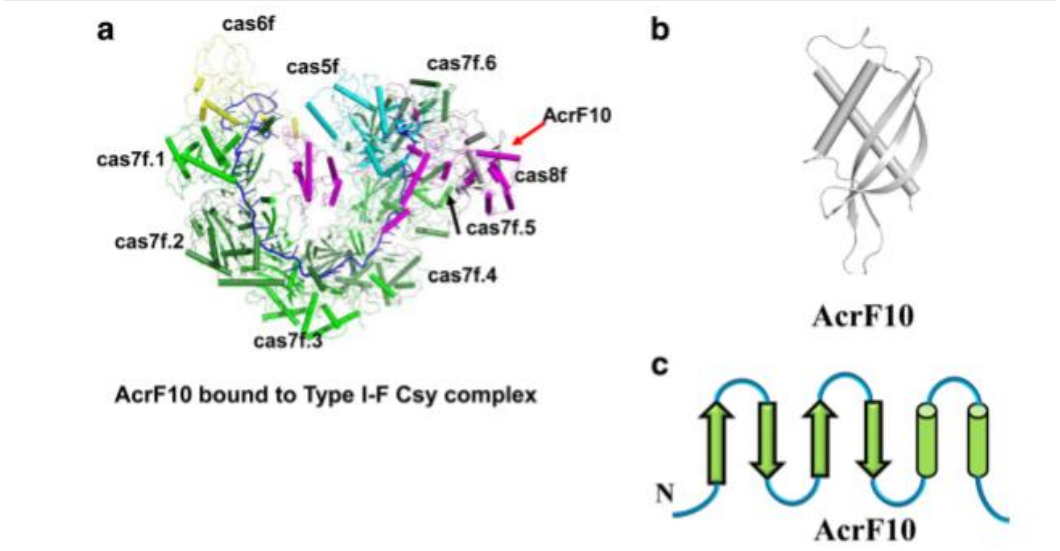
AcrIIA4 does have a "triangle" bind that consists of 3 anti-parallel strands and 3 helices (α1-β1↑-β2↓-β3↑-α2-α3). These 3 anti-parallel strands create a -sheet containing three -helices over one end. AcrIIA4 has the same topological structure as AcrF1, but the B-strand has replaced the Alpha-helix Stand. The PAMinteracting region in the AcrIIA4–SpyCas9–sgRNA structures are held by AcrIIA4, which binds to the spyCas9 TOPO, CTD, and RuvC domains (Dong et al., 2017).

Structure of AcrIIA1

A common type II-A Acr is AcrIIA1, a 149-amino-acid Acrderived from an *L. monocytogenes* prophage. AcrIIA1's geometry was recently established using X-ray diffraction with a magnification of 2.0Å. Two AcrIIA1 compounds combine to produce a homodimer, each with an all-helical 2-domain architecture (Ka et al., 2018).

Structure of AcrF10

X-ray diffraction was used to determine the structure of AcrF10 (Guo et al., 2017).



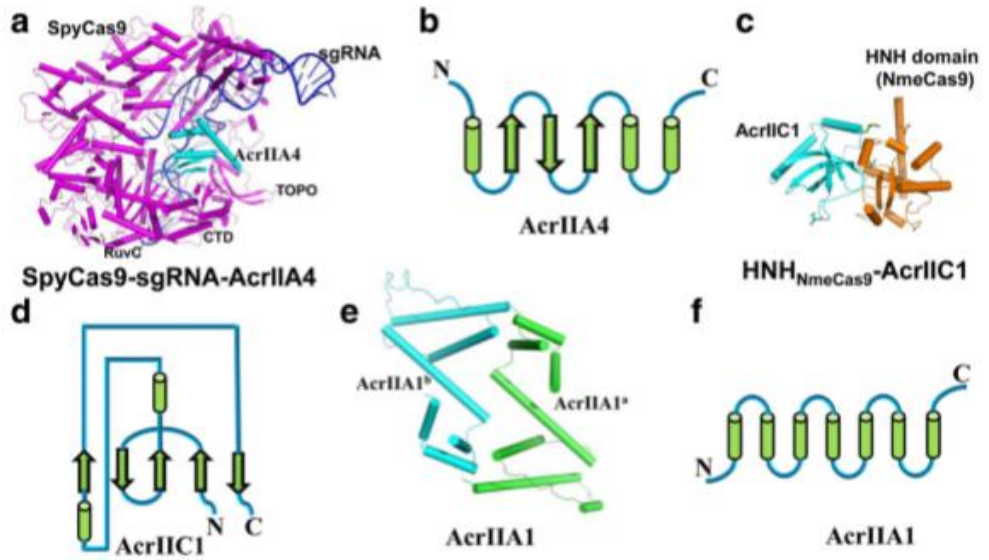
View of the I-F Csy complex linked to AcrF10 from a structural standpoint (a) Csy complex of type I-F connected to AcrF10 structure The type I-F Csy complex's cas5f, cas6f, cas7f, and cas8f subunits are colored as shown in the 3D structure of AcrF10. AcrF10 (b)and topological view (c)

Structure of CrllA4

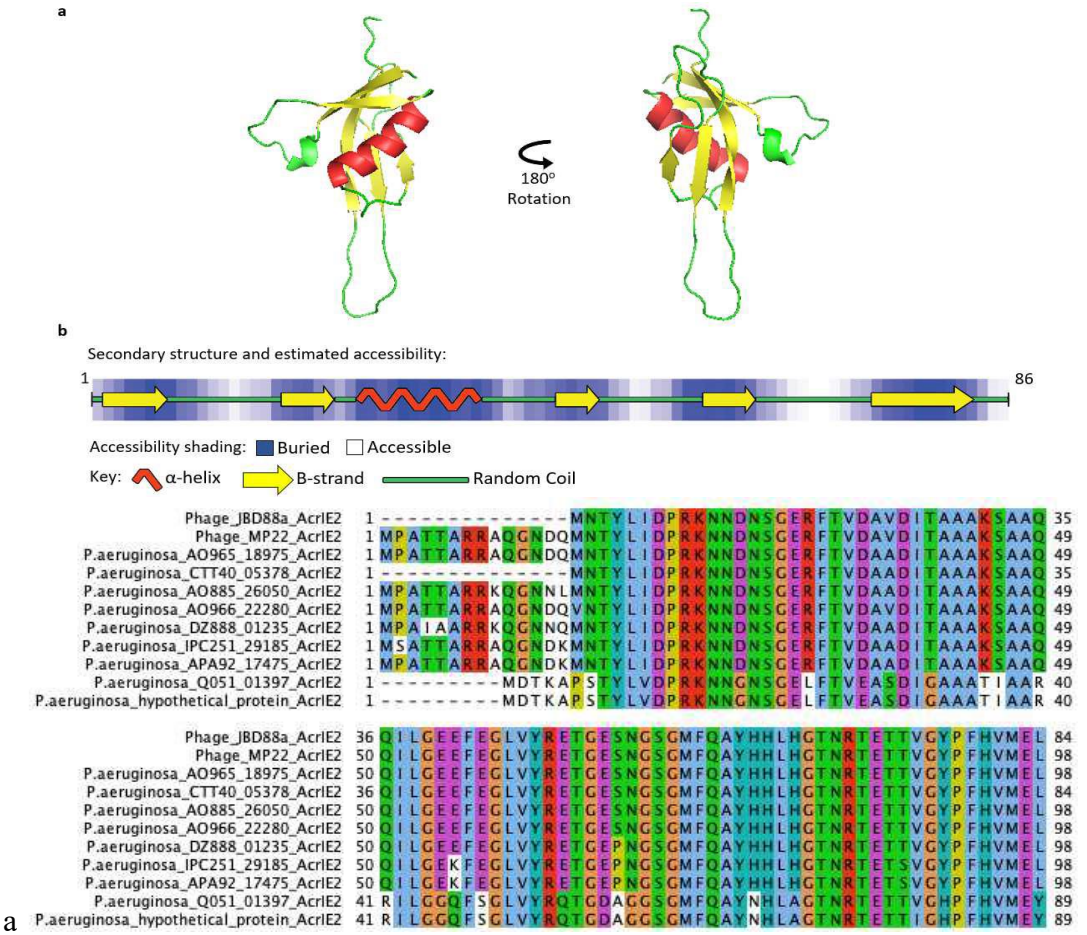
CrIIA4 is a bacteriophage from *L. monocytogenes* that contains 87 amino acids.

Structure of AcrII2

The structure identified for AcrIE2 is depicted in Figure 9a. The structure of AcrIE2 is an 84- residue amide with a core alpha-helix and a five-strand antiparallel β-sheet (Figure 9a, b). The α-helix packs against one side of the β-sheet, generating a compact hydrophobic core with 10 residues >90 percent buried. The geometry of AcrIE2 is not related to any previously known Acr protein, and research utilizing the Dali server did not indicate structural similarities to another protein (Holm & Rosenstrï¿ ½m, 2010).



Type II Acr proteins seen structurally. In the AcrIIA4-SpyCas9-sgRNA complex, SpyCas9 and AcrIIA4 are magenta and cyan, respectively. Blue is the color of crRNA. RuvC CTD and TOPO domains of SpyCas9 have been identified. (b)AcrIIA4 from a topological standpoint AcrllC1 and the HNHnmecas9 domain are colored cyan and orange, respectively, in this structure of the AcrIICl-HNHnmecas9 domain complex. AcrIIC1 from a topological standpoint (e)AcrIIA1dimer structure with its cyan and green colored ids. f)Kf AcrIIA1 topological perspective



a

AcrIE2 NMR structure (Figure 9). () The backbone atoms in ribbon representation (N, C, and C') in the NMR solution structure of AcrIE2. (b) AcrIE2 secondary structure with different shades of blue indicating relative residue accessibility. Blastp alignment of the JBD88a-AcrIE2 protein's amino acids.

Inhibition mechanisms of Acr proteins

AcrIIA1-A21

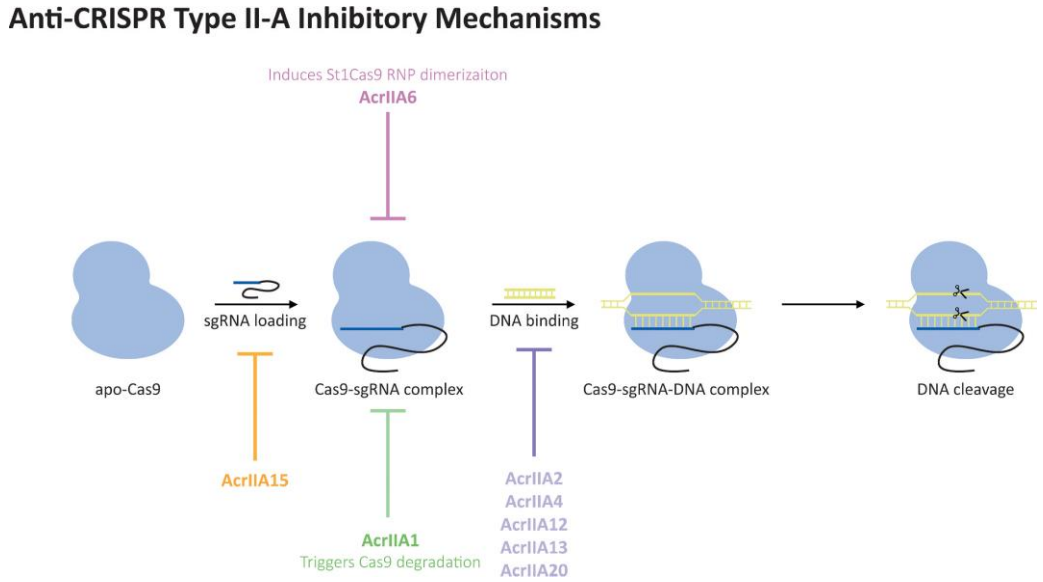
Listeria monocytogenes prophages encode AcrIIA1-A4, which suppresses the type II-A CRISPR-Cas system among *L. monocytogenes* strain (Rauch et al., 2017). The seeming self-targeting of CRISPR-Cas systems in *L. monocytogenes* prompted about finding of AcrIIA1-A4. this bacterial genome demonstrates the coexistence of the protospacers (derived from MGE) and excellent matching spacer, which is thought to be accountable for apoptosis when the CRISPR array is expressed. As a result, a bioinformatics technique was used to search *L. monocytogenes* DNA for a specific inhibitor — of that viral origin – which may disable this CRISPR-Cas system resulting in enabling cell viability (Rauch et al., 2017). The mostly co of acrIIA1 with different acrIIA genes (for example AcrIIA2-A4 also AcrIIA12 (Osuna et al., 2020; Rauch et al., 2017) identified in *L. monocytogenes* prophage revealed how AcrIIA1 suppresses gene editing from LmoCas9, implying that AcrIIA1 needs an Acr companion for inactivation of Cas9. Cas9 disintegration is aided by AcrIIA1, whilst DNA attachment is hindered by the other Acr. As a result, the collaboration of the two Acr is required to efficiently disable Cas9 at the time of the invasion of phage (Osuna et al., 2020). AcrIIA1 has been found to build two helical-domain Dimer architectures and a motif of helix-turn-helix (HTH) at the N terminus (Ka et al., 2018). When AcrIIA1 is highly expressed in E. coli, it may also bind with RNAs (Ka et al., 2018). In E. coli and human cells, AcrIIA1 did not demonstrate any inhibition of SpyCas9-guided gene editing (Rauch et al., 2017), but it did show strong inhibitory effectiveness in Saccharomyces cerevisiae (Nakamura et al., 2019).

Among human cells, E. coli, and also S. cerevisiae, AcrIIA2, and AcrIIA4 act well against SpyCas9-driven DNA cleavage (Basgall et al., 2018; Nakamura et al., 2019; Rauch et al., 2017). AcrIIA4 inhibits SpyCas9 nuclease action fully (Rauch et al., 2017), but AcrIIA2 inhibits SpyCas9 nuclease function less effectively in mammalian cells than in S. cerevisiae (Nakamura et al., 2019). Modification to AcrIIA2, as attaching the GFP (green fluorescent protein) to the N or C terminals, seems to significantly reduce its inhibitory capacity, while AcrIIA4 appears to be more resistant (Basgall et al., 2018). in their unbound form AcrIIA2 and AcrIIA4 are monomers, unlike AcrIIA1 (Yang & Patel, 2017). AcrIIA2 and AcrIIA4 have a functional similarity in that they both use DNA mimicry to suppress the type II-A CRISPR-Cas mechanism. AcrIIA2 and AcrIIA4 have a greater affinity for the SpyCas9-sgRNA dual complex than DNA when produced in bacterial cells. They engage SpyCas9-sgRNA as a result block their site of DNA-binding (Dong et al., 2017), inhibiting the recognition of PAMs and so the development of the SpyCas9-sgRNA-DNA ternary complexes. Subsequently, a SpyCas9-sgRNA-DNA ternary complex is formed, even not AcrIIA2 not AcrIIA4 could remove the DNA of that the SpyCas9-sgRNA complexes. In the lack of sgRNA, AcrIIA2 and AcrIIA4 do not interfere with SpyCas9. SpyCas9 does undergo a conformational shift after engaging the sgRNA, which produces the attaching furrow of the DNA substrate or Acr proteins (Dong et al., 2017; Jiang et al., 2015; Jinek et al., 2014; Yang & Patel, 2017). the structural modifications generated by AcrIIA2 and AcrIIA4 are somewhat different. AcrIIA2

establishes a hydrogen bond (H) with the PI (PAM-interacting), HNH (His-Asn-His), WED (wedge-like), and REC2 (RNA be familiar with) domain of that SpyCas9 when it links SpyCas9-sgRNA (Liu et al., 2019). AcrIIA4 forms hydrogen bonds with the PI, WED, and RuvC domains rather than the HNH domain of SpyCas9 (Dong et al., 2017; Liu et al., 2019). A nuclease domain HNH and RuvC are essential for that SpyCas9-dependant RNP action. The former cleave the non-target strands of the dsDNA substrates, The former cleaves the target strand, but the latter does not (Jinek et al., 2014)That after the DNA substrate has indeed placed against SpyCas9-sgRNA besides base-pairing among DNA substrate also sgRNA has been finished, an HNH domain experiences a conformation reorganization which is hypothesized for promoting the inactivity to vigorous alignment transition (Jiang et al., 2015). The hydrogen bond(H) in between AcrIIA2 and the HNH domains of SpyCas9 prevents any endorsement change in HNH, preventing the RNP from acting as a nuclease. In comparison, AcrIIA4 interacts along with the RuvC dominion active site and prevents it. (Dong et al., 2017; Jiang et al., 2015; Shin et al., 2017; Yang & Patel, 2017), provide comprehensive research on both construction of SpyCas9-sgRNA-AcrIIA2 and also of the SpyCas9-sgRNA-AcrIIA4. The aggressive phages of *Streptococcus thermophilus* encode AcrIIA5 and AcrIIA6 (Hynes et al., 2018; Hynes et al., 2017). AcrIIA5 is anticipated to have a unique motif which is coiled-coil which is thought to operate as a binding motif of nucleic acid, but AcrIIA6 should not (Hynes et al., 2018; Hynes et al., 2017).

So far, only a few research have looked into the process of AcrIIA5 inhibition (An et al., 2020; Garcia et al., 2019; Hynes et al., 2017; Song et al., 2019). However, it's unclear whether AcrIIA5 works via cleaving sgRNA (Garcia et al., 2019) or by inhibiting RuvC domain nuclease action (Song et al., 2019). AcrIIA5 has been shown to correlate to the Cas9-sgRNA than apo-Cas9 (i.e. short of sgRNA) in a new analysis, suggesting the Acr proteins might use an initially unidentified approach to restrict a variety of Cas9 homologs (that makes usage of its N-terminal fundamentally muddled region – IDR) (An et al., 2020). AcrIIA5 overpowers genetic excision intermediated by several Cas9 homologs after type II-A (e.g., SpyCas9), type II-B (e.g., Francisella novicida Cas9 – Fn AcrIIA6 creates a dimer with two St1Cas9-binding sites that are homologous. AcrIIA6 inhibits St1Cas9 by causing St1Cas9 RNP dimer formation by intermolecular contacts (Fuchsbauer et al., 2019). As a result, the AcrIIA6 dimer lowers the St1Cas9 RNP's interaction efficiency for the aim DNA. AcrIIA7-A10 were identified using a unique in elevation amount transmission method relying on a mock genetic track in E. coli cells, anywhere the action of fresh Acr protein is linked to antibiotic choice. Human gut metagenomes had AcrIIA7, AcrIIA8, and AcrIIA9, whereas a soil metagenomic collection contained AcrIIA10. These four Acrs' operating mechanism is currently unclear. AcrIIA7, on the other hand, is thought to work in a novel manner, disrupting the assembly of the SpyCas9-sgRNA binary complexes (Uribe et al., 2019). AcrIIA11 is discovered comparably, using protein efficient action and high-throughput metagenomic library transmission (Forsberg et al., 2019). However, the mechanism by which AcrIIA11 performs its role remains uncertain. This suggests that AcrIIA11 interacts with a SpyCas9 area which conformation does not vary whether SpyCas9 forms complexes with equally the solitary sgRNA or both the sgRNA and the target DNA. Furthermore, when AcrIIA11 binds SpyCas9, it may experience a conformationals shift which made it more likely to attach dsDNA (Forsberg et al., 2019). As previously reported, AcrIIA12 was discovered in *L. monocytogenes* prophage, wherever it co-occurs with AcrIIA1. Cas9 is likely prevented from attaching to DNA by AcrIIA12. There are preserved HTH-

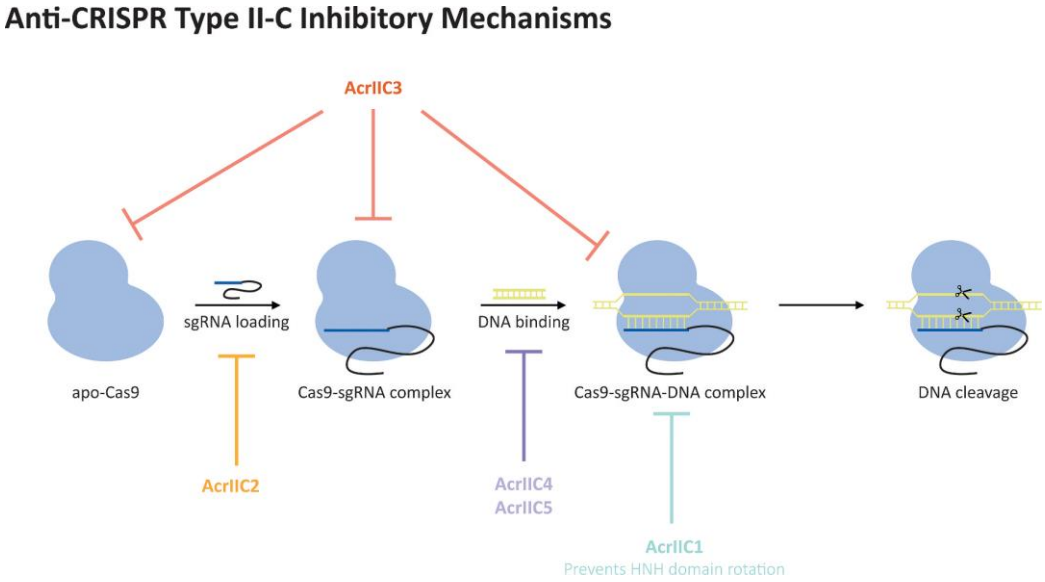
encoding aca in the proximity of acr genes, which is significant. This discovery led to the hypothesis that the presence of HTH might indicate the presence of acr genes (Bondy-Denomy et al., 2015; Pawluk, Staals, et al., 2016; Stanley et al., 2019). A bioinformatics technique known as 'guilt-by-association' has been developed based on this concept to find possible acr genes in a variety of bacterial species (Bondy-Denomy et al., 2013; Pawluk, Amrani, et al., 2016). The anticipated HTH motif is retained in the N-terminal region of all three Acrs. They do, however, have distinct C-terminal dominions which play a role in the Acr action that disrupts SauCas9's function. The SauCas9-sgRNA complex is prevented from attaching to the target DNA by AcrIIA13, while sgRNA loading onto SauCas9 is hampered by AcrIIA15. AcrIIA14 doesn't prevent sgRNA packing or target DNA engagement, but it does reduce SauCas9 DNA breakage (Watters et al., 2020). In human cells, AcrIIA13 and AcrIIA14 are particularly effective suppressors of SauCas9-guided gene-altering, with AcrIIA13 demonstrating the greatest efficacy (almost complete suppression). AcrIIA15's inhibitory effectiveness is substantially lower than AcrIIA13-A14's. This was prompted by the discovery that acrIIA1, which contains the HTH motif, was often found down-streams of acrIIA2-A4 [38]. Although its action method has yet to be determined, AcrIIA16-A19 had the capacity to block the activity of SpyCas9 and stop DNA breakage in humanoid cell. Significantly, AcrIIA16 and AcrIIA17 inhibit type II Cas9 homologs throughout a wide range. Machine-learning methods had recently being used for the search for new Acr (Eitzinger et al., 2020; Gussow et al., 2020). AcrIIA20 is the smallest type II Acr discovered so far, with just 64 amino acids. In vitro, AcrIIA20 was shown to be a strong SinCas9 inhibitor over DNA mockery, although it was fewer efficient in contradiction of SpyCas9. AcrIIA21 was very effective against SinCas9 nuclease movement, still only moderately effective against SpyCas9 and SauCas9. Its methods of operation are yet unclear (Eitzinger et al., 2020).



AcrIIC1-5

Using the 'guilt-by-association' bioinformatics technique, 5 types of II-C Acr protein called AcrIIC1-C5, are found then are characterized so far (Lee et al., 2018; Pawluk, Amrani, et al., 2016). AcrIIC1-C3 was detected within *N.meningitidis* and MGE, while AcrIIC4 too AcrIIC5 were detected in Haemophilus parainfluenzae then also in Simonsiella muelleri strain, respectively. Nme1Cas9 which is also present in humanoid cells (Lee et al., 2018; Pawluk, Amrani, et al., 2016), HpaCas9, also

SmuCas9 both of them are prevented from cleaving DNA by each five type II-C Acrs. AcrIIC1-C4 is efficient against Nme2Cas9, a homolog of Nme1Cas9 (Edraki et al., 2019; Lee et al., 2018; Pawluk, Amrani, et al., 2016). (two NmeCas9s differently, essential, just for the PI domains, that result in different PAM arrangement recognitions). On the other hand, AcrIIC5 performs best at Nme1Cas9 (Lee et al., 2018; Pawluk, Staals, et al., 2016). Other type II-C Cas9 homologs, like GeoCas9 and CjeCas9, are likewise inhibited by AcrIIC1. As a result, it is classified as a broad-spectrum inhibitor of type IIC (Harrington et al., 2017). It also engages Cas9 analogs by creating hydrogen bonds along with its largely preserved HNH nuclease domains. The HNH nuclease domain's conformational variations are limited by these hydrogen bonds, which reduces DNA breakage while allowing the loading of RNA and binding of DNA (Harrington et al., 2017). When the Cas9-sgRNA complex of surveillance engages the DNA substrate, the HNH nucleases domains rotate and the conformational shift to the catalytically activated state occurs. The target-strand cleavage is preconditioned by a catalytically active HNH, which leads to RuvC nuclease domain initiation. Both strands' target and non-target breakage is eventually achieved by the two catalytically active nuclease domains (Harrington et al., 2017; Jinek et al., 2014; Sternberg et al., 2015; Sun et al., 2019). The HNH nuclease domains are no longer able to spin after AcrIIC1 engagement, keeping Cas9 inactive (Harrington et al., 2017). AcrIIC3 is also engaged by the HNH nucleases domains of Nme1Cas9 as a monomer and inhibits their rotations(Sun et al., 2019). Furthermore, DNA-binding is not entirely abolished but just decreased following the synthesis of Nme1Cas9-sgRNA-AcrIIC3 (Harrington et al., 2017; Sun et al., 2019). Now Two AcrIIC3 monomer capture which causes the dimer formation of two different Nme1Cas9s in more detail. Every AcrIIC3 monomer binds to the HNH nuclease domains of a Nme1Cas9 and the REC2 domains of the second Nme1Cas9 (they are spatially opposite each other) (Kim et al., 2019; Sun et al., 2019; Zhu et al., 2019). As a component of a Nme1Cas9-sgRNA-AcrIIC3 dimer by or devoid of DNA mark, AcrIIC3 inhibits HNHs nucleases domains spinning and confines Nme1Cas9 in an inactivated state, preventing the cleavage of DNA (Sun et al., 2019). AcrIIC2 is exclusively active as a dimer and acts on Nme1Cas9 in an unusual way. The negatively charged surface of the AcrIIC2 dimer links with Nme1Cas9's positively charged arginine-rich bridging helix (BH). That hinders the loading of RNA and also the development of the Nme1Cas9-sgRNA complexes as a result. As a result, Nme1Cas9 is rendered inactive and becomes vulnerable to proteolytic enzyme or protein (Thavalingam et al., 2019; Zhu et al., 2019). AcrIIC2 has lesser reactivity for the establishing Nme1Cas9-sgRNA surveillance complex, resulting in decreased inhibition effectiveness (Thavalingam et al., 2019; Zhu et al., 2019). Surprisingly, two BH alterations (T62R and T73R) provide the same AcrIIC2-reaction site to SpyCas9 as Nme1Cas9. As a result, AcrIIC2 inhibits like SpyCas9 mutants, indicating that AcrIIC2 that interacts largely along with the BH that of Nme1Cas9 (Thavalingam et al., 2019; Zhu et al., 2019). Nonetheless, AcrIIC2 develops the stable complex (along with varying affinities) in vitro and Cas9 develop several subtypes of type II: Nme1Cas9 and CjeCas9 (II-C subtype); SauCas9 and SpyCas9 (II-A); and FnoCas9 (II-B) (II-B). In any case, only Nme1Cas9 (Zhu et al., 2019) has high inhibitory efficiency. Nme1Cas9 cannot attach to DNA when AcrIIC4 and AcrIIC5 are present (Lee et al., 2018). However, the Acr protein's structural structures are absent, and there mode of action has yet to be discovered (see Fig. 2 for an summary on Acr type II-C means of action).



Acr protein metabolism

Chains of peptides were normally totally stable in solution, and nonenzymatic hydrolysis may take years (Kahne & Still, 1988). While there are several routes inside the body of a human that break down proteins, Acr protein digestion must be fairly expected if it is considered to be transcribed or translated, which is then destroyed, within cells. The autophagy-lysosomal route and the ubiquitin-proteasomal route are the two most common pathways for intracellular proteolysis. Protein is uptake into autophagic vacuole inside the cell in a nonselective manner. These entities may subsequently combine with lysosomes, which contain proteases that destroy the proteins that have been trapped (Meijer & Codogno, 2004). The ubiquitin-proteasomal mechanism, on the other hand, is more selective. In a nutshell, ubiquitin polypeptides are covalently linked to the target protein to mark it for destruction (ubiquitination). The proteasome subsequently degrades the ubiquitinated proteins. Proteolysis releases amino acids that may be utilized by biological activities, such as protein synthesis (Glickman & Ciechanover, 2002). Actually fusion related to Acrs with the cell division-controlled proteins Cdt1 was utilized to show that Cas9 inhibition through the ubiquitin-proteasomal pathway is cell cycle-dependent (Matsumoto et al., 2020). One disadvantage of using Acr protein is that the half-life of Acr protein is affected by cellular metabolic parameters for example type of cell, stress, and health of the cell.

Uses for gene-editing technologies

The usage of Acr proteins that is on leave during genetic modification is one of their most important applications. It's critical for CRISPR-Cas9 technology to have as bit off-target activity as possible, especially for therapeutic applications. Even though efforts are being made in a big way have resulted in Cas9 variations with improved specificity. extreme or severe Cas9 action may enhance the risk of off-target editing or toxicity, requiring a method to stop Cas9 action once the required outcome has been achieved. Acr proteins, when used in conjunction with modified Cas9 variations and methods to regulate Cas9 production, can provide an additional defence against Cas9's potentially harmful consequences. For example, in minimizing the interval of Cas9 function and going to take advantage of kinetics changes in Cas9 editing at on- and off-target sites (Shin et al., 2017).

The timed release of AcrIIA4 lowered the degree of off-target editing in cell inserted genes which are hereditary components that induce superMendelian heredity to spread desirable features in a population, have also been developed using CRISPR technology. The continuous advancement of female sterility-inducing gene drives in mosquitos to remove vector-borne illnesses like malaria is a good example (Nateghi Rostami, 2020). As a safety precaution, effective awareness of the distribution of a gene drive after its first discharge is a very valuable property. After the parental driving microorganisms are discharged into the essential ecosystem, Acr proteins could be used to put a stop to the proliferation of CRISPR base drive (Basgall et al., 2018).

Uses for dCas9-based applications

Various effector proteins could be connected or recruited to genomic regions of interest using nuclease-inactive dCas9. dCas9 was given recombinant proteins (FPs) as well as transcriptionational stimulators and downregulation, for instance, chromosomal imaging and modulation of a specific gene can be obtained. The use of dCas9-based technologies allows not only genome manipulation but also epigenome manipulation by the fusing of DNA demethylation enzymes (e.g., TET)or histone-modifying effectors (e.g., LSD1 or p300) (Adli, 2018). Acrs which control the actions of these functional categories can likewise be utilized to inhibit DNA binding. Type II Acr proteins, for example, have been utilized to regulate chromosome labeling by dCas9-FPs and demethylation by dCas9-Tet1 fusions in created pluripotent stem cells (Basgall et al., 2018; Bubeck et al., 2018; Rauch et al., 2017). Furthermore, by modulating CRISPRi and CRISPRa, Acr proteins have permitted programmed and flexible gene regulation (Hoffmann et al., 2019; Nakamura et al., 2019).

Engineering Acr proteins:

Acr proteins can commonly survive epitope tag and FP fusion without losing their inhibitory effectiveness. Inserting an external region, A fluorescence peptide, also including mCherry, at precisely AcrIIC1 surfaces that has been chosen locations greatly increased Nme1Cas9 inhibitory (Mathony et al., 2020). This opens up the possibility of modifying Acr proteins by inserting additional domains while retaining the inhibitory action. The inducible destabilization domain, which degrades the proteins within the absence of an exterior binding referred to as Shield1, was fused to Acr proteins to achieve post-translational regulation From a synthetic biology standpoint,(Nakamura et al., 2019) Acrs can be further modified to change their selectivity and efficacy (Aschenbrenner et al., 2020; Osuna, 2019). AcrIIC1 gene that could be changed AcrIIC1X evolved from a Type II-C regulator which restricts class II-A SauCas9, depends on the structure of the Nme1Cas9 interacting contact with AcrIIC1 (Osuna, 2019). The suppressive efficacy of Cas9 activity could be fine-tuned by utilizing intentionally weakened Acr proteins to achieve an ideal-target editor and some off-target editor events are in a kinetic equilibrium (Aschenbrenner et al., 2020).

Other uses of Acr proteins;

Inhibitory Cas9 is also a beneficial strategy for creating vectors of viral infection with a "self-cleaving" chromosome. By design, a (helper-dependent adenovirus) HDAd vector for temporary SpyCas9 as well as guiding are encoded by Cas9 activation in specific receptors Cas9 manifestation in the intended recipient cells encodes SpyCas9 and breakage among the vector's genetic code later target cell modulation allowing transitory SpyCas9 expression and function (Palmer et al., 2019). Self-cleavage can

also occur throughout the viral generation, resulting in rearrangements in the genome that are impossible to develop and render virus production. SpyCas9 was 6inhi2 bitten from starting vector self-cleavage during viral generation using Acr proteins, considerably boosting yield (Palmer et al., 2019). Acr proteins could also be used to produce phage treatments as a replacement for antibiotics used to diagnose bacterial infections (Nobrega et al., 2015).

Cas9-pDBD fusion proteins improve the activity and precision of Cas9;

The pDBD linked to a PAM attenuated SpyCas9 provides an extended facility for high specificity and targeted range (Bolukbasi et al., 2015; Bolukbasi et al., 2018). Similarly, changes in some PID residues reduce Nme1Cas9's ability to bind to specific DNA (Amrani et al., in construction). We can use a simple variant (R1025A) and a dual variant (K1013A/R1025A) that demonstrate the more pronounced decrease of Nme1Cas9 intrinsically good DNA binding capacity with the definitive PAM identified as simple variant (SM) and daul variant (DM, as shown] (Amrani et al., in preparation). Due to Cas9 variations' decreased ability for PAM recognition, an extra DNA creating a component is required to engage and cleave target DNA, bolstering the nuclease's stronger affinity and precision. Nme1Cas9 mutants were linked to ZFPs, that are DNA-binding proteins (Amrani et al., 2018).

Inhibition of Cas9-pDBD proteins by Acr proteins;

The HEK293T molecules that had been co-transfected including vectors displaying the Acr protein, Nme1Cas9, and plasmid are use to target the NTS25 genomic region Type II-C Acr proteins could suppress Nme1Cas9-pDBD genome editing. T7E1 and TIDE were then utilised to determine genome editing efficacy. In the existence of Nme1Cas9WT, the appearance of Acrs significantly inhibited Nme1Cas9-induced mutations confirming our prior findings. They do not see any modification without any kind of Acr proteins, as indicated for Nme1Cas9SM because Nme1Cas9SM cannot attach to the target DNA without the pDBD. In the absence of Acrs, connecting the ZFP to Nme1Cas9SM (Zif268-Nme1Cas9SM) restores and even enhances Cas9 cleavage activity. In the Acr proteins, the increase in editing efficiency is nearly erased except for AcrIIC2Nme, all Type II-C Acrs reduced the breakage action of the forest-type and the ZFP-Nme1Cas9 merging protein. AcrIIC2Nme inhibits Nme1Cas9WT but not Nme1Cas9WT merged to a ZFP, which is surprising. This finding prompted us to see if AcrIIC2Nme can inhibit Nme1Cas9WT fused to a pDBD from off-targeting while not interfering with on-target site editing.

Cas9-Cas9 fusion proteins expand the utility of Cas9 genome editing

The pDBD adds a second step to the specific site licensing before to cross-linking in the chimaera between PAM-association suppressed Cas9 and pDBD, improving the targeted area, efficacy, and specificity (Amrani et al., 2018; Bolukbasi et al., 2015). However, generating operational pDBD mergers is more difficult than programming Cas9 with guide RNAs. In order to enable the adoption of Cas9's RNA-programmable binding platform over pDBD, antagonistic Cas9-Cas9 fused amino acids have been designed. Cas9-Cas9 fusions,(Bolukbasi et al., 2018) whether single- or dual-nuclease, have a wider targeting range and large specific surface area for genome editing. A couple of complementary mutations Cas9s have been coupled in particular to provide the specific lateral reductions used Cas9 (Bolukbasi et al., 2018).

CRISPR/Cas9 Therapeutic Application:

Most pharmaceutical industries and smaller biomedical begin are working on

Clustered regularly interspaced short palindromic therapeutics. CRISPR genome editing is expected to be quicker, lower expensive, and maybe more secure than the conventional gene therapy process. Autologous CRISPR gene cures, which use genome editing to correct an abnormality in a patient's own tissues, could eliminate the disapproval risks involved with provider transplantation therapies. CRISPR genome editing has special potential for disorders that can be treated by changing tissues which can be easily managed by an individual, according to researchers. Enabling for additional monitoring to ensure that no off-target genome modifications arise as a result of the editing procedure.

Reducing off-target effects:

When given to living cells at small doses and at the right time, AcrIIA4 was demonstrated to significantly minimize SpyCas9 off-target impacts while maintaining on-target SpyCas9-mediated gene editing (Shin et al., 2017). SpyCas9-sgRNA activity is vir Controlling tually completely reduced when AcrIIA4 has produced 24 hours ago SpyCas9 RNP stimulation. When AcrIIA4 is introduced to cells 6 hours through the SpyCas9-sgRNA compound is formed, genetic manipulation is only reduced by roughly fifty percent (Shin et al., 2017).

The activity of SpyCas9 RNPs can be monitored rather than putting into consideration several of these parameters, according to Aschenbrenner and coauthors simply combining SpyCas9 to mutant (weaker) forms of AcrIIA4 (Aschenbrenner et al., 2020). SpyCas9 will have too much time to alter – only – the center of intrest (higher affinity) Before being deactivated in this way. In theory, this technique reduces off-target effects significantly while having no observable negative effects on ontarget editing. However, with different sgRNAs, the method's performance varies

Controling gene drive

CRISPR-Cas9 is a dependable mechanism for achieving gene drive, or the spread of a specific gene throughout a population (Hammond et al., 2016). Basgall and co-authors (Basgall et al., 2018) used AcrIIA2 and AcrIIA4 to completely abolish gene drive in S. cerevisiae. They also created a gene drive mechanism that can be induced in which SpyCas9 and AcrIIA2/A4 were controlled by two distinct regulated promoters, GAL1 and MET25, roughly. MET25 promoter is blocked by methionine, but the GAL1 promoter is stimulated by galactose. SpyCas9 was produced by yeast cells starting to grow in a medium containing both galactose and methionine which when bound to a suitably designed sgRNA, resulted in gene drive. Anti CRISPR amino acids were created and the gene drive was turned off when yeast cells were grown in a material that lacked methionine. Importantly, this research included a thorough examination of both AcrIIA2 and AcrII4, to determine which metabolites are critical for the suppressive action of the two proteins. An impartial alanine scan revealed how distinct mutational sites influenced Acractivity to vary degrees. 22 AcrIIA2 and 19 AcrIIA4 mutations were created: 8 completely shed their disruptive action (6 AcrIIA2 and 2 AcrIIA4 variations), and five people are still employed but at a lower grade than wild-type proteins (two AcrIIA2 and three AcrIIA4 mutants). All of these mutations as well as those created through simple and multiple mutations on AcrIIA4 were studied when it comes to the gene drive. This experiment proved that Acrmutations can be used to fine-tune gene drive effects.

Nme1Cas9-guided programmable RNA editing;

Nme1Cas9 is unique among Cas9 family members in that it may target single-stranded RNA (ssRNA) without the presence of a PAM pattern. Without the need for any RNA-

PAM, ssRNA-crRNA complementary base-pairing is used to recognize and select the target ssRNA (or rPAM). As a result, Nme1Cas9 may execute Unique to the location ssRNA cleavage without causing damage to the dsDNA.AcrIIC1-C3 also inhibits ssRNA cleavage (Rousseau et al., 2018). Using Nme1Cas9 and AcrIIC1-C3, this discovery provides a novel platform for manipulating ssRNA (Rousseau et al., 2018).

Summary

In certain situations, the CRISPR-Cas adaptive immune defense organizations seen in bacteria and Archaea may give discrete sequence defense in contradiction to invading DNA or RNA. Acr genes produce proteins that attach to the unit facilitating the defense process and so counteract CRISPR-Cas immune mechanisms in bacteria, despite the emergence of defense mechanisms in bacterial hosts. In the future, more Acrs are expected to be identified, and their activities and responsibilities should be extensively researched. Acrs increased understanding might contribute to their extensive use as efficient controllers of CRISPR-Cas system-associated genome editing, which could aid mankind in overcoming diseases. As CRISPR becomes more commonly employed in human medicine, techniques for hindering Cas enzymes will become increasingly useful. In the upcoming days, blockers might be applied to lower CRISPR activity in non-target tissues during gene therapy, added proactively to diminish off-target action, or even employed in an emergency when CRISPR has been misused or has generated poor results.

Acknowledgments

The authors concede the support of the Cholistan University of Veterinary & Animal Sciences-Bahawalpur, Pakistan, during the write-up.

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Brito, I.L., *Examining horizontal gene transfer in microbial communities.* Nature Reviews Microbiology, 2021. **19**(7): p. 442-453.
2. Soucy, S.M., J. Huang, and J.P. Gogarten, *Horizontal gene transfer: building the web of life.* Nature Reviews Genetics, 2015. **16**(8): p. 472-482.
3. Mejdani, M., *Acr and CRISPR-Cas: Characterization and Biotechnology*. 2019, University of Toronto (Canada).
4. Tang, X.-D., et al., *Methods for enhancing clustered regularly interspaced short palindromic repeats/Cas9-mediated homology-directed repair efficiency.* Frontiers in genetics, 2019. **10**: p. 551.
5. Davidson, A.R., et al., *Acr: protein inhibitors of CRISPR-Cas systems.* Annual review of biochemistry, 2020. **89**: p. 309-332.
6. Hampton, H.G., B.N. Watson, and P.C. Fineran, *The arms race between bacteria and their phage foes.* Nature, 2020. **577**(7790): p. 327-336.

7. Jia, N. and D.J. Patel, *Structure-based functional mechanisms and biotechnology applications of* Acr proteins. Nature Reviews Molecular Cell Biology, 2021. **22**(8): p. 563-579.
8. Eaglesham, J.B. and P.J. Kranzusch, *Conserved strategies for pathogen evasion of cGAS–STING immunity.* Current opinion in immunology, 2020. **66**: p. 27-34.
9. Cheng, Z., et al., *The interactions between cGAS-STING pathway and pathogens.* Signal transduction and targeted therapy, 2020. **5**(1): p. 1-15.
10. Deveau, H., et al., *Phage response to CRISPR-encoded resistance in Streptococcus thermophilus.* Journal of bacteriology, 2008. **190**(4): p. 1390-1400.
11. Mendoza, S.D., et al., *A bacteriophage nucleus-like compartment shields DNA from CRISPR nucleases.* Nature, 2020. **577**(7789): p. 244-248.
12. Malone, L.M., et al., *A jumbo phage that forms a nucleus-like structure evades CRISPR–Cas DNA targeting but is vulnerable to type III RNA-based immunity.* Nature microbiology, 2020. **5**(1): p. 48-55.
13. Samson, J.E., et al., *Revenge of the phages: defeating bacterial defences.* Nature Reviews Microbiology, 2013. **11**(10): p. 675-687.
14. Bondy-Denomy, J., et al., *Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system.* Nature, 2013. **493**(7432): p. 429-432.
15. Makarova, K.S., et al., *Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants.* Nature Reviews Microbiology, 2020. **18**(2): p. 67-83.
16. Makarova, K.S., et al., *An updated evolutionary classification of CRISPR–Cas systems.* Nature Reviews Microbiology, 2015. **13**(11): p. 722-736.
17. Van Der Oost, J., et al., *Unravelling the structural and mechanistic basis of CRISPR–Cas systems.* Nature Reviews Microbiology, 2014. **12**(7): p. 479-492.
18. Marraffini, L.A., *CRISPR-Cas immunity in prokaryotes.* Nature, 2015. **526**(7571): p. 55-61.
19. Andersson, A.F. and J.F. Banfield, *Virus population dynamics and acquired virus resistance in natural microbial communities.* Science, 2008. **320**(5879): p. 1047-1050.
20. Hwang, S. and K.L. Maxwell, *Meet the Acr: widespread protein inhibitors of CRISPR-Cas systems.* The CRISPR journal, 2019. **2**(1): p. 23-30.
21. Marino, N.D., et al., Acr protein *applications: natural brakes for CRISPR-Cas technologies.* Nature methods, 2020. **17**(5): p. 471-479.
22. Jinek, M., et al., *A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity.* science, 2012. **337**(6096): p. 816-821.
23. Mali, P., et al., *RNA-guided human genome engineering via Cas9.* Science, 2013. **339**(6121): p. 823-826.
24. Cong, L., et al., *Multiplex genome engineering using CRISPR/Cas systems.* Science, 2013. **339**(6121): p. 819-823.
25. Qi, L.S., et al., *Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression.* Cell, 2013. **152**(5): p. 1173-1183.
26. Hsu, P.D., E.S. Lander, and F. Zhang, *Development and applications of CRISPR-Cas9 for genome engineering.* Cell, 2014. **157**(6): p. 1262-1278.
27. Adli, M., *The CRISPR tool kit for genome editing and beyond.* Nature communications, 2018. **9**(1): p. 1-13.

28. Kempton, H.R. and L.S. Qi, *When genome editing goes off-target.* Science, 2019. **364**(6437): p. 234-236.
29. Li, C., et al., *HDAd5/35++ adenovirus vector expressing Acrpeptides decreases CRISPR/Cas9 toxicity in human hematopoietic stem cells.* Molecular Therapy-Methods & Clinical Development, 2018. **9**: p. 390-401.
30. Li, C., et al., *In vivo HSC gene therapy using a bi-modular HDAd5/35++ vector cures sickle cell disease in a mouse model.* Molecular Therapy, 2021. **29**(2): p. 822-837.
31. Pawluk, A., et al., *Disabling a type IE CRISPR-Cas nuclease with a bacteriophage-encoded* Acr protein. MBio, 2017. **8**(6): p. e01751-17.
32. Meaden, S., et al., *Phage gene expression and host responses lead to infection-dependent costs of CRISPR immunity.* The ISME journal, 2021. **15**(2): p. 534-544.
33. Pawluk, A., A.R. Davidson, and K.L. Maxwell, *Anti-CRISPR: discovery, mechanism and function.* Nature Reviews Microbiology, 2018. **16**(1): p. 12-17.
34. Touchon, M. and E.P. Rocha, *The small, slow and specialized CRISPR and Acrof Escherichia and Salmonella.* PloS one, 2010. **5**(6): p. e11126.
35. Bondy-Denomy, J., et al., *Multiple mechanisms for CRISPR–Cas inhibition by* Acr proteins. Nature, 2015. **526**(7571): p. 136-139.
36. Borges, A.L., A.R. Davidson, and J. Bondy-Denomy, *The discovery, mechanisms, and evolutionary impact of Acr.* Annual review of virology, 2017. **4**: p. 37-59.
37. Stanley, S.Y., et al., *Anti-CRISPR-associated proteins are crucial repressors of Acrtranscription.* Cell, 2019. **178**(6): p. 1452-1464. e13.
38. Peng, X., et al., Acr proteins *in archaea.* Trends in Microbiology, 2020. **28**(11): p. 913-921.
39. Liu, Q., H. Zhang, and X. Huang, *Anti-CRISPR proteins targeting the CRISPR-Cas system enrich the toolkit for genetic engineering.* The FEBS journal, 2020. **287**(4): p. 626-644.
40. e Pacheco, B.I.C., *Physiological and genomic characterization of six virulent bacteriophages of Shiga toxin-producing Escherichia coli O157: H7 for biocontrol and detection applications.* 2019.
41. Derrien, T., et al., *The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression.* Genome research, 2012. **22**(9): p. 1775-1789.
42. Shehreen, S., *The impact of Acr on Horizontal Gene Transfer (HGT) and their regulation in mobile genetic elements*. 2021, University of Otago.
43. Yin, Y., B. Yang, and S. Entwistle, *Bioinformatics identification of Acrloci by using homology, guilt-by-association, and CRISPR self-targeting spacer approaches.* Msystems, 2019. **4**(5): p. e00455-19.
44. Walker, F.C., *CRISPR Regulation and RNA Biogenesis in Staphylococcus epidermidis*. 2017: The University of Alabama.
45. Pawluk, A.I.E., *Prevalence, Diversity, Impact, and Applications of CRISPR-Cas Inhibitors*. 2016, University of Toronto (Canada).
46. Zhu, Y., et al., *Diverse mechanisms of CRISPR-Cas9 inhibition by type IIC* Acr proteins. Molecular cell, 2019. **74**(2): p. 296-309. e7.
47. Porto, E.M., et al., *Base editing: advances and therapeutic opportunities.* Nature Reviews Drug Discovery, 2020. **19**(12): p. 839-859.
48. Bondy-Denomy, J., *Protein inhibitors of CRISPR-Cas9.* ACS chemical biology, 2018. **13**(2): p. 417-423.

49. Yaung, S.J., K.M. Esvelt, and G.M. Church, *CRISPR/Cas9-mediated phage resistance is not impeded by the DNA modifications of phage T4.* PloS one, 2014. **9**(6): p. e98811.
50. Reinshagen, C., et al., *CRISPR-enhanced engineering of therapy-sensitive cancer cells for self-targeting of primary and metastatic tumors.* Science translational medicine, 2018. **10**(449): p. eaao3240.
51. Pinilla-Redondo, R., et al., *Discovery of multiple Acr highlights anti-defense gene clustering in mobile genetic elements.* Nature communications, 2020. **11**(1): p. 1-11.
52. Hille, F. and E. Charpentier, *CRISPR-Cas: biology, mechanisms and relevance.* Philosophical transactions of the royal society B: biological sciences, 2016. **371**(1707): p. 20150496.
53. Osuna, B.A., et al., *Listeria phages induce Cas9 degradation to protect lysogenic genomes.* Cell host & microbe, 2020. **28**(1): p. 31-40. e9.
54. Zhang, Y. and M.A. Marchisio, *Type II* Acr proteins *as a new tool for synthetic biology.* RNA biology, 2021. **18**(8): p. 1085-1098.
55. Leon, L.M., *CRISPR-Cas3: Studying the molecular interactions that drive adaptation & engineering novel bacterial editing tools*. 2021: University of California, San Francisco.
56. Yi, H., et al., *AcrFinder: genome mining Acroperons in prokaryotes and their viruses.* Nucleic acids research, 2020. **48**(W1): p. W358-W365.
57. Birkholz, N., et al., *The autoregulator Aca2 mediates Acrrepression.* Nucleic acids research, 2019. **47**(18): p. 9658-9665.
58. Forsberg, K.J., et al., *The novel AcrAcrIIA22 relieves DNA torsion in target plasmids and impairs SpyCas9 activity.* PLoS biology, 2021. **19**(10): p. e3001428.
59. Thavalingam, A., *The Functional Characterization of AcrAcrIIC2 Nme*. 2019, University of Toronto (Canada).
60. Pawluk, A., et al., *Naturally occurring off-switches for CRISPR-Cas9.* Cell, 2016. **167**(7): p. 1829-1838. e9.
61. Webber, B.L., S. Raghu, and O.R. Edwards, *Opinion: Is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat?* Proceedings of the National Academy of Sciences, 2015. **112**(34): p. 10565-10567.
62. Scheben, A., et al., *Towards CRISPR/Cas crops–bringing together genomics and genome editing.* New Phytologist, 2017. **216**(3): p. 682-698.
63. Huang, C.-H., K.-C. Lee, and J.A. Doudna, *Applications of CRISPR-Cas enzymes in cancer therapeutics and detection.* Trends in cancer, 2018. **4**(7): p. 499-512.
64. Lino, C.A., et al., *Delivering CRISPR: a review of the challenges and approaches.* Drug delivery, 2018. **25**(1): p. 1234-1257.
65. Karimian, A., et al., *CRISPR/Cas9 technology as a potent molecular tool for gene therapy.* Journal of cellular physiology, 2019. **234**(8): p. 12267-12277.
66. Babačić, H., et al., *CRISPR-cas gene-editing as plausible treatment of neuromuscular and nucleotide-repeat-expansion diseases: A systematic review.* PloS one, 2019. **14**(2): p. e0212198.
67. Yasaka, M., et al., *Correction of INR by prothrombin complex concentrate and vitamin K in patients with warfarin related hemorrhagic complication.* Thrombosis research, 2002. **108**(1): p. 25-30.
68. CR, L., *The neutralization of heparin by protamine.* Surgery, 1948. **24**(1): p. 97-99.

69. Hammond, A., et al., *A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae.* Nature biotechnology, 2016. **34**(1): p. 78-83.
70. Zhang, F., G. Song, and Y. Tian, *Anti-CRISPRs: The natural inhibitors for CRISPR-Cas systems.* Animal models and experimental medicine, 2019. **2**(2): p. 69-75.
71. Barboni, M., et al., *Early formation of the Moon 4.51 billion years ago.* Science advances, 2017. **3**(1): p. e1602365.
72. Naeem, M., et al., *Latest developed strategies to minimize the off-target effects in CRISPR-Cas-mediated genome editing.* Cells, 2020. **9**(7): p. 1608.
73. Zuccaro, M.V., et al., *Allele-specific chromosome removal after Cas9 cleavage in human embryos.* Cell, 2020. **183**(6): p. 1650-1664. e15.
74. Shen, C.-C., et al., *Synthetic switch to minimize CRISPR off-target effects by self-restricting Cas9 transcription and translation.* Nucleic acids research, 2019. **47**(3): p. e13-e13.
75. Shin, J., et al., *Disabling Cas9 by an AcrDNA mimic.* Science advances, 2017. **3**(7): p. e1701620.
76. Kosicki, M., K. Tomberg, and A. Bradley, *Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements.* Nature biotechnology, 2018. **36**(8): p. 765-771.
77. Meltzer, H., et al., *Tissue-specific (ts) CRISPR as an efficient strategy for in vivo screening in Drosophila.* Nature communications, 2019. **10**(1): p. 1-9.
78. Wang, X.-W., et al., *A microRNA-inducible CRISPR–Cas9 platform serves as a microRNA sensor and cell-type-specific genome regulation tool.* Nature cell biology, 2019. **21**(4): p. 522-530.
79. Wei, T., et al., *Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing.* Nature communications, 2020. **11**(1): p. 1-12.
80. Pawluk, A., et al., *A new group of phage* Acr genes *inhibits the type IE CRISPR-Cas system of Pseudomonas aeruginosa.* MBio, 2014. **5**(2): p. e00896-14.
81. Rauch, B.J., et al., *Inhibition of CRISPR-Cas9 with bacteriophage proteins.* Cell, 2017. **168**(1-2): p. 150-158. e10.
82. Hynes, A.P., et al., *An Acrfrom a virulent streptococcal phage inhibits Streptococcus pyogenes Cas9.* Nature microbiology, 2017. **2**(10): p. 1374-1380.
83. Garcia, B., et al., *AcrAcrIIA5 potently inhibits all Cas9 homologs used for genome editing.* Cell reports, 2019. **29**(7): p. 1739-1746. e5.
84. Song, G., et al., *AcrIIA5 inhibits a broad range of Cas9 orthologs by preventing DNA target cleavage.* Cell reports, 2019. **29**(9): p. 2579-2589. e4.
85. Watters, K.E., et al., *Potent CRISPR-Cas9 inhibitors from Staphylococcus genomes.* Proceedings of the National Academy of Sciences, 2020. **117**(12): p. 6531-6539.
86. Watters, K.E., et al., *Systematic discovery of natural CRISPR-Cas12a inhibitors.* Science, 2018. **362**(6411): p. 236-239.
87. Chowdhury, S., et al., *Structure reveals mechanisms of viral suppressors that intercept a CRISPR RNA-guided surveillance complex.* Cell, 2017. **169**(1): p. 47-57. e11.
88. Maxwell, K.L., et al., *The solution structure of an* Acr protein. Nature communications, 2016. **7**(1): p. 1-5.

89. Guo, T.W., et al., *Cryo-EM structures reveal mechanism and inhibition of DNA targeting by a CRISPR-Cas surveillance complex.* Cell, 2017. **171**(2): p. 414-426. e12.
90. Wang, X., et al., *Structural basis of Cas3 inhibition by the bacteriophage protein AcrF3.* Nature structural & molecular biology, 2016. **23**(9): p. 868-870.
91. Ka, D., et al., *Crystal structure of an* Acr protein, *AcrIIA1.* Nucleic acids research, 2018. **46**(1): p. 485-492.
92. Dong, D., et al., *Structural basis of CRISPR–SpyCas9 inhibition by an* Acr protein. Nature, 2017. **546**(7658): p. 436-439.
93. Harrington, L.B., et al., *A broad-spectrum inhibitor of CRISPR-Cas9.* Cell, 2017. **170**(6): p. 1224-1233. e15.
94. Mallon, J. and S. Bailey, *A molecular arms race: New insights into Acrmechanisms.* Nature structural & molecular biology, 2016. **23**(9): p. 765-766.
95. Wang, J., et al., *A CRISPR evolutionary arms race: structural insights into viral anti-CRISPR/Cas responses.* Cell research, 2016. **26**(10): p. 1165-1168.
96. Holm, L. and P.i. Rosenstrï¿ ½m, *Dali server: conservation mapping in 3D.* Nucleic acids research, 2010. **38**(suppl_2): p. W545-W549.
97. Nakamura, M., et al., *Anti-CRISPR-mediated control of gene editing and synthetic circuits in eukaryotic cells.* Nature communications, 2019. **10**(1): p. 1-11.
98. Basgall, E.M., et al., *Gene drive inhibition by the* Acr proteins *AcrIIA2 and AcrIIA4 in Saccharomyces cerevisiae.* Microbiology, 2018. **164**(4): p. 464.
99. Yang, H. and D.J. Patel, *Inhibition mechanism of an Acrsuppressor AcrIIA4 targeting SpyCas9.* Molecular cell, 2017. **67**(1): p. 117-127. e5.
100. Jinek, M., et al., *Structures of Cas9 endonucleases reveal RNA-mediated conformational activation.* Science, 2014. **343**(6176): p. 1247997.
101. Jiang, F., et al., *A Cas9–guide RNA complex preorganized for target DNA recognition.* Science, 2015. **348**(6242): p. 1477-1481.
102. Liu, L., et al., *Phage AcrIIA2 DNA mimicry: structural basis of the CRISPR and Acrarms race.* Molecular cell, 2019. **73**(3): p. 611-620. e3.
103. Hynes, A.P., et al., *Widespread* Acr proteins *in virulent bacteriophages inhibit a range of Cas9 proteins.* Nature communications, 2018. **9**(1): p. 1-10.
104. An, S.Y., et al., *Intrinsic disorder is essential for Cas9 inhibition of AcrAcrIIA5.* Nucleic acids research, 2020. **48**(13): p. 7584-7594.
105. Fuchsbauer, O., et al., *Cas9 allosteric inhibition by the* Acr protein *AcrIIA6.* Molecular cell, 2019. **76**(6): p. 922-937. e7.
106. Uribe, R.V., et al., *Discovery and characterization of Cas9 inhibitors disseminated across seven bacterial phyla.* Cell host & microbe, 2019. **25**(2): p. 233-241. e5.
107. Forsberg, K.J., et al., *Functional metagenomics-guided discovery of potent Cas9 inhibitors in the human microbiome.* elife, 2019. **8**: p. e46540.
108. Pawluk, A., et al., *Inactivation of CRISPR-Cas systems by* Acr proteins *in diverse bacterial species.* Nature microbiology, 2016. **1**(8): p. 1-6.
109. Gussow, A.B., et al., *Machine-learning approach expands the repertoire of* Acr protein *families.* Nature communications, 2020. **11**(1): p. 1-12.
110. Eitzinger, S., et al., *Machine learning predicts new* Acr proteins. Nucleic acids research, 2020. **48**(9): p. 4698-4708.
111. Lee, J., et al., *Potent Cas9 inhibition in bacterial and human cells by AcrIIC4 and AcrIIC5* Acr proteins. MBio, 2018. **9**(6): p. e02321-18.

112. Edraki, A., et al., *A compact, high-accuracy Cas9 with a dinucleotide PAM for in vivo genome editing.* Molecular cell, 2019. **73**(4): p. 714-726. e4.
113. Sun, W., et al., *Structures of Neisseria meningitidis Cas9 complexes in catalytically poised and anti-CRISPR-inhibited states.* Molecular cell, 2019. **76**(6): p. 938-952. e5.
114. Sternberg, S.H., et al., *Conformational control of DNA target cleavage by CRISPR–Cas9.* Nature, 2015. **527**(7576): p. 110-113.
115. Kim, Y., et al., *Anti-CRISPR AcrIIC3 discriminates between Cas9 orthologs via targeting the variable surface of the HNH nuclease domain.* The FEBS journal, 2019. **286**(23): p. 4661-4674.
116. Thavalingam, A., et al., *Inhibition of CRISPR-Cas9 ribonucleoprotein complex assembly by AcrAcrIIC2.* Nature communications, 2019. **10**(1): p. 1-11.
117. Kahne, D. and W.C. Still, *Hydrolysis of a peptide bond in neutral water.* Journal of the American Chemical Society, 1988. **110**(22): p. 7529-7534.
118. Meijer, A.J. and P. Codogno, *Regulation and role of autophagy in mammalian cells.* The international journal of biochemistry & cell biology, 2004. **36**(12): p. 2445-2462.
119. Glickman, M.H. and A. Ciechanover, *The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction.* Physiological reviews, 2002.
120. Matsumoto, D., H. Tamamura, and W. Nomura, *A cell cycle-dependent CRISPR-Cas9 activation system based on an* Acr protein *shows improved genome editing accuracy.* Communications biology, 2020. **3**(1): p. 1-10.
121. Nateghi Rostami, M., *CRISPR/Cas9 gene drive technology to control transmission of vector-borne parasitic infections.* Parasite immunology, 2020. **42**(9): p. e12762.
122. Bubeck, F., et al., *Engineered* Acr proteins *for optogenetic control of CRISPR–Cas9.* Nature methods, 2018. **15**(11): p. 924-927.
123. Hoffmann, M.D., et al., *Cell-specific CRISPR–Cas9 activation by microRNA-dependent expression of* Acr proteins. Nucleic acids research, 2019. **47**(13): p. e75-e75.
124. Mathony, J., et al., *Computational design of* Acr proteins *with improved inhibition potency.* Nature Chemical Biology, 2020. **16**(7): p. 725-730.
125. Aschenbrenner, S., et al., *Coupling Cas9 to artificial inhibitory domains enhances CRISPR-Cas9 target specificity.* Science advances, 2020. **6**(6): p. eaay0187.
126. Osuna, B., *Surveillance and Defense Mechanisms in Microbes*. 2019: University of California, San Francisco.
127. Palmer, D.J., D.L. Turner, and P. Ng, *Production of CRISPR/Cas9-mediated self-cleaving helper-dependent adenoviruses.* Molecular Therapy-Methods & Clinical Development, 2019. **13**: p. 432-439.
128. Nobrega, F.L., et al., *Revisiting phage therapy: new applications for old resources.* Trends in microbiology, 2015. **23**(4): p. 185-191.
129. Bolukbasi, M.F., et al., *DNA-binding-domain fusions enhance the targeting range and precision of Cas9.* Nature methods, 2015. **12**(12): p. 1150-1156.
130. Bolukbasi, M.F., et al., *Orthogonal Cas9–Cas9 chimeras provide a versatile platform for genome editing.* Nature communications, 2018. **9**(1): p. 1-12.
131. Amrani, N., et al., *NmeCas9 is an intrinsically high-fidelity genome-editing platform.* Genome Biology, 2018. **19**(1): p. 1-25.

132. Rousseau, B.A., et al., *Programmable RNA cleavage and recognition by a natural CRISPR-Cas9 system from Neisseria meningitidis.* Molecular cell, 2018. **69**(5): p. 906-914. e4.

About the Authors

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkiye. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 68 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.
E-mail: msafdar@cuvas.edu.pk, ORCID: 0000 0002 3720 2090

Shafeeq ur Rehman graduated from the Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur. He continues to work on FMD and PPR viruses as his area of interest. He is still working as a Research Assistant in FMDV and PPRV project in Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan.
E-mail: shafeequrrehmanbhutta@gmail.com, ORCID: 0000-0003-3571-8226

Furqan Shafqat graduated from Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in medical biotechnology from University of Wollongong Australia.
E-mail: furqanshafqat007@gmail.com, ORCID: 0000-0002-2563-913X

Muhammad Younus completed did his Graduation from Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan. He continues his post graduate study in Zoology at Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan. He is working on Biodiversity of species along with DNA barcoding of species as his main areas of interest.
E-mail: younus31302@gmail.com, ORCID: 0009-0005-5392-7031

To Cite This Chapter:
Rehman, S et al., (2023). **Anti crispr proteins as a new tool for synthetic biology**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 59-85). ISRES Publishing.

IN VTRO BIOSYNTHESIS OF NANOPARTICLES AND THEIR POTENTIAL APPLICATIONS AS NANOMEDICINE ARE SUBJECTS OF SIGNİFICANT ACADEMIC INTEREST

Aman QAMMAR
Nageen HUSSAİN
Muhammad WASEEM

1. Introduction

In the quest for knowledge, man is developing the most beautiful physical world. The components of this world are in biggest as well as in smallest size. This difference is in the view of many dimensions like length mass and times (Mohanraj and Chen, 2006). The importance of the smallest physical structures was unknown until the discovery of the nanoparticles. Nanotechnology is a very important and vast field of science. Bio-nanotechnology which is the combination of biotechnology and nanotechnology for the development of biosynthetic and environmentally friendly nanomaterial synthesis technology is emerging day by day. Nanotechnology and nanoscience have been burgeoned as on the most exciting and vast field of study with remarkable revolution in the current century by exploring this mysterious area of research due to its plenty of awesome opportunities. One of a lectures entitled" There is plenty of room at the bottom" was credited with discovering nanotechnology. Some researchers contemplate that Nanotechnology is implemented in all disciplines of science including engineering, information technology, material science and life science along with clarifying astonishing facts regarding human health, specifically in cancer treatment. (Paleel et al., 2020; Naveed et al., 2022a; Ayesha et al., 2022; Naveed et al., 2022b). This field deals with the development and synthesis of various types of nanoparticles. These are the objects which have a size ranging from 1-100nm (Shang *et al.*, 2014) and these array of atoms on a 1-100 nm scale, nanodevices and structures makes them feasible in the research area (Iqbal et al., 2022). Keeping in view the size of these important particles, these are far different from bulk materials. Another unique feature of nanoparticles is their biomolecule nature such as poly nucleic acid and proteins. They also have unique physio-chemical properties (Rampino *et al.*, 2013).

In the present times, nanoparticles are being used in a lot of the world. They are being used in the medical field as treatment and have a lot of applications in industry such as oxide fuel batteries and solar systems. They are also being used in wide incorporations such as into the diverse material thing of everyday use like clothes and cosmetics (Stark *et al.*, 2015). In the present era, different types of nanoparticles are being produced by using different materials like copper, titanium, zinc, magnesium, alginate, gold, and silver. The growth and development of nanoparticles are complex enough processes. It depends upon many conditions like viscosity, temperature, the concentration of the medium, etc (Rajput, 2015). This review article will depict various methods for nanoparticle production and their applications.

1.1 Types of Nanoparticles:

Inorganic and organic nanoparticles are further subcategorized into two primary subcategories. Inorganic nanoparticles are metallic, magnetic, ceramic, and nano shell. In a magnetic field, superparamagnetic iron oxide particles known as

magnetic nanoparticles exhibit strong magnetic moments. These are inexpensive to produce, competitive, incompatible, and chemically stable. These are mostly employed in thermotherapy for the targeted delivery of medications or genes (Agata *et al*., 2018). Metallic nanoparticles constitute of iron, silver, gold, silver, copper, and some other metallic nanoparticles as shown in Figure 1 and Figure 2. These are larger in surface area, contain higher medication dosages, and are smaller (50 nm), however, they have no clear purpose when utilized in vivo and have poor biocompatibility (Wang *et al*., 2018).

Nano shell nanoparticle- Silica nanoparticles are nano shells with imaging potential comparable to quantum. They are more substantial than quantum dots and less poisonous. These are employed in the photothermal ablation of tumours (Yen *et al*., 2013). Ceramic nanoparticles are composed of inexpensive, stable non-metallic materials. They are generally adaptable, simple to make, soluble in water, and stable in biological systems. At low temperatures, they can produce bulk material and form a coating (Yen *et al*., 2013).

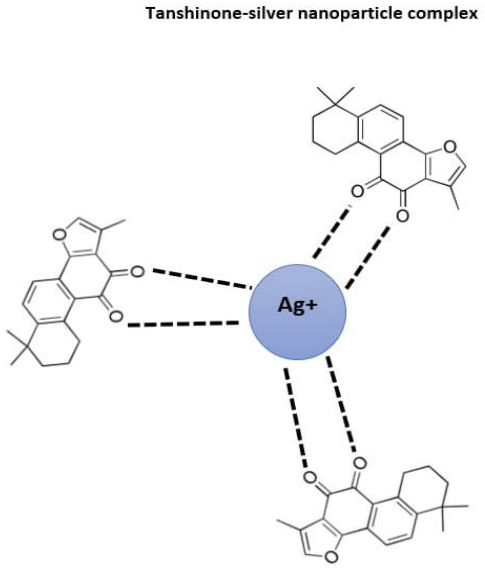


Figure 1. Tanshinone-silver nanoparticle complex

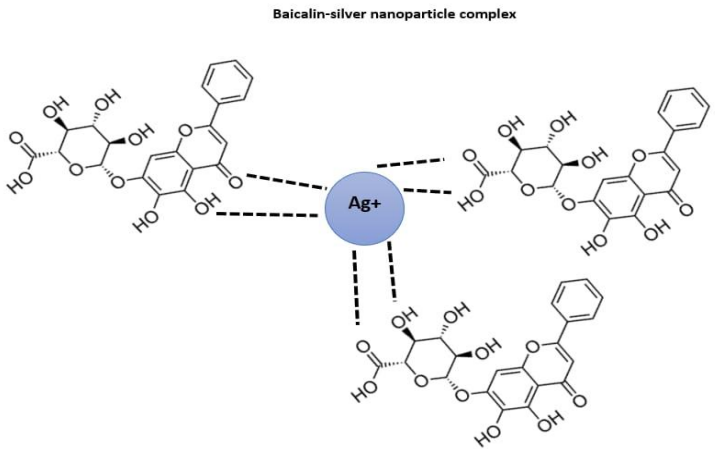


Figure 2. Baicalin-silver nanoparticle complex

1.2 Synthesis of Nanoparticles

One can use two methods of nanoparticle synthesis procedures that can be chemical and biological. As there is a danger of the presence of some toxic chemicals, the chemical method may have some adverse effects. Biological methods are environment-friendly alternatives to both chemical and physical processes. This branch is a very important and evolving branch of nanoparticles. Especially silver nanoparticles are prepared by this method which finds huge applications. The biosynthesis mechanism of nanoparticle preparation is an eco-friendly and green method (Figure 3). Metallic biosynthesis involves a wide range of prokaryotic and eukaryotic organisms that aid in the production of gold, zirconium, silver, platinum, iron, cadmium, palladium, etc. The microbes involved in this process are actinomycetes, bacteria, algae, and fungi. (Li *et al.*, 2011) In this method, ions transport takes place into the microbial cells which take place in the presence of enzymes. These particles that form in the organism's body are comparatively smaller in size. The size limit depends upon the organism in which nanoparticles are being formed (Kitching *et al.*, 2015).

1.2.1 Fungal intracellular synthesis of the nanoparticles:

Fungi can secrete a wide range of components. These secretary components are evolved and reduced in the capping of nanoparticles. Extracellularly produced nanoparticles have a wide range of applications because there are no adjoining cellular components that come from the cells in the case of intracellular ones. (Jeevanandam *et al.*, 2016).

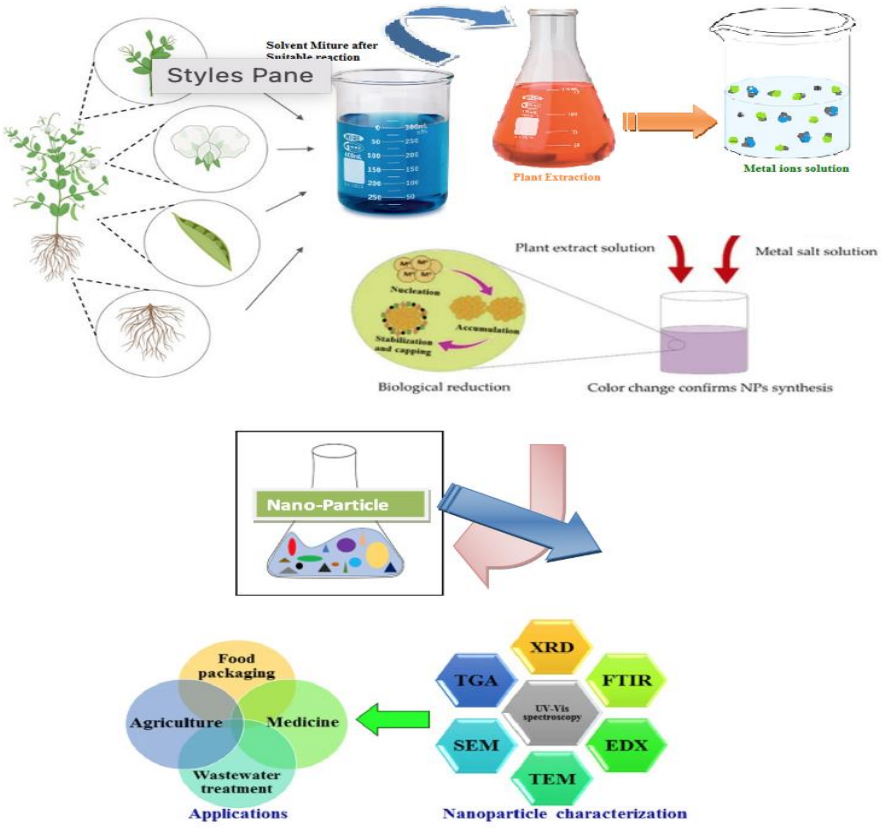


Figure 3. Synthesis of Nanoparticles

For nanoparticle production in an inorganic way. The plant samples were collected, washed, and dried. Heated the sample, filtered it, and centrifuged it to get extract material. A metallic salt solution was added and incubated for 10 minutes; with a pH range of 8-10. Changes in colours indicate the production of nanoparticles by reducing the metal ions into metal atoms. Some other compounds such as sugar, water, polyphenol, and vitamins are found to be effective for the synthesis of nanoparticles. Polyphenols are responsible for the reduction process while other compounds act as capping agents. The spectrophotometer is also involved in the absorption spectrum. (Ahmad *et al.*, 2017) Nanoparticle production by the "green synthesis" method is mass production low-cost rate and is ecologically beneficial due to its working capacity at low pressure, less temperature required for the complete reaction, and consume less amount of less energy. In opposite to plants, the nanoparticles extracted from plants are found to be more stable. The major application of NPs is considered to be in the field of medicine as antimicrobial agents. The world is facing resistivity of microbial activity against medicine, but the discovery of NPs has exuded a glimmer of problems. (Bhardwaj *et al.*, 2020)

A physical system to alter the properties of drugs especially pharmacokinetics and pharmacodynamics in the in-vitro mode of action. Diverse polymers are being used in the constitution of nanoparticles while the commencement of drugs in restorative of illness with prohibiting the negative effects. This new mode of working included the field of physical, chemical, material sciences, mechanical and electrical engineering. The alternate name of nanoparticles is a quantum confined atom, hydrocarbon sulphate (H_2S) emits the energy range from $50nm < D_p < 1000nm$. In some cases, while manufacturing nanoparticles it shows structural changes during the nucleation and coagulation process in the dilution method which makes it difficult to collect sampling for designing nanoparticles. A significant role of these particles is they can be used as an anti-oncology agent as silver particles. (Rajput *et al.*, 2015) They also show antitumor cells *in-vivo* and *ex-vivo* against a minacious disease Ehrlich's ascites carcinoma in animal modelling research work. The antimicrobial activities in-vitro assess the opposite agent to resign extraction of silver nitrate and zinc nitrate as nanoparticles in biosynthesis reactions. A huge number of agricultural products are present in nature which is no need for the application of complexity of artificial products for its growth and can be used as biosynthetic nanoparticles. Among all the *Haloarchaea* found to be more effective due to its peculiar character quickly lyses even at low concentrations of salt, which gives a pathway to S-protein to recover more efficiently by acting as building blocks for syntheses of main biomolecules. (Mohanraj and Chen, 2006).

1.2.1 Fungal Biosynthesis of Gold Nanoparticles

Due to their stability, biocompatibility, and resistance to oxidation, gold nanoparticles (AuNPs) are used as burgeoning research tool. AuNP is frequently synthesised chemically, which generally leaves harmful by-products that may raise various environmental concerns. Biosynthesis of AuNps, whether intracellular or extracellular by the use of viable microorganisms, however, has been regarded as the most inexpensive and eco-friendly. Fungi can tolerate greater metal concentrations than bacteria and release larger amounts of extracellular redox proteins to convert soluble metal ions to their insoluble form, which is then converted to nanocrystals. New metal reductases for metal detoxification and bio reduction may be found in fungi, which also have undiscovered biological variety. To shorten the duration of biosynthesis and

scale up the production of AuNPs, a detailed knowledge of the biosynthetic mechanism of AuNPs in fungi is required (Figure 4).

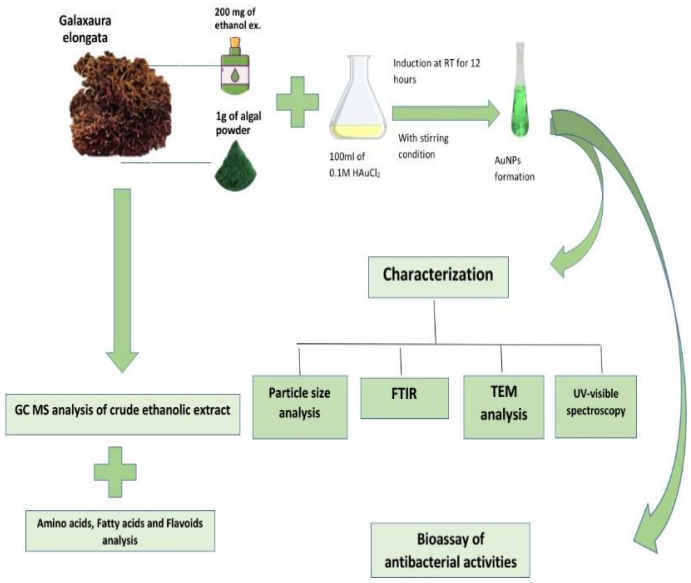


Figure 4. Biosynthesis of Gold Nanoparticles

Fungus is challenging to define because of their complex structure, which makes microscopic and mechanistic investigations that are necessary for nanoparticle characterisation. As a result, the microbiology of fungi is often considerably less studied. Fungi and bacteria both grow at a comparable biomass density in laboratory. For instance, an Escherichia coli culture grown with glucose in a stirred tank reactor had a biomass yield of 0.31 g g1, but a Rhizopus oryzae culture grown with glucose as a carbon/energy source had a biomass output of 0.55 g g1 (Taherzadeh *et al*., 2003; Xu *et al*., 1999).

Fungi, however, holds several advantages over bacteria in several bioprocesses, including AuNP production. Large quantities of extracellular proteins with a variety of activities are secreted by fungi. All proteins released into the extracellular space are collectively referred to as the "secretome" (Girard *et al*., 2013). The industrial manufacture of heterologous and homologous proteins has made use of the high content of fungal secretome. For instance, it has been documented that the entomopathogenic fungus *Beauveria bassiana* expresses a functionally active class I fungal hydrophobic (Kirkland and Keyhani, 2011). In addition to being a well-known reducing agent for metal reduction, tripeptide glutathione is also known to contribute to the manufacture of cadmium sulphide (CdS) in yeasts and fungi. For instance, it has been documented that the entomopathogenic fungus *Beauveria bassiana* expresses a functionally active class I fungal hydrophobic (Kirkland and Keyhani, 2011). In addition to being a well-known reducing agent for metal reduction, tripeptide glutathione is also known to contribute to the manufacture of cadmium sulphide (CdS) in yeasts and fungi. CdS nanoparticles were produced as a result of glutathione's recombinant expression in E. coli (Chen *et al*., 2009).

Au Reduction Process:

In the biosynthetic process, there are two primary precursors of AuNPs: (i) HAuCl4, which dissociates to Au3+ ions, and (ii) AuCl, which dissociates to Au+ (Zeng et al.,

2010). Because Au3+ ions are more soluble than Au+ ions, the study of the Au+ precursor is less extensively studied. However, by complexing with the proper ligands, such as alkenes, alkylamines, alkyl phosphines, alkanethiols, and halide ions, Au+ solubility may be boosted (Zeng et al., 2010). Whereas, the conversion of Au^{3+} to Au^{0} via the three-electron reduction process presumably involves a number of chemical steps, the conversion of Au^{+} to Au^{0} , however, involves the single-electron reduction process (Das *et al*., 2012b).

AuNP production might take place in the extracellular or intracellular region. It is frequently reported that extracellular AuNP production occurs in fungi when trapped Au^{3+} ions are reduced by proteins in the cell wall. Previous research with the fungus *Verticillium sp.* disproved the idea that the reduction of Au^{3+} ions is caused by reduced sugars in the cell wall and further suggested that $AuCl^{4-}$ ions are adsorbed on the cell-wall enzymes through electrostatic interaction with positively charged groups (such as lysine) (Mukherjee *et al*., 2002; Durán *et al*., 2011). Intracellular production of AuNP, diffusion of Au^{3+} ions permeate across the cell membrane and are reduced by systolic redox mediators in the case of intracellular production (Das *et al*., 2012b).

Due to their biocompatibility, stability, and oxidation resistance gold nanoparticles are used as a burgeoning research tool and find tremendous application in the field of medicine. AuNP is frequently synthesized chemically, which generally leaves harmful by-products that may raise various environmental concerns. Biosynthesis of AuNPs, whether intracellular or extracellular, using viable microorganisms, however, has been regarded as the most inexpensive and eco-friendly. Fungi are well-known for their metal-tolerance level and release of larger amounts of redox extracellular proteins, and for the conversion of soluble metal ions into their insoluble form, which is then converted to nanocrystals. Fungi may have undiscovered metal reductases for detoxification and bio-reduction. To abbreviate biosynthesis and scale up AuNP production, a comprehensive understanding of fungal biosynthesis is needed. This study examines the optimal bioreactors for commercial AuNP production and the known processes in living fungus and fungal protein extracts. Fungus' complicated structure requires microscopic and mechanistic studies to characterize nanoparticles. Fungi microbiology is typically understudied. Lab-grown fungi and bacteria have similar biomass densities. In a stirred tank reactor, Escherichia coli grew 0.31 g g1 of biomass on glucose, but *Rhizopus oryzae* grew 0.55 g g1 on glucose (Taherzadeh et al., 2003; Xu et al., 1999).

1.2.2 Biosynthesis of Nanoparticles by microorganisms

Both multicellular and unicellular organisms are involved in the production of nanoparticles both intracellularly and intercellularly. The capability of controlling the biosynthesis of different metallic nanoparticles of different microbes such as fungi and bacteria is used in search of new materials. Due to their high tolerance and metallic bioaccumulation capabilities, fungi have proved to play a tremendous role in the formation of nanoparticles (Park *et al.*, 2016). An easily cultivated and manipulated agent bacteria were the first microorganism that was taken for nanoparticle synthesis. The microorganisms involved in synthesizing nanoparticles include *Shewanella oneidensis, Cupriavidus metallidurans*, *Bacillus subtilis, Shewanella algae*, *Pseudomonas aeruginosa*, *Rhodopseudomonas capsulate* (Jiang *et al*., 2021). A chemical silver ion that is highly dangerous and toxic can be converted to silver nanoparticles by a reduction process using microorganisms like *Lactobacillus* sp. and *Pseudomonas stutzeri* (Jomehzadeh *et al*., 2021). Some others are listed in Table 1.

Table 1. Biosynthesis of metal nanoparticles

Microorganism	Location	Shape (Size)	Culture Temperature (˚C)	Nano-Product Type	References
Phoma sp.	Extracellular	N/A (71.06–74.46nm)	-	Ag	Hasan, 2015; Tag *et al*., 2021
Plectonemaboryanum	Intracellular	Cubic (<10–25nm)	25-100˚C	Au	Lengke, et al., 2006
Plectonema boryanum UTEX485	Extracellular	Octahedral (10 nm–6 μm)	25 ˚C	Au	Lengke, et al., 2006
Rhodococcus sp.	Intracellular	Spherical (5-15nm)	37 ˚C	Au	Ahmad et al., 2003
Sargassum wightii	Extracellular	Planar (8–12 nm)	Not available	Au	Singaraveluet al., 2007
Candida utilis	Intracellular	N/A N/A	37	Au	Gericke and Pinches, 2006
Yarrowia lipolytica	Extracellular	Triangles (15 nm)	30	Au	Agnihotri et al., , 2009.
Pseudomonas aeruginosa	Extracellular	N/A (15-30 nm)	37	Au	Husseiny et al., 2007
V. luteoalbum	Intracellular	N/A N/A	37	Au	Gericke and Pinches, 2006
Escherichia coli	Extracellular	Triangles, hexagons (20–30 nm)	37	Au	Du et al., 2007
Trichoderma asperellum	Extracellular	N/A (13-to-18 nm)		Ag	Hasan, 2015; Tag *et al*., 2021
Rhodopseudomonas capsulate	Extracellular	Spherical (10-to–20 nm)	30	Au	He et al., 2007
Trichoderma viride	Extracellular	Spherical	27	Ag	

		(5–40 nm)			Fayaz et al., 2010
Trichoderma viride	Extracellular	N/A (2–4 nm)	10–40	Ag	Fayaz et al., 2009
Brevibacterium casei	Intracellular	Spherical (10–50 nm)	37	Au, Ag	Kalishwaralal et al., 2010
Bacillus licheniformis	Extracellular	N/A (50nm)	37	Ag	Kalimuthu, et al., 2008
Phaenerochaete chrysosporium	Extracellular	Pyramidal (50–200 nm)	37	Ag	Vigneshwaran, et al., 2006
Phaenerochaete chrysosporium	Extracellular	N/A (50–200 nm)		Ag	Hasan, 2015 and Tag *et al*., 2021)
Corynebacterium glutamicum	Extracellular	Irregular (5–50 nm)	30	Ag	Sneha et al., 2010
Escherichia coli	Extracellular	N/A (50 nm)	37	Ag	Gurunathan et al., 2009
Bacillus cereus	Intracellular	Spherical (4-5 nm)	37	Ag	Babu and Gunasekaran, 2009
Ureibacillus thermosphaericus	Extracellular	N/A (50–70 nm)	60–80	Au	Juibari et al., 2011
Fusarium oxysporum	Extracellular	Spherical (8–14 nm) (5–50 nm)	25 25	Alloy Au-Ag Ag	Senapati et al., 2004; 2005
Fusarium oxysporum	Extracellular	N/A (20–40 nm)		Au	Hasan, 2015; Tag *et al*., 2021)
Aspergillus flavus	Extracellular	Spherical (8.92 ± 1.61nm)	25	Ag	Vigneshwaran et al.,2007

		Spherical (5–25 nm)		Ag	Bhainsa and D'Souza, 2006
Aspergillus fumigatus	Extracellular	N/A (5–25 nm)	25	Ag	Hasan, 2015; Tag *et al*., 2021)
Verticillium sp.	Extracellular	Spherical (25 ± 8 nm)	25	Ag	Senapati et al., 2004
Verticillium sp.	Intracellular	N/A (25 ± 12 nm)		Ag	Hasan, 2015; Tag *et al*., 2021)
Shewanella sp	Extracellular	Spherical (181 ± 40)	30	Se	Lee et al., 2007
Neurospora crassa	Extra, / intra-cellular	Spherical (20–25, 32nm)	28	Au, Au/Ag	Castro-Longoria et al., 2011
Shewanella oneidensis	Extracellular	Spherical (12 ± 5 nm)	30˚C	Au	Suresh et al., 2011
Enterobacter sp..	Intracellular	Spherical (2-5 nm)	30	Hg	Sinha and Khare, 2011
Shewanella algae	Intracellular	N/A (10–20 nm)	25	Au	Konishi et al., 2007
Shewanella algae	Intracellular	N/A (5 nm)	25	Pt	Konishi et al., 2007
yeast	Extracellular	Polygonal, Irregular (9–25 nm)	30	Au/Ag	Zheng et al., 2010
Desulfovibrio desulfuricans	Extracellular	Spherical (50 nm)	25	Pd	Lloyd et al., 1998
Pyrobaculum islandicum	Extracellular	Spherical (N/A)	100	U(VI), Co(III), Cr(VI), Tc(VII), Mn(IV)	Kashefi and Lovley, 2000
Escherichia coli	Extracellular	Spherical (2.0–3.2 nm)	37	Cd, Te	Bao et al., 2010

Bacterial Vs. Fungal Biosynthetic production of AuNP:

Fungus is challenging to define because of their complex structure, which makes microscopic and mechanistic investigations that are necessary for nanoparticle characterisation. As a result, the microbiology of fungi is often considerably less studied. Fungi and bacteria both grow at a comparable biomass density in laboratory. For instance, an Escherichia coli culture grown with glucose in a stirred tank reactor had a biomass yield of 0.31 g g1, but a Rhizopus oryzae culture grown with glucose as a carbon/energy source had a biomass output of 0.55 g g1 (Taherzadeh *et al*., 2003; Xu *et al*., 1999). Fungi, however, hold several advantages over bacteria in several bioprocesses which also involve AuNP production. Large quantities of extracellular proteins with a variety of activities are secreted by fungi as depicted in Table 2 and Figure 2. All proteins released into the extracellular space are collectively referred to as the "secretome" (Girard et al., 2013). High fungal secretome concentration finds its application in the industrial manufacturing of heterologous and homologous proteins. For instance, it has been documented that the entomopathogenic fungus *Beauveria bassiana* expresses a functionally active class I fungal hydrophobin (Kirkland and Keyhani, 2011). In addition, being a reducing agent involved in metal reduction, tripeptide glutathione, is also well-known for its contribution in the manufacturing of cadmium sulphide (CdS) in yeasts and fungi. For instance, it has been documented that the entomopathogenic fungus *Beauveria bassiana* expresses a functionally active fungal hydrophobin (Kirkland and Keyhani, 2011). In addition to being a well-known reducing agent for metal reduction, tripeptide glutathione is also known to contribute to the manufacture of cadmium sulphide (CdS) in yeasts and fungi. CdS nanoparticles were produced as a result of glutathione's recombinant expression in *E. coli* (Chen et al., 2009).

Table. 2. Bacterial Vs. Fungal Biosynthetic production of AuNP

	Bacteria	**Fungi**
AuNP Location	Both Extracellular and Intracellular (Lengke and Southam, 2006)	Mostly extracellular (Duran et al., 2011)
Resistance To Metal Toxicity	Medium or High (Rajapaksha et al., 2004)	High or Very High (Rajapaksha et al., 2004)
Biosynthesis Rate	(>24 h) Relatively slow (Du et al., 2007)	(<1hr) Fast in the cell-free filtrate (Du et al., 2011)
Bio-Reducing Agent	Redox mediators, cytochromes, membrane proteins, inorganic, and Redox Compounds (Marshall et al., 2008; V o n - Canstein et al., 2008; Mukherjee *et al.,* 2002)	NADH-reductases, manin, phyocheins, (Mukherjee et al., 2001)

AuNP Size and Shape	Most Prevalently small and spherical AuNPs (Wen et al., 2009) ,	Shape and size depend on biomass and/or AU ratio (Pimprikar et al., 2009)
Stability To Industrial Processes	Less Likely	More Likely
Bio-Caping Agents	Not identified Yet	Un-identified surface-bound Proteins (Shankar et a/t al., 2003; Das et al., 2009)

Physical and chemical synthesis

Nanoparticles can also be synthesized by using physical or chemical methods. Many methods can be used in the production of these particles. The three most important methods that can be used effectively, are listed below

(1) Dispersion of preformed polymers

(2) Polymerization of monomers

(3) Ionic gelation or coacervation of hydrophilic polymers.

Dispersion of preformed polymers:

This is a common technique that is used in the biodegradable preparation of nanoparticles from lactic acid, D, L-glycolide, D, L-lactide-co-glycolide, and cyanoacrylate. Various techniques can be used for these methods. One of the important techniques is the solvent **evaporation** method. (Nikam *et al.*, 2014) The solvent evaporation method is a method, in which the polymer is dissolved into the organic solvent. Organic solvents could be chloroform, ethyl acetate, and dichloromethane. Then an aqueous solution is used for the emulsification of this mixture. The stable emulsion is formed after some time and the organic solvent is evaporated by continuous stirring or by reducing pressure. (Ahlin Grabnar and Kristl, 2011)

Polymerization method:

In this process, the polymerization of monomers is done to form nanoparticles in the aqueous medium. The drug could be dissolved into the medium or it could be on the nanoparticle. The purification is done to remove the surfactants and various stabilizers (Rao and Geckeler, 2011).

Coacervation or ionic gelation method

Biodegradable hydrophobic polymers for example gelatin, chitosan, and sodium alginate, etc. can be used for the preparation of nanoparticles. In this method, two aqueous methods are mixed. One of them is polymer chitosan and the other is polyanion sodium tripolyphosphate. Positive ions interact with each other and coacervate. Their size range of them is in nanometers (Pedroso-Santana and Fleitas-Salazar, 2020).

Applications:

Nanoparticles have a wide range of applications in the diagnosis and treatment of drugs. Nanoparticles have several medical uses and have replaced traditional drug delivery techniques as the primary tools in nanomedicine. For the proper production of functional nanocarriers, it is crucial to take into account how the nanocarriers function. The key difficulty is choosing and creating suitable ligands that will finally perform the task of targeting and homing. As its presence on the nanocarriers helps generate the active nanocarriers with active ligands that aid in the proper target delivery of the carriers, polyethylene glycol is a good choice for chemical functionalization These offer significant benefits for drug delivery, targeting, and release. Additionally, their added potential can be used to combine treatment and diagnosis, making them useful developing tools in nanomedicine. They are an excellent means of delivering medication to biological systems for the safer treatment of numerous neurodegenerative and viral disorders. These extremely effective drug-delivery devices have a wide range of potential uses, including radiation, gene therapy, AIDS treatment, and anticancer therapy. Some of the applications of nanoparticles are enlisted in Figure 5 below:

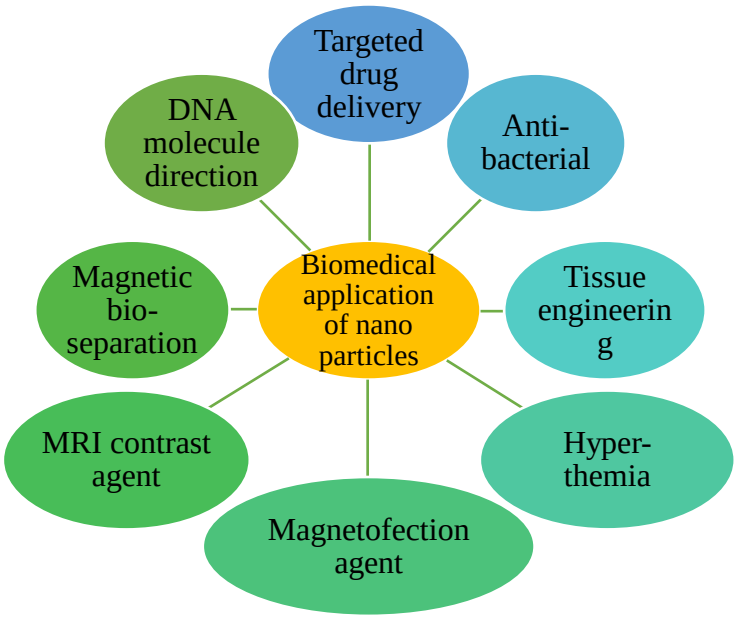


Figure 5. Biomedical Applications of nanoparticles

These are also utilized as carriers or vesicles to cross the blood-brain barrier and to deliver proteins, antibiotics, virostatics, and vaccinations (Figure 6). These drug delivery methods could also be used to introduce molecular and immunological agents into the biological system. Additionally, they help convey genes and create recombinant therapeutic peptides by fusing new genes into existing cells. To stop neurodegenerative illnesses, it is capable of transferring neurotrophic agents. Thus, after one or two weeks of injection, nanoparticle penetration enables secure and continuous medication release at the desired spot. More precisely, nanoparticles have a larger range of applications in the treatment of cancer, Alzheimer's disease, and brain tumors. The nanocarriers' top qualities for use as drug carriers or vehicles are not able to induce an immune response, blood shouldn't clump together or divide (Blood

should be stable). There should be a focused BBB moiety, parent drug consistency needs to be maintained. Further medication release patterns that are adjustable should be used, acceptable for the transport of peptides, proteins, and other tiny particles, maintain blood flow over a continuous period of time. Such nanoparticles should be safe, recyclable, and biocompatible materials. The minimum element size is 100nm (excluding the delivery through macrophages and monocytes) (Comoglu et al., 2017).

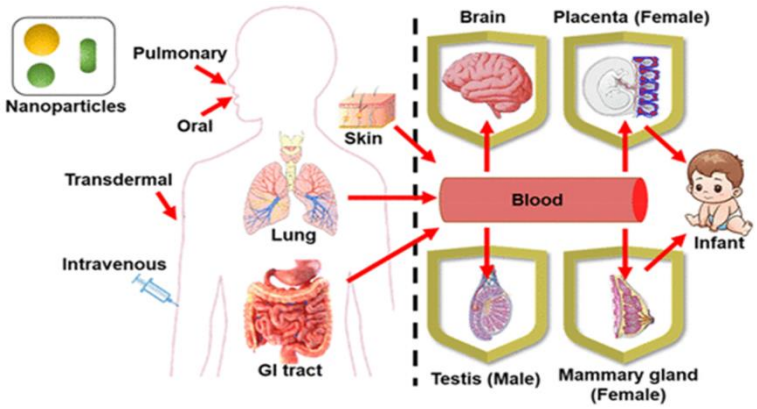


Figure 6. Various nanoparticles crossed biological barriers to enter vital animal organs.

Tumour and nanoparticles

Due to the mechanism of resistance in the tumor cells, anticancer drugs have limited efficacy. Due to the resistance mechanism tumors can evade the process of chemotherapy. Multidrug resistance (MDR) is another important phenomenon in chemotherapy. In this case, nanoparticles are used as a colloidal carrier to improve the efficacy of anticancer drugs (Stark, 2011). Some noble nanoparticles such as Au have some silent features such as Surface Plasmon Resonance (SPR), high adsorption, and enhanced light scattering. Au conjugation with specific ligands enhances the treatment available chances and diagnosis of the disease. They are also helpful in the "selective laser photothermal therapy of cancer" as they can convert the absorbed light into heat energy (Jain *et al.*, 2007).

Oral delivery of proteins and peptides

In these days, various types of vaccines are based on proteins and peptides. For this type of vaccine, the carrier plays a vital and crucial rule (Ruoslahti, 2012). Nanoparticles are effective carriers because they can protect these particles from hydrolytic and enzymatic degradation. Mucosa has important properties to degrade the peptides by using different phenomenon. (Petros and DeSimone, 2010) So colloidal particle carriage system is compulsory. So, nanoparticles have important properties to enhance the effect system of drug delivery in GI tract (Stark, 2011).

Nanoparticles and gene delivery

Some vaccines work with the delivery of genes that encode specific antigens. These types of vaccines are involved both in humoral and cell-mediated immunity. There are a few issues in the delivery of these genes or polypeptides (Sun *et al.*, 2014). Efficient delivery of them in the body is one of the major issues. Nanoparticles can play a vital role in the sustained released delivery of these genes in the body. These types of

methods can also be used in the bone healing processes (Stark, 2011) (Chen *et al.*, 2016). Delivery System for Cationic Solid Lipid Nanoparticles Mediated by Cholesterol**.** Medication carriers for peptides, proteins, and oral drug administration are lipid-based nanoparticles. Transdermal medication administration and immunostimulatory RNA adjuvant are further applications for lipid-derived nanoparticles. Numerous techniques, such as cationic lipid or DNA lipoplexes, PLGA-based nanoparticulate systems, etc., are employed for cancer therapy. For more effective gene delivery to tumor cells, cyclen-based cationic lipids and polylipid nanoparticles are utilized. Lipoplexes and functional lipids are employed for enhanced nonviral vector gene delivery (Suñé-Pou *et al.*, 2018).

Nanocarrier and drug delivery into the brain

Blood arteries are a source of nutrition for all body parts. They also do for the CNS via the blood-brain barrier (BBB). BBB is a natural control of all the nutrients in the system. In this way, the human blood works in normal healthy conditions. In normal physiological conditions, BBB plays a pivotal role in the homeostasis of the brain and other pathological conditions (Teleanu *et al.*, 2018). As this barrier allows only the selective passage of molecules, so it can make obstacles in drug delivery to the brain. The transcellular and paracellular processes of molecular transit are both significant. Small molecules can dissolve into tight junctions (TJ) and pass across the membranes. Macromolecules, on the other hand, need carrier proteins to do so. But there are several obstacles that prevent the transport of drugs to the brain in case of pathological brain conditions. This physical barrier is comprised of adherent and tight junctions. Claudins and occluding transmembrane proteins are mainly connected with the TJ which assures the selective permeability of the sieve. Some cytoplasmic proteins are also involved in the permeability of the TJ including 7H6 phosphoprotein and cingulin with weights of 155kDa and 140 kDa. Glycoproteins in adhesion junctions (AJ) link to cytoplasmic proteins below the tight junction (Tosi *et al.*, 2020). The combination of TJ and AJ makes a very selective paracellular transport system. Medical science, hence, faced difficulty in drug delivery to the brain, in case of pathological conditions of the brain (Wang *et al.*, 2020).

Drug delivery via nanocarriers is successful in many areas of the body, including the brain. Scientists have been more interested in using them via the BBB in recent years. Due to their tiny size, macromolecules and more drug payload may be carried by nanocarriers, which has fundamentally transformed the field of interest. They also have advanced drug-releasing capabilities (Yu *et al.*, 2019). Since they are poor immunogens, these nanocarriers allow for the maintenance of medication consistency. Additionally, their pharmaceutical release schedules can also be changed. Moreover, they are safe, reliable, biocompatible, and recyclable materials. These qualities make them the most effective alternative to other techniques (Wang *et al.*, 2020).

Nanocarrier and drug delivery into kidney

The kidney plays a vital role in the removal of waste from the human body which is a crucial need of life. Some blood-to-kidney barriers regulate the supply of molecules in between the both. These layers are three in number. The first one is made up of endothelial cells and is porous. The second barrier layer is the glomerular basement membrane. The third layer is made up of podocytes and it has foot-like functions. Collectively these layers are called glomerular barriers. This barrier functions in the permeability of molecules. It allows a significant amount of fluid filtration into Bowman's capsule (Du *et al.*, 2018). There are a lot of pathological

complications of the kidney such as Neuropathic syndrome (Proteinuria), Nephrotic Syndrome diet, Proteinuria Albuminuria, Chlorothiazide, Acetazolamide, Spironolactone, and amiloride, etc (Ibrahim *et al.*, 2018). The barrier in the system can hinder the delivery of drugs in the process of treatment. To solve the problem of drug delivery, one can use nanoparticles as they are good carriers in treatment. They can reduce renal toxicity. According to research, the nanoparticles having a diameter of 75±25 nm can also target the renal mesangial (Gómez-Vallejo *et al.*, 2018).

Nanocarrier and drug delivery into the heart

The heart is the lifeline of a human being. It is made up of corn-shaped muscles. The heart has epithelial layers and values in it which provide it a selective barrier. There are a lot of complications of the human heart like heart failure, chambers malfunction and valve problems (Stampfl *et al.*, 2011). In case of pathological conditions, the drug delivery vehicle plays an important role. Liposomes, nanoparticles, genes and stem cells can be the carrier vehicles in case of complications (Figure 7). One of the most important vehicles in all of them are nanoparticles. They have unique features like their size, drug retention time, high payoff drug load and many more. It is crucial to pay off the drug into the heart with minimal effect on other organs which they perform in best way. Due to these reasons, they are considered best in few nanoscale drug delivery systems in the heart problem (Nguyen *et al.*, 2015).

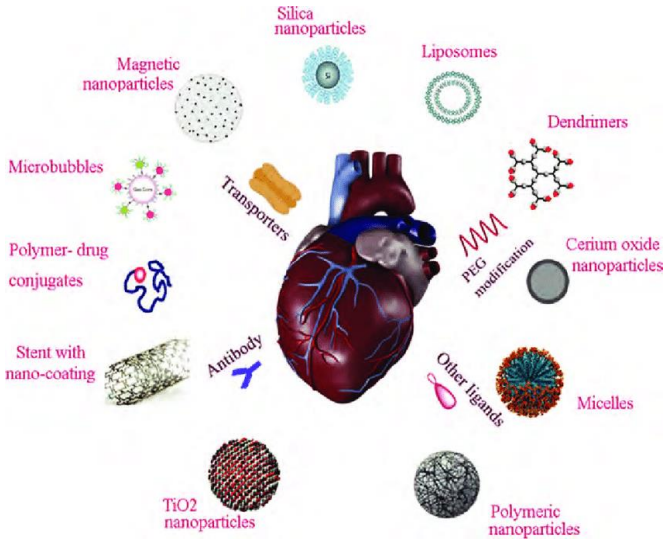


Figure 7. Carrier vehicles used in various heart complications.

Barrier function of the cell membrane:

The majority of macromolecular genes and medicines have actions inside the cell. Therefore, it is essential to get through the cell's outer membrane. Their transporter is accountable. In order for the NP to be internally processed, contact with the cell membrane is crucial. Different models have been used to study these interactions, and it has been found that size, surface charge, and hydrophobicity play the most important effects. We are aware that interactions with membrane are increased by charged particles and decreased by uncharged particles, including pegylated NPs, due to their steric hindrance. As a result, NP may collect around the membrane and block the admission of more NPS. According to its charge, NP adsorbs proteins to their surface, as indicated in this review. Their entry into the cell is well controlled by receptor-mediated endocytosis. It has been discovered that smaller particles internalised

preferentially via clathrin-mediated endocytosis are those that are less than 200 nm in size. Beyond 200 nm-sized particles are connected to the caveolin-mediated endocytosis. Additionally, it was shown that internalisation requires energy and that the loss of cholesterol inhibits particle uptake. Cancer cells overexpress a number of cell-surface receptors, including integrins, glucose, LDL, and folic acid transporters, among others. A excellent strategy to promote NP internalisation is to use their active targeting. Since this idea has been around for a while, it won't be covered here in order to stay current with advancements. There have already been numerous reviews written. However, just increasing the density of the targeted ligands will not improve the internalisation. By binding highly affine targeting ligands to peripheral cancer cells, a "binding barrier" is created that helps stop NPs from penetrating farther. The use of cell-penetrating peptides (CPPs) to target the heparan sulphate chains resulted in large clusters, but these findings did not correspond with an increase in NP uptake. This is also feasible because the cell only supplies a limited quantity of energy for particle uptake. The study is a key element in determining the density of the ligand and the NP dosing regimens, which is crucial for preventing oversaturation of the receptors and accelerating receptor recycling. For more details, consult the references (Simkiss, 1998).

Organellar and vesicular barriers:

Once internalised, NP arrives at its intracellular target and begins to dump its cargo. Endosomal vesicles facilitate the movement of numerous macromolecules, including those found in internal organelles including the Golgi, endoplasmic reticulum, nucleus, mitochondria, and lysosomes (Heald and Cohen-Fix, 2016)].

The intracellular medication delivery system was the foundation upon which the single department predetermined the trafficking of cargo. Endocytosis mediates a number of pathways and functions, including clathrin-dependent endocytosis, caroline-dependent endocytosis, micropinocytosis, and phagocytosis. Different approaches need internalising various pathways. However, a significant number of these tracks can obstruct an untargeted/unmarked organelle location and the lysosomes where later NP debasement occurs. e.g., Gene and peptide medications must be delivered via this way since they are more unstable than other pharmaceuticals. It is generally known that cationic lipids and polymers can help drugs escape from endosomes. Because of their capacity to fuse with endosomal membrane and release their contents into the cytoplasm, branching polymers like polyethyleneimine and PAMAM and lipids like DOTMA and DOTAP have been employed extensively. Internal acidification is brought on by ATPase action as endosomes mature from early to late and then become lysosomes. It is blown up by a variety of NPs that aid in endosomal escape into the cytoplasm. Famous fusogenic lipid-like DOPE molecules also go through a shift from layer to hexagonal phase based on pH variation, which makes them easier to fuse with endosomal membrane. Additionally, DOPE 133 and 134 have been coupled to low molecular weight polyethyleneimine to enhance escape and subsequent DNA and SiRNA delivery (Z. Yang *et al.*, 2013). If additional information is needed, use C references. By actively targeting the NPS of ceramide in receptor-mediated endocytosis, a further method for enhancing intracellular drug delivery, compartmentalization of the lysosome is improved by transferrin targeting of lysosome ceramide. Ceramide grows sufficiently inside the lysosome to penetrate the membrane and trigger caspase-dependent apoptosis. Folic acid is necessary for the synthesis or production of nucleotides.

Because it targets liposomal doxorubicin, the medication can accumulate in the nucleus and then interact with triggered apoptosis. -140 (Ewers and Helenius, 2011; Gil, *et al*., 1998).

Efflux Transporter for Drugs

A tiny portion of the given dose begins to exercise its cytotoxic effects intracellularly as NP draws to a close. As a result, solid tumours can use specialised equipment known as drug-efflux pumps to efflux themselves out of these medications. Clinical resistance to chemotherapy is connected with P-GP expression, and P-glycoprotein (P-GP) is the mechanism of drug efflux transporter overexpression. Drug-efflux pumps are surrounded by specific targeting NPs. The use of tumour vasculature-specific peptides to target paclitaxel nanoparticles was successful. To treat malignancies that are resistant to chemotherapy, anti-cancer medications like paclitaxel have been combined with molecules inhibitors like tariquidar and benzyl dihydropyridines as well as bacterial-derived substances like H6. In BBB, P-GP is also expressed. Target tariquidar and paclitaxel were employed to reduce drug toxicity. The MDR gene family controls P-GP expression, which is also triggered by transcription factors (NF-KB). MicroRNA transfection resensitizes cancer cells to the main therapeutic approach. Doxorubicin resistance in human breast cancer cells has been successfully overcome by anti-surviving administered in combination with doxorubicin (Sun *et al*., 2003).

Antibacterial and Antiproliferative activities of green synthesized and metal nanoparticles

The antibacterial and antiproliferative effects of Zn NPs with folic acid and Ag/Ni NPs with *Punica granatum* have great importance. By using the well diffusion method, the antibacterial activity against multiple bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Acinetobacter baumanii* and *Proteus vulgaris* has been evaluated. In millimetres, the zone of inhibition is being observed in may cases. These zones demonstrate that tested microorganisms are better inhibited by commercially available antibiotics. In contrast to Ag/Ni NPs, it is believed that Zn NP found to have no antimicrobial action. While MTT, Crystal Violet, and Trypan Blue viability assays is being found good element used to measure the antiproliferative effectiveness against the HepG2 cancer cell line. At 570 nm, the absorbance was measured

Challenges in Treating Cancer due to Unregulated Cell Proliferation

In living organisms, each cell serves a specific purpose. In general, the cells within us multiply in an organized way. If cells are injured in any way, they die and are replaced by new cells. Cancer can be caused by uncontrolled cell division. It is the world's second most lethal and aggressive disease, distinguished by abnormal cell growth (Fawzy et al., 2020). There are many different types of cancer, but the main cause of all cancers is the uncontrolled growth of abnormal cells. Cancer cells have the unique ability to penetrate other tissues, which normal cells are unable to do. Many synthetic drugs have been developed to treat cancer; these drugs are effective in treating cancer, but their side effects cannot be avoided (Madhuri & Pandey, 2009).

The antiproliferative effect of the prepared nanoparticles on HepG-2 cell lines is examined at different level. The Hep G2 cell line can be created by using minces

from human liver biopsies that originally layered on feeder cultures of radioactively treated mouse cell layers (STO). A growing, feeder-independent cell line can be produced after several months of passage of isolated colonies with constrained expansion. The Hep G2 cell line's morphology meets the requirements for a hepatoblastoma, which is a type of liver tumor (Javitt, 1990).

Targeted Therapies and Green Synthesis from Punica granatum

The field of nano medicine has so far seen a large number of investigations on improved materials at the nano scale (often less than 100 nm). Nano materials have the capacity to pass through cell membranes and thus interact with intracellular organelles to provide the required therapy to particular aberrant cells. Electronics, biomaterials and medicine are just a few of the transdisciplinary fields that fall under the umbrella of nanotechnology. The creation of metallic nanoparticles utilizing the green synthesis method, which has various plant parts from predetermined plants, is a significant technology. Due to its high concentration of a number of crucial phytochemicals, the pomegranate plant (*Punica granatum*) is regarded as a significant traditional source for treating dangerous diseases (Sarkar & Kotteeswaran, 2018)

Basic biological processes depend on zinc (Zn), which is the second-most common trace metal in eukaryotic species. This shows how effective Zn is as a targeted delivery mechanism for eliminating faulty cells while preserving the viability of healthy cells. Zn-NPs are an intriguing inorganic substance with a wide range of applications in numerous industries. Folic acid-linked nanoparticle components might be an example of a sort of carrier system that has the potential to suppress both cancer and microbial cell proliferation. They offer a variety of biological applications and are non-toxic, biocompatible, and affordable, such as targeted drug delivery, anti-inflammatory, wound healing, antibacterial agents, anti-cancer, and bio imaging (Heinlaan et al., 2008).

These days, silver and nickel nanoparticles (Ag/Ni NPs) with surface functionalization have novel applications in consumer goods. The biotechnology and medical industries mostly use these noble MNP nanoparticles. Due to its compatibility with in vivo testing, biosynthesized conjugated bimetallic nanoparticles are now used in the biomedical industry for imaging, luminescence tagging, labeling, and drug delivery. As a result of the surface plasmin resonance wavelength shifting to a longer wavelength based on particle size and the potential for Ag and Ni NPs to have high antibacterial activity against some pathogenic species, as well as their optical qualities (Akinsiku et al., 2018).

Nanoparticles utilized for Escherichia coli infection therapy

A variety of NPs have been utilized to treat bacterial infections, including silver NPs, zinc oxide NPs, and cationic surfactant NPs. The particle size, shape, and surface modification of silver nanoparticles all have an impact on their antibacterial properties. Positively charged cationic surface NPs can kill bacteria by destroying their cell membrane or wall and releasing free radicals in the process. Gold, silver, magnetic NPs, and quantum dots (QDs) are examples of nanotechnology-based techniques that exhibit selective target-binding properties. They are the best candidates for the diagnosis and bio sensing of *E. coli* infections because of these qualities (Gupta et al., 2019).

Use of nanoparticles in treatment of *Klebsiella pneumonia* infections

Prior research has been done on nanoparticle drug delivery methods for the treatment of *K. pneumoniae*. One of these is the use of metal nanoparticles with built-in antimicrobial characteristics, such as gold or silver. To improve pulmonary delivery of the antibiotics, systems with antibiotic-loaded nanoparticles have also been investigated, such as those with ceftazidime-loaded liposomes or those with gentamicin-loaded chitosan/fucoidan nanoparticles (Pareek et al., 2021). In the past, antibacterial compounds such as silver and other metals like copper and zinc have been utilised. Although direct contact with mammalian cells is said to have hazardous side effects, Ag+ is said to have better antibacterial activity. This limits the use of Ag+ in therapy. Ag NPs, on the other hand, offer a larger surface area, resulting in a more regulated release of Ag+. The potential for this form as an antibacterial chemical thus exists (Alexander, 2009).

Use of nanoparticles in treatment of *Acinetobacter baumanni* infections

Interest in using nanoparticles as treatment regimens has grown during the past several years. The synthesis processes used to create nanoparticles and the conditions, under which they are formed, such as temperature, solvent type, concentration, and the type of reducing agent, determine whether or not they have antibacterial or biomedical capabilities (Wintachai et al., 2019). Nanoparticles are now being considered as a different strategy for combating antimicrobial resistance as a result of their green manufacturing. Silver nanoparticles (AgNps) and gold nanoparticles are the most extensively researched metal nanoparticles (AuNps). For instance, silver nanoparticles (AgNPs), which have a high rate of surface capacity and low ecotoxicity, can prevent the development of biofilm components necessary for evasion and protection (Mba & Nweze, 2021).

Use of nanoparticles in treatment of *Pseudomonas aeruginosa* infections

Metal nanoparticles (MNPs) and metal nanocomposite materials (MNCs) have been shown to have extraordinary antibacterial properties and can thus be employed in alternative therapy. Similar to this, antibacterial, anti-inflammatory, and anticancer drugs made of nickel, copper, and zinc nanoparticles (NPs) are crucial in biomedicine (JAMILA et al., 2022). However, very little research on nanoparticles has been done on *P. aeruginosa* strains that are resistant to multiple drugs (Palanisamy et al., 2014).

Use of nanoparticles in treatment of *Proteus vulgaris* infections

Due to the antimicrobial properties of metals, nanotechnology has made significant advancements in the treatment of UTIs. For example, the use of metallic nanoparticles (NPs) or surface-tailored nano carriers has allowed for the elimination of multidrug resistance, the prevention of biofilm formation, and the suppression of cytotoxic processes in healthy cells (Dance et al., 1987). Metal nanoparticles are now one of the potential remedies for MDR bacteria's microbial resistance. These inorganic agents include silver nickel nanoparticles (Ag/Ni NPs), which are non-toxic, made from the metals silver and nickel, and which, at low concentrations, have bactericidal, antiviral, and antifungal effects (Saleh et al., 2019).

Use of nanoparticles in liver cancer

Compared to chemotherapy and radiation, which are more well-established treatments, targeted and immune therapy has increased the therapeutic capacity. However, this progress can only be evaluated in months, and not all patients will react

to these therapies. Better treatment alternatives are therefore desperately needed for liver cancer patients who cannot have surgery. Many researchers have looked to the science of nanotechnology to solve this problem. Nanotechnology pioneer Norio Taniguchi coined this phrase in 1974, and K. Eric Drexler used it once more in 1981. Nanomedicine is now a potential area of nanotechnology in the twenty-first century. Most NP types can serve as carriers in a medication delivery system, and some NPs have inherent therapeutic benefits. Because many malignancies, including breast, colorectal, and liver cancers, are known to overexpress folic acid receptors in their cell membranes, folic acid has been utilised to target cancer cells and tumors (Mintz & Leblanc, 2021). Metallic nanoparticles have a wide range of applications due to their exceptional chemical and physical characteristics, such as their high surface-to-volume ratio and strong thermal conductivity. Gold nanoparticles (AuNps) exhibit unique optical, physical, and chemical properties, are reasonably resistant to germs, have a long history dating back to ancient times, and have a bright future in the fields of biological and chemical sciences (Arzumanian et al., 2021).

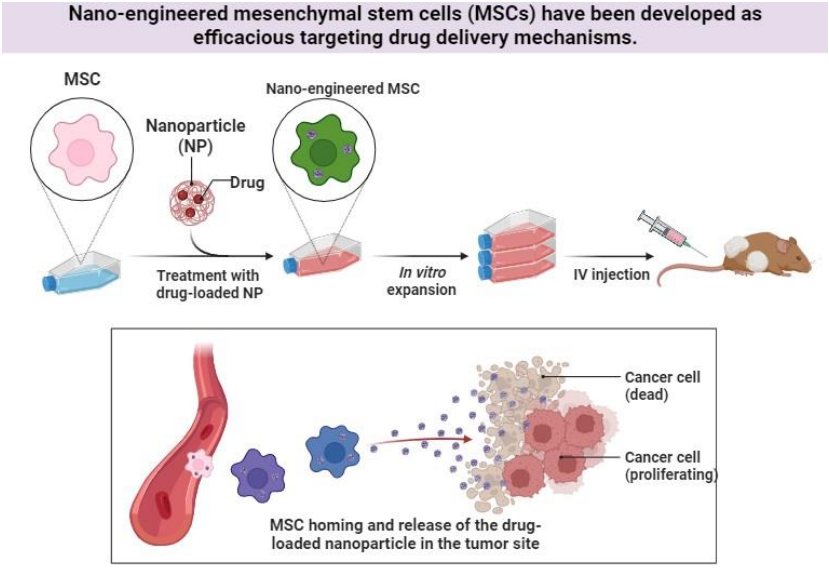


Figure 8. Drug delivery mechanism of nano particle

The development of nano-engineered mesenchymal stem cells (MSCs) has resulted in the creation of highly effective targeting drug delivery mechanisms. These highly sophisticated mechanisms are capable of precisely identifying and delivering drugs to specific cells and tissues within the human body, thus improving the effectiveness of drug therapy. The nano-engineered MSCs are able to navigate through the complex and dynamic environment of the human body, and can be engineered to respond to specific stimuli, such as changes in temperature or pH levels. Furthermore, these advanced delivery mechanisms have shown great promise in the field of regenerative medicine, as they can be used to deliver stem cells directly to damaged tissues, promoting tissue repair and regeneration. Overall, the development of nano-engineered MSCs represents a significant advancement in the field of drug delivery, with the potential to greatly improve the effectiveness and precision of drug therapy.

HepG-2

Fresh human hepatocytes are hard to come by, hence hepatoma cell lines like HepG2 cells are routinely employed as in vitro alternatives. HepG2 cells were the first hepatic cell line to display the essential traits of hepatocytes. However, although being

regarded as a model of HCC, the older cell line SK-Hep1, which was developed in 1971, lacks important liver cell markers such the expression of albumin and alpha- and gamma-fibrinogen (Arzumanian et al., 2021). The most popular human hepatoma cell line in pharmaco-toxicological studies is HepG2. The liver biopsies of a 15-year-old Caucasian male with differentiated hepatocellular carcinoma provided the material for the creation of this cell line. There has been a lot of research done on HepG2 cells' functional properties. They exhibit a variety of distinct liver processes, including the production and secretion of plasma proteins, the metabolism of cholesterol and triglycerides, the transport and metabolism of lipoproteins, the production of bile acids and glycogen, and the signaling of insulin (Donato et al., 2015).

Use of nanoparticles in HeG-2

The goal of the current study is to examine the potential cytotoxicity mechanisms of nanoparticles in HepG2 cells, which are a type of liver cancer (Ahmadian et al., 2018). To lessen the intrinsic toxicity of silver NPs, bio-reduction is a typical method of preparation. To create silver nanoparticles, Benelli and colleagues employed an extract from the earthworm *E. eugeniae*. Although silver nanoparticles (NPs) have a variety of anti-cancer effects, they frequently lack specificity. They are frequently harmful to healthy, normal cells as well, albeit occasionally to a lesser extent, and their cytotoxicity has been researched in relation to a variety of organisms. Another metal oxide that has been studied against liver cancer cells is zinc nanoparticles, albeit their mode of action is very different from that of the other examples. Iswarya et al. created zinc nanoparticles (NPs) using a bio decomposition method. They used a protein that binds to 3-glucan to decrease Zn ions cover the surface of the NPs with a coating to promote cellular uptake (Mintz & Leblanc, 2021).

Treatment of HepG-2 cell line with Nanoparticles

On a 96-well plate, HepG-2 cell lines at their second passage are cultured to test for cell proliferation. HepG-2 cell lines are cultured and split into 5 groups for the Ag/Ni NPs with Punica granatum and 5 groups for the Zn NP with folic acid, respectively. Out of the five groups, one is regarded as normal for both NPs. The remaining 4 are administered with 10µg, 50µg, 100µg, and 200µg of Ag/Ni NPs with Punica granatum, and the other 4 with 50µg, 100µg, 200µg, and 400µg of Zn NP with folic acid. The therapy is applied to cultured cells for 24 hours. After 24 hours, the harvested media from all experimental groups is used for additional biochemical experiments on 96-well plates used for cell proliferation assays, trypan blue, and crystal violet assays (Faedmaleki, Shirazi, Salarian, Ashtiani, & Rastegar, 2014).

Cell Proliferation Assay:

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is carried out on the cells cultivated in 96-well plates to examine the proliferation potential of several HepG-2 cell lines. After washing the cells with phosphate buffer saline (PBS; Invitrogen Inc., USA), the monolayer of cells is incubated for 2 hours in 100µl of complete media containing 25µl of MTT solution. Living cells convert MTT into purple-colored formazan, which is then dissolved in 10% sodium dodecyl sulphate (SDS) from Invitrogen Inc. in the United States, and the solution's absorbance is measured at 570 nm (Senthilraja & Kathiresan, 2015).

Trypan Blue Assay:

Trypan blue, a dye that prevents both living and dead cells from coexisting, is used to measure cell viability. After three PBS washes, the cells from the various experimental groups are incubated in trypan blue (Invitrogen Inc., USA) for 15 minutes. After three PBS washes, cells are examined under a microscope. Trypan blue-stained cells are considered dead. Cells lacking staining are divided by the total number of cells and multiplied by 100 to determine the percentage (Felice, Sun, & Liu, 2009).

Crystal Violet Assay:

Crystal violet staining is used to measure cell viability as well. This procedure is carried out in a 96-well plate. The medium from a different experimental group is removed from the plate's wells and rinsed with PBS. Following cleaning, the wells are coated with 0.1 percent crystal violet dye mixed with 2 percent ethanol. It is incubated for 15 minutes at room temperature. Wells are thoroughly cleaned, and dye is disposed of carefully to prevent cells from lifting out of the well. The stain is then solubilized by adding 100µl of 1 percent SDS to each well, and this process takes 5 to 10 minutes. A microplate is used to measure absorbance at 540 or 595 nm at the conclusion (Feoktistova, Geserick, & Leverkus, 2016).

Scratch Assay:

A basic, quantifiable way for evaluating fundamental cell movement is the scratch experiment. HepG-2 cell line is cultured in a 6 well plate using this approach. To make a "scratch," scrape a straight line across the cell monolayer using a p10-l pipet tip. For 10µl of Ag/Ni NPs with Punica granatum and 80µl of Zn with folic acid, respectively, 1 and 2 are regarded as normal. After a 24-hour incubation period, scan the wells in the imaging station (Liang, Park, & Guan, 2007).

Antibacterial Results

The antibacterial results of Ag/Ni NPs with *Punica granatum* and Zn NP with folic acid by well diffusion method against all bacterial strains.

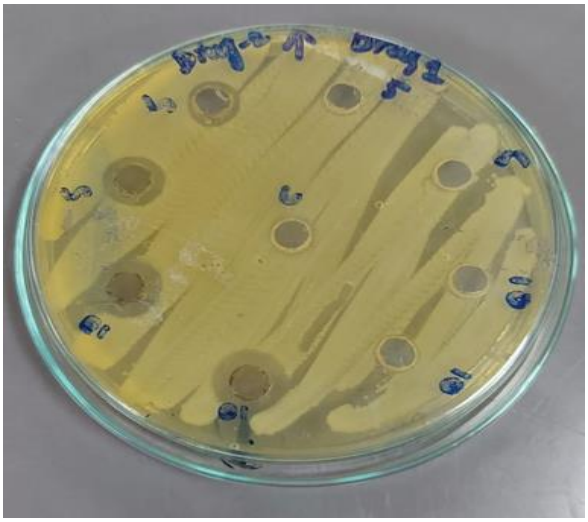


Figure 9. Antibacterial activity of Zn NP functionalized by folic acid and Ag/Ni bimetallic NPs with *Punica granatum* against *Acinetobacter baumannii*

Figure shows inhibition zone on MHA containing *Acinetobacter Baumannii*, 24 hours after application of nanoparticles. The mean diameter of the zone of

duplicates for 5µl, 10µl Zn NP functionalized by folic acid is 9.5±0.5, 9.5±0.5 respectively and for 5µl, 10µl is Ag/Ni bimetallic NPs with *Punica granatum* is 13.5±0.5, 15±1. The results suggest that Ag/Ni bimetallic NPs with *Punica granatum* are inhibiting the growth of *Acinetobacter baumannii*.

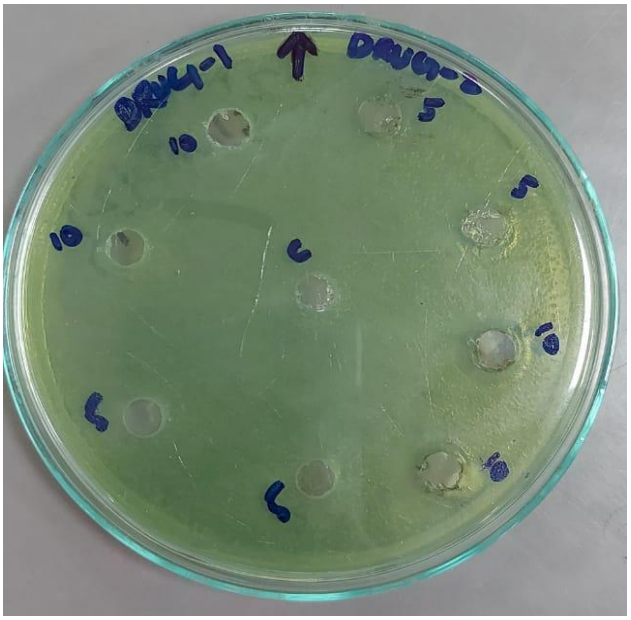


Figure 10. Antibacterial activity of Zn NP functionalized by folic acid and Ag/Ni bimetallic NPs with *Punica granatum* against *Pseudomonas aeruginosa*

Figure shows inhibition zone on MHA containing *Pseudomonas aeruginosa*, 24 hours after application of nanoparticles. The mean diameter of the zone of duplicates for 5µl, 10µl Zn NP functionalized by folic acid is 9.5±0.5, 9.5±0.5 respectively and for 5µl, 10µl is Ag/Ni bimetallic NPs with Punic Granite is 15±1, 17±1. The results suggest that Ag/Ni bimetallic NPs with *Punica granatum* are inhibiting the growth of *Pseudomonas aeruginosa*.

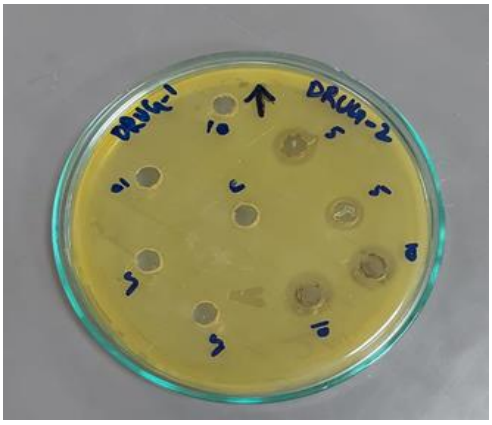


Figure 11. Antibacterial activity of Zn NP functionalized by folic acid and Ag/Ni bimetallic NPs with *Punica granatum* against *Proteus vulgaris*

Figure shows inhibition zone on MHA containing Proteus, 24 hours after application of nanoparticles. The mean diameter of the zone of duplicates for 5µl, 10µl Zn NP functionalized by folic acid is 9.5±0.5, 9.5±0.5 respectively and for 5µl, 10µl is Ag/Ni

bimetallic NPs with *Punica granatum* is 13±1, 15±1. The results suggest that Ag/Ni bimetallic NPs with *Punica granatum* are inhibiting the growth of *Proteus vulgaris.*

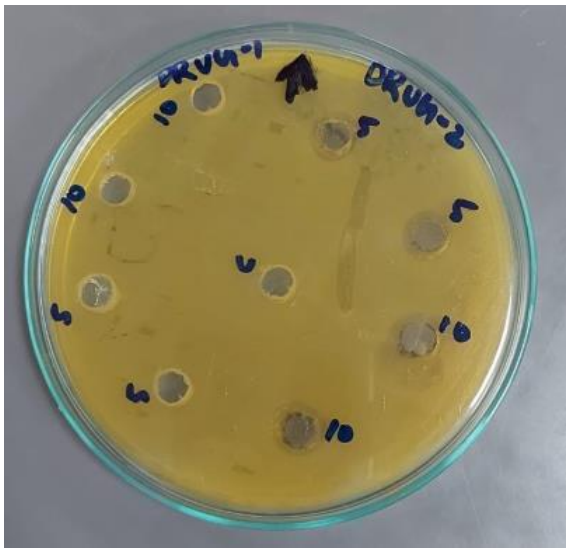


Figure 12. Antibacterial activity of Zn NP functionalized by folic acid and Ag/Ni bimetallic NPs with *Punica granatum* against *Escherichia coli*

Figure shows inhibition zone on MHA containing Proteus, 24 hours after application of nanoparticles. The mean diameter of the zone of duplicates for 5µl, 10µ Zn NP functionalized by folic acid is 9.5±0.5, 9.5±0.5 respectively and for 5µgl, 10µl is Ag/Ni bimetallic NPs with *Punica granatum* is 11±1, 14±1. The results suggest that Ag/Ni bimetallic NPs with *Punica granatum* are inhibiting the growth of *Escherichia coli***.**

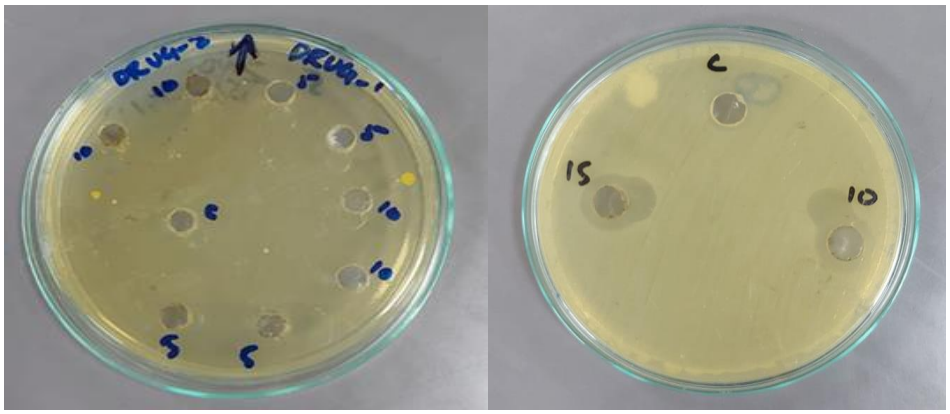


Figure 13. a. Antibacterial activity of Zn NP functionalized by folic acid and Ag/Ni bimetallic NPs with *Punica granatum* against *Klebsiella pneumonia*. b. Ag/Ni bimetallic NPs with *Punica granatum* against *Klebsiella pneumonia* for 3rd trial by Antibacterial activity

Conclusion

Nanoparticles have revolutionary changed the research fields and had opened new eras in research. They can be produced and prepared by using biological, chemical as well as physical methods. They have a wide range of applications in many fields of life, especially in medicine and industry. Major developments have been made in the realm of drug delivery behind various blood-tissue barriers, including the blood-brain barrier, for the treatment of cancer and HIV/AIDS, respectively, employing

nanotechnology. Nanocarriers can be used effectively for drug delivery in many parts of the body. These nanocarriers are bad immunogens thus consistency of the drug can be maintained in their case, they are safe, reliable, biocompatible, and recyclable materials. These characteristics made them the best replacement for other therapeutic methods. There are still many ethical questions surrounding nanoparticles once they reach the human body where they will reside. Risk management and risk assessment are thus the most crucial ethical problems at play. Despite all of these warnings about risks, the public continues to want nanotherapeutics due to their numerous advantages.

Discussion

The World Health Organization (WHO) named antimicrobial resistance (AMR), or the capacity of microorganisms (bacteria, parasites, viruses, and fungi) to withstand antimicrobial treatments, as one of the top ten global health problems in 2019. Innovative technologies appear to be viable supplemental approaches to make up for the paucity of new antibiotic medications. Nanotechnology by definition entails the use of nanomaterials for particular purposes, in this case the control of bacteria and other microorganisms. Many inorganic NP have built-in antibacterial properties, but AgNP in particular has been utilized for centuries (Şen Karaman et al., 2020). A previous study's findings demonstrated the potential cytotoxicity of the biosynthesized AgNPs against human hepatocellular carcinoma HepG2 cells, and they suggested that they might be useful in pharmacological, medical, and therapeutic applications. (Priya et al., 2020).

Nanoparticles are anti-proliferative, anti-cancerous, and anti-bacterial, according to prior studies. Due to the potential for particular ingredients to be effective for treating cancer, medicinal plants and nanoparticles are evaluated (Qadir et al., 2014).

In this study metallic nanoparticles are accessed to evaluate the antibacterial and antiproliferative activity. Ag/Ni Bimetallic NPs with *Punica granatum* and Zn NP with folic acid were evaluated for antibacterial activity against *E. coli*, *K. pneumonia*, *P. vulgaris*, *P. aeruginosa* and *A. baumannii.* The results of Ag/Ni NPs showed inhibition zones against Gram negative strains similar to the observation of Bushra Al Edhar who stated that Ag/NiNPs showed more effective antibacterial activity than other mixed NPs (Al Edhari et al., 2021). Another supplementary report indicated that the antibacterial effectiveness of nanomaterial against *E. coli* was close to 100%. Numerous studies have revealed Ag NPs to be potent antibacterial agents. (Alavi & Rai, 2019).

On the other hand Zn NP with folic acid had no inhibition activity against any of the strain in my study by well diffusion method. All the strains were found to be ineffective against Zn nanoparticle, but according to previous studies High antibacterial efficiency at low concentrations (0.16–5.00 mmol/L), activity against a variety of strains, and comparatively inexpensive cost is only a few benefits of ZnO nanoparticles (Gudkov et al., 2021).

According to earlier research, metallic nanoparticles have been shown to exhibit strong toxicity and anti-proliferative effects on a variety of malignancies (Daduang et al., 2015). Samah A Loutfy stated that, Standard chromogenic tests were used to measure cell viability. Because it's possible that metallic nanoparticles will disrupt redox-based tests, including the delicate MTT cell viability assay (Loutfy et al., 2015).

As according to this study Ag/Ni and Zn NPs shows the results in accordance with the present study where both nanoparticles were significantly antiproliferative against HepG2 cancer cells.

Conflicts of interest: Declare no potential conflicts of interest.

Funding: All the author found no funding or financial support for this study.

References:

1. Ahlin Grabnar, P. and Kristl, J. (2011). The manufacturing techniques of drug-loaded polymeric nanoparticles from preformed polymers. *Journal of microencapsulation*, **28**:323-335.
2. Ahmad, N., Bhatnagar, S., Saxena, R., Iqbal, D., Ghosh, A. K., & Dutta, R. (2017). Biosynthesis and characterization of gold nanoparticles: kinetics, in vitro and in vivo study. *Materials Science and Engineering: C*, **78**:553-564.
3. Bhardwaj, K., Dhanjal, D. S., Sharma, A., Nepovimova, E., Kalia, A., Thakur, S., Kuča, K. (2020). Conifer-derived metallic nanoparticles: Green synthesis and biological applications. *International Journal of Molecular Sciences*, **21**(23):9028.
4. Campani, V., Salzano, G., Lusa, S.,and G. de Rosa, Lipid nanovectors to deliver RNA oligonucleotides in cancer, *Nanomaterials*, vol. 6, no. 7, pp. 1–22, 2016, doi: 10.3390/nano6070131.
5. Chen, J., Guo, Z., Tian, H. and Chen, X. (2016). Production and clinical development of nanoparticles for gene delivery. *Molecular Therapy-Methods & Clinical Development*, **3**:16023.
6. T. Comoglu, S. Arisoy, and Z. B. Akkus, "Nanocarriers for Effective Brain Drug Delivery.," *Curr. Top. Med. Chem.*, vol. 17, no. 13, pp. 1490–1506, 2017, doi: 10.2174/1568026616666161222101355.
7. Du, B., Yu, M. and Zheng, J. (2018). Transport and interactions of nanoparticles in the kidneys. *Nature Reviews Materials*, **3**:358-374.
8. Ewers, H., and Helenius, A. Lipid-mediated endocytosis, *Cold Spring Harb. Perspect. Biol.*, vol. 3, no. 8, pp. 1–14, 2011, doi: 10.1101/Csh Perspect.a004721.
9. F. D'Agata *et al.*, (2018). Magnetic nanoparticles in the central nervous system: Targeting principles, applications and safety issues *Molecules*, 23(1):1–25.
10. Gómez-Vallejo, V., Puigivila, M., Plaza-García, S., Szczupak, B., Piñol, R., Murillo, J. L., Sorribas, V., Lou, G., Veintemillas, S. and Ramos-Cabrer, P. (2018). PEG-copolymer-coated iron oxide nanoparticles that avoid the reticuloendothelial system and act as kidney MRI contrast agents. *Nanoscale*, **10**:14153-14164.
11. Gil, J., Silage, D.A., and McNiff, J.M., Distribution of vesicles in cells of air-blood barrier in the rabbit., *J. Appl. Physiol.*, vol. 50, no. 2, pp. 334–340, Feb. 1981, doi: 10.1152/jappl.1981.50.2.334.
12. Hamblin, "Chapter 8 - Drug efflux pumps in photodynamic therapy," in *Cancer Sensitizing Agents for Chemotherapy*, vol. 7, A. Sosnik and R. B. T.-D. E. P. in C. R. P. F. M. R. and C. to P. I. S. in C. Bendayan, Eds. Academic Press, 2020, pp. 251–276.
13. Hasan, S. (2015). A review on nanoparticles: their synthesis and types. *Res. J. Recent Sci*, **2277**:2502.

14. Heald, R and O. Cohen-Fix, "Morphology and function of membrane-bound organelles," *Curr. Opin. Cell Biol.*, vol. 26, pp. 79–86, 2014, doi: https://doi.org/10.1016/j.ceb.2013.10.006.
15. Ibrahim, K. E., Al-Mutary, M. G., Bakhiet, A. O. and Khan, H. A. (2018). Histopathology of the liver, kidney, and spleen of mice exposed to gold nanoparticles. *Molecules*, **23**:1848.
16. Jain, P. K., El-Sayed, I. H. and El-Sayed, M. A. (2007). Au nanoparticles target cancer. *nano today*, **2**:18-29.
17. Jeevanandam, J., Chan, Y. S. and Danquah, M. K. (2016). Biosynthesis of metal and metal oxide nanoparticles. *ChemBioEng Reviews*, **3**:55-67.
18. Jiang, C., Jiang, Z., Zhu, S., Amulraj, J., Deenadayalan, V. K., Jacob, J. A., & Qian, J. (2021). Biosynthesis of silver nanoparticles and the identification of possible reductants for the assessment of in vitro cytotoxic and in vivo antitumor effects. *Journal of Drug Delivery Science and Technology*, **63:**102444.
19. Jomehzadeh, N., Koolivand, Z., Dahdouh, E., Akbari, A., Zahedi, A., & Chamkouri, N. (2021). Investigating in-vitro antimicrobial activity, biosynthesis, and characterization of silver nanoparticles, zinc oxide nanoparticles, and silver-zinc oxide nanocomposites using Pistacia Atlantica Resin. *Materials Today Communications*, **27:** 102457.
20. Kitching, M., Ramani, M. and Marsili, E. (2015). Fungal biosynthesis of gold nanoparticles: mechanism and scale up. *Microbial biotechnology*, **8**:904-917.
21. Li, X., Xu, H., Chen, Z.-S. and Chen, G. 2011. Biosynthesis of nanoparticles by microorganisms and their applications. *Journal of Nanomaterials*, **2011**.
22. M. Suñé-Pou *et al.*, (2018). Cholesteryl oleate-loaded cationic solid lipid nanoparticles as carriers for efficient gene-silencing therapy. *Int. J. Nanomedicine*, 13:3223–3233.
23. Mohanraj, V. and Chen, Y. (2006). Nanoparticles-a review. *Tropical journal of pharmaceutical research*, **5**:561-573.
24. Mohanraj, V. J., & Chen, Y. (2006). Nanoparticles-a review. *Tropical journal of pharmaceutical research*, **5**(1), 561-573.
25. Nguyen, M. M., Carlini, A. S., Chien, M. P., Sonnenberg, S., Luo, C., Braden, R. L., Osborn, K. G., Li, Y., Gianneschi, N. C. and Christman, K. L. (2015). Enzyme-responsive nanoparticles for targeted accumulation and prolonged retention in heart tissue after myocardial infarction. *Advanced materials*, **27**:5547-5552.
26. Nikam, A. P., Ratnaparkhiand, M. P. and Chaudhari, S. P. (2014). Nanoparticles–an overview. *International Journal of Research and Development in Pharmacy & Life Sciences*, **3**:1121-1127.
27. Park, T. J., Lee, K. G. and Lee, S. Y. (2016). Advances in microbial biosynthesis of metal nanoparticles. *Applied microbiology and biotechnology*, **100**:521-534.
28. Pedroso-Santana, S. and Fleitas-Salazar, N. (2020). Ionotropic gelation method in the synthesis of nanoparticles/microparticles for biomedical purposes. *Polymer International*, **69**:443-447.
29. Petros, R. A. and DeSimone, J. M. (2010). Strategies in the design of nanoparticles for therapeutic applications. *Nature reviews Drug discovery*, **9**:615-627.
30. Rajput, N. (2015). Methods of preparation of nanoparticles-a review. *International Journal of Advances in Engineering & Technology*, *7*(6), 1806.

31. Rajput, N. 2015. Methods of preparation of nanoparticles-a review. *International Journal of Advances in Engineering & Technology*, **7**:1806.
32. Rampino, A., Borgogna, M., Blasi, P., Bellich, B. and Cesàro, A. 2013. Chitosan nanoparticles: Preparation, size evolution and stability. *International journal of pharmaceutics*, **455**:219-228.
33. Rao, J. P. and Geckeler, K. E. (2011). Polymer nanoparticles: Preparation techniques and size-control parameters. *Progress in polymer science*, **36**:887-913.
34. Ruoslahti, E. (2012). Peptides as targeting elements and tissue penetration devices for nanoparticles. *Advanced materials*, **24**:3747-3756.
35. Simkiss, K. "Cell membranes; barriers, regulators and transducers?," *Comp. Biochem. Physiol. A. Mol. Integer. Physiol.*, vol. 120, no. 1, pp. 17–22, May 1998, doi: 10.1016/s1095-6433(98)10004-1.
36. Shang, L., Nienhaus, K. and Nienhaus, G. U. (2014). Engineered nanoparticles interacting with cells: size matters. *Journal of nanobiotechnology*, **12**:1-11.
37. Stampfl, A., Maier, M., Radykewicz, R., Reitmeir, P., Göttlicher, M. and Niessner, R. (2011). Langendorff heart: a model system to study cardiovascular effects of engineered nanoparticles. *ACS nano*, **5**:5345-5353.
38. Stark, W. J., Stoessel, P. R., Wohlleben, W. and Hafner, A. (2015). Industrial applications of nanoparticles. *Chemical Society Reviews*, **44**:5793-5805.
39. Sun, N.f., Liu, Z.a., Huang, W.b., Tian, A.l. and Hu, S.y. (2014). The research of nanoparticles as gene vector for tumor gene therapy. *Critical Reviews in Oncology/Hematology*, **89**:352-357.
40. Tag, H. M., Saddiq, A. A., Alkinani, M., & Hagagy, N. (2021). Biosynthesis of silver nanoparticles using Haloferax sp. NRS1: image analysis, characterization, in vitro thrombolysis and cytotoxicity. *AMB Express*, **11**(1), 1-12.
41. Teleanu, D. M., Chircov, C., Grumezescu, A. M., Volceanov, A. and Teleanu, R. I. (2018). Impact of nanoparticles on brain health: An up to date overview. *Journal of clinical medicine*, **7**:490.
42. Tosi, G., Duskey, J. and Kreuter, J. (2020). Nanoparticles as carriers for drug delivery of macromolecules across the blood-brain barrier. *Expert opinion on drug delivery*, **17**:23-32.
43. Wang, C., Wu, B., Wu, Y., Song, X., Zhang, S. and Liu, Z. (2020). Camouflaging nanoparticles with brain metastatic tumor cell membranes: a new strategy to traverse blood–brain barrier for imaging and therapy of brain tumors. *Advanced Functional Materials*, **30**:1909369.
44. Y. C. Wang, É. Rhéaume, F. Lesage, and A. Kakkar. (2018). Synthetic methodologies to gold nanoshells: An overview. *Molecules*, **23(**11):1–28.
45. Yu, S., Xu, X., Feng, J., Liu, M. and Hu, K. (2019). Chitosan and chitosan coating nanoparticles for the treatment of brain disease. *International journal of pharmaceutics*, **560**:282-293.
46. Z. Yang *et al.*, (2013). A review of nanoparticle functionality and toxicity on the central nervous system. *Nanotechnology, Brain, Futur.* **10:**313–332.
47. Fawzy, N. M., Ahmed, K. M., Abo-Salem, H. M., & Aly, M. S. (2020). New furochromone derivatives as promising in-vitro anti-proliferative agents toward HepG-2 and MCF-7 cell lines with molecular docking studies. *Journal of Heterocyclic Chemistry, 57*(7), 2748-2761.
48. Madhuri, S., & Pandey, G. (2009). Some anticancer medicinal plants of foreign origin. *Current science*, 779-783.

49. Javitt, N. B. (1990). Hep G2 cells as a resource for metabolic studies: lipoprotein, cholesterol, and bile acids. *The FASEB Journal, 4*(2), 161-168.
50. Sarkar, S., & Kotteeswaran, V. (2018). Green synthesis of silver nanoparticles from aqueous leaf extract of Pomegranate (Punica granatum) and their anticancer activity on human cervical cancer cells. *Advances in Natural Sciences: Nanoscience and Nanotechnology, 9*(2), 025014.
51. Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H.-C., & Kahru, A. (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus. *Chemosphere, 71*(7), 1308-1316
52. Akinsiku, A. A., Dare, E. O., Ajanaku, K. O., Ajani, O. O., Olugbuyiro, J. A. O., Siyanbola, T. O., . . . Emetere, M. E. (2018). Modeling and synthesis of Ag and Ag/Ni allied bimetallic nanoparticles by green method: Optical and biological properties. *International journal of biomaterials, 2018*.
53. Guerrero-Pepinosa, N. Y., Cardona-Trujillo, M. C., Garzon-Castano, S. C., Veloza, L. A., & Sepúlveda-Arias, J. C. (2021). Antiproliferative activity of thiazole and oxazole derivatives: A systematic review of in vitro and in vivo studies. *Biomedicine & Pharmacotherapy, 138*, 111495.
54. Gupta, A., Bhardwaj, S. K., Sharma, A. L., & Deep, A. (2019). A graphene electrode functionalized with aminoterephthalic acid for impedimetric immunosensing of Escherichia coli. *Microchimica Acta, 186*(12), 1-7.
55. Alexander, J. W. (2009). History of the medical use of silver. *Surgical infections, 10*(3), 289-292.
56. Anwanwan, D., Singh, S. K., Singh, S., Saikam, V., & Singh, R. (2020). Challenges in liver cancer and possible treatment approaches. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1873*(1), 188314
57. Wintachai, P., Paosen, S., Yupanqui, C. T., & Voravuthikunchai, S. P. (2019). Silver nanoparticles synthesized with Eucalyptus critriodora ethanol leaf extract stimulate antibacterial activity against clinically multidrug-resistant Acinetobacter baumannii isolated from pneumonia patients. *Microbial pathogenesis, 126*, 245-257.
58. Mba, I. E., & Nweze, E. I. (2021). Nanoparticles as therapeutic options for treating multidrug-resistant bacteria: Research progress, challenges, and prospects. *World Journal of Microbiology and Biotechnology, 37*(6), 1-30.
59. JAMILA, N., KHAN, N., KHAN, K., BIBI, N., ULLAH, F., MINHAZ, A., . . . BIBI, H. S. (2022). MONOTROPA HYPOPITYS MEDIATED METAL (Ag, Ni AND Cu) NANOPARTICLES IN MICROBIAL INHIBITION AND MERCURY (II) ION DETECTION. *Pak. J. Bot, 54*(2), 743-750.
60. Palanisamy, N. K., Ferina, N., Amirulhusni, A. N., Mohd-Zain, Z., Hussaini, J., Ping, L. J., & Durairaj, R. (2014). Antibiofilm properties of chemically synthesized silver nanoparticles found against Pseudomonas aeruginosa. *Journal of nanobiotechnology, 12*(1), 1-7.
61. Dance, D., Pearson, A., Seal, D., & Lowes, J. (1987). A hospital outbreak caused by a chlorhexidine and antibiotic-resistant Proteus mirabilis. *Journal of Hospital Infection, 10*(1), 10-16.
62. Saleh, T. H., Hashim, S. T., Malik, S. N., & AL-Rubaii, B. A. L. (2019). Down-regulation of flil gene expression by Ag nanoparticles and TiO2 nanoparticles in pragmatic clinical isolates of Proteus mirabilis and Proteus vulgaris from urinary tract infection. *Nano Biomed. Eng, 11*(4), 321-332.
63. Mintz, K. J., & Leblanc, R. M. (2021). The use of nanotechnology to combat liver cancer: Progress and perspectives. *Biochimica et Biophysica Acta (BBA)-*

Reviews on Cancer, 1876(2), 188621.
64. Arzumanian, V. A., Kiseleva, O. I., & Poverennaya, E. V. (2021). The curious case of the HepG2 cell line: 40 years of expertise. *International Journal of Molecular Sciences, 22*(23), 13135.
65. Donato, M. T., Tolosa, L., & Gómez-Lechón, M. J. (2015). Culture and functional characterization of human hepatoma HepG2 cells. In *Protocols in In Vitro Hepatocyte Research* (pp. 77-93): Springer.
66. Faedmaleki, F., Shirazi, F. H., Salarian, A.-A., Ashtiani, H. A., & Rastegar, H. (2014). Toxicity effect of silver nanoparticles on mice liver primary cell culture and HepG2 cell line. *Iranian journal of pharmaceutical research: IJPR, 13*(1), 235.
67. Senthilraja, P., & Kathiresan, K. (2015). In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast. *Journal of applied pharmaceutical science, 5*(3), 080-084.
68. Felice, D. L., Sun, J., & Liu, R. H. (2009). A modified methylene blue assay for accurate cell counting. *Journal of Functional Foods, 1*(1), 109-118.
69. Liang, C.-C., Park, A. Y., & Guan, J.-L. (2007). In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nature protocols, 2*(2), 329-333.
70. Şen Karaman, D., Ercan, U. K., Bakay, E., Topaloğlu, N., & Rosenholm, J. M. (2020). Evolving technologies and strategies for combating antibacterial resistance in the advent of the postantibiotic era. *Advanced Functional Materials, 30*(15), 1908783.
71. Priya, K., Vijayakumar, M., & Janani, B. (2020). Chitosan-mediated synthesis of biogenic silver nanoparticles (AgNPs), nanoparticle characterisation and in vitro assessment of anticancer activity in human hepatocellular carcinoma HepG2 cells. *International journal of biological macromolecules, 149*, 844-852.
72. Qadir, M. I., Ali, M., & Ibrahim, Z. (2014). Anti-cancer activity of Morus nigra leaves extract. ||| *Bangladesh Journal of Pharmacology, 9*(4), 496-497.
73. Al Edhari, B., Mashreghi, M., Makhdoumi, A., & Darroudi, M. (2021). Antibacterial and antibiofilm efficacy of Ag NPs, Ni NPs and Al2O3 NPs singly and in combination against multidrug-resistant Klebsiella pneumoniae isolates. *Journal of Trace Elements in Medicine and Biology, 68*, 126840.
74. Alavi, M., & Rai, M. (2019). Recent advances in antibacterial applications of metal nanoparticles (MNPs) and metal nanocomposites (MNCs) against multidrug-resistant (MDR) bacteria. *Expert review of anti-infective therapy, 17*(6), 419-428.
75. Gudkov, S. V., Burmistrov, D. E., Serov, D. A., Rebezov, M. B., Semenova, A. A., & Lisitsyn, A. B. (2021). A mini review of antibacterial properties of ZnO nanoparticles. *Frontiers in Physics, 9*, 641481.
76. Daduang, J., Palasap, A., Daduang, S., Boonsiri, P., Suwannalert, P., & Limpaiboon, T. (2015). Gallic acid enhancement of gold nanoparticle anticancer activity in cervical cancer cells. *Asian Pacific Journal of Cancer Prevention, 16*(1), 169-174.
77. Loutfy, S. A., Al-Ansary, N. A., Abdel-Ghani, N. T., Hamed, A. R., Mohamed, M. B., Craik, J. D., . . . Hasanin, M. (2015). Anti-proliferative activities of metallic nanoparticles in an in vitro breast cancer model. *Asian Pacific Journal of Cancer Prevention, 16*(14), 6039-6046.
78. Hussain, N., Adil, M., Mumtaz, M., & Waseem, M. (2022). The biological causes and consequences of COVID-19: ACE I/D polymorphism and in-silico

screening of potential bioactive phytochemicals against COVID-19. *Bioinformatics and Biology Insights*, *16*, 11779322221139061.

79. Hussain, K., Waseem, M., Mumtaz, I., & Riaz, S. (2022). Molecular Characterization of Deciphering Fungal Community Structure in Zea Mays L. and Triticum Aestivum L. *International Journal of Innovations in Science & Technology*, *4*(3), 727-737.
80. Adil, M., Waseem, M., Haider, A. Q., & Hussain, N. (2023). Variations in genomic epidemiology and in-silico screening of potential phytochemicals to cure Monkeypox. *Advancements in Life Sciences*, *10*(2), 161-166.
81. Tahir, M. S. B., Waseem, M., Ullah, M. S., Hussain, N., Kazmi, F., Rasheed, M., & Qammar, A. (2023). The Epidemiological and Clinical Manifestations of COVID-19 Variants in the Punjab Tertiary Care Hospitals. *BioScientific Review*, *5*(1), 29-43.

About The Authors

Miss Aman Qammar: Aman Qamar is doing MS in Molecular Genetics Department Biosciences, Comsat University Islamabad, Pakistan. She has interest in Genetics and Molecular in human biology. She is doing research on ramen spectrometer and oxidative stress level in nano-particles.
E-mail: amanqamar2000@gmail.com, ORCID: 0009-0008-7025-6913

Dr. Nageen Hussain: Dr. Nageen Hussain earned her PhD in Microbiology and Molecular Genetics from University of the Punjab, Lahore-Pakistan. She is an Assistant Professor in the Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore-Pakistan. Her research interests are immunology, genetics, pathology, and environmental ethics. She has published more than 64 research articles in National and International journals. She has written two books; one is on PCR and the other one is on Diabetes.
Email: nageen.mmg@pu.edu.pk, ORCID: 0000-0002-4944-1881

Muhammad Waseem: Muhammad Waseem has completed his Master of Science degree from the Institute of Microbiology and Molecular Genetics at the University of the Punjab in Lahore, Pakistan. He has conducted extensive research in the realm of Genetics and Bioinformatics, having authored numerous published articles. Moreover, he is contemplating delving further into the intricate domain of Genetics.
Email: waseem.mmg.pu@gmail.com, ORCID: 0000-0002-0819-129X

To Cite This Chapter:
Qammar, A., Hussain, N., Waseem, M., (2023). **In vitro biosynthesis of nanoparticles and their potential applications as nanomedicine are subjects of significant academic interest.** In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 86-116). ISRES Publishing

DRUG BASED SYNTHESIZED SILVER NANOPARTICLES AGAINST PATHOGENIC BACTERIA

SHAHNAZ
Yasmeen JUNEJO
Muhammad SAFDAR

Introduction

Pathogenic bacteria are microorganisms that have the ability to cause disease in their hosts. Unlike many bacteria that are harmless or even beneficial, pathogenic bacteria can lead to infections and illnesses in humans, animals, and plants. These bacteria have evolved various mechanisms to evade the host's immune system and establish infections. They can produce toxins, adhere to host tissues, invade cells, and manipulate host cell functions. Pathogenic bacteria are causative agent of various diseases, from minor infections to serious, potentially fatal diseases. Examples of pathogenic bacteria include:

1. ***Escherichia coli (E. coli):*** Certain strains of *Escherichia coli* can lead to gastrointestinal tract illnesses and food poisoning.
2. ***Salmonella*:** Salmonella species are known to cause foodborne illnesses and can lead to symptoms such as diarrhea, fever, and abdominal cramps.
3. ***Staphylococcus aureus:*** This bacterium can cause skin infections, respiratory infections, and more severe conditions such as pneumonia and sepsis.
4. ***Clostridium perfringens:*** This bacterium is associated with food poisoning and can cause gas gangrene in wounds.
5. ***Listeria monocytogenes:*** Listeria can lead to severe infections, particularly in pregnant women, newborns, and individuals with weakened immune systems.
6. ***Vibrio cholerae:*** Responsible for cholera, a waterborne disease characterized by severe diarrhea and dehydration.
7. ***Shigella*:** Causes shigellosis, leading to symptoms like diarrhea, fever, and stomach cramps.
8. ***Bacillus cereus:*** Associated with food poisoning, producing toxins that cause gastrointestinal symptoms.
9. ***Legionella*:** Legionnaires' disease is a severe form of pneumonia that can be caused by *Legionella pneumophila*.
10. ***Campylobacter jejuni:*** Commonly implicated in bacterial gastroenteritis.

Understanding the characteristics and behavior of pathogenic bacteria is crucial for developing effective strategies for prevention, diagnosis, and treatment of bacterial infections. Researchers continually explore new methods, such as advanced nanomaterials, for the rapid and accurate detection of these harmful microorganisms to mitigate the impact of infectious diseases on human health.

Many diseases caused by harmful germs and toxins have a significant detrimental impact on the human body. Among these, pathogenic bacteria play a crucial role as the primary contributors to epidemic infections, posing a contagious threat to

individuals as they can be present in various sources such as food, water, and other biological samples. The swift, selective, highly sensitive, qualitative, and quantitative detection of single or multiple harmful bacteria has been recognized as a challenging undertaking in recent years (Wu et al., 2023). Major pathogenic microorganisms responsible for epidemics include *Clostridium perfringens, Salmonella, Escherichia coli, Vibrio, Shigella, Bacillus cereus, Listeria monocytogenes, Legionella, Staphylococcus aureus,* and *Campylobacter jejuni* (Sharma & Sharma, 2023). The pathogenic bacterium *Escherichia coli (E. coli)* is considered opportunistic, commonly inhabiting the vertebrate gut and increasingly associated with various intestinal and sub-intestinal illnesses. Diverse pathotype groups with distinct pathogenic traits have been identified based on intricate criteria. Whole-genome sequencing has facilitated a population phylogenomic perspective on the origin of virulence, revealing that a small number of *E. coli* lineages, distinguished by gained virulence genes on mobile elements, are responsible for illnesses (Cabezas-Cruz & Bermúdez-Humarán, 2023). The genus Escherichia comprises bacteria present in the intestinal flora of both animals and humans, functioning as symbionts aiding in digestion and vitamin synthesis. *E. coli* serological types, including those linked to illnesses such as meningitis, hemolytic-uremic syndrome, sepsis, and pneumonia (Ilieva et al., 2023).

Staphylococcus aureus, a Gram-positive human commensal, can become pathogenic and negatively impact health and the economy (Pal et al., 2023). Nasal carriage of *S. aureus* poses an increased risk of infection, with a variety of illnesses caused by this bacterium, including osteoarticular infections, surgical site infections, infectious endocarditis, pneumonia, and infections associated with medical devices. *S. aureus* produces a range of virulence factors, including exotoxins, contributing to its effectiveness as a pathogen. Understanding these mechanisms is crucial for managing staphylococcal infections, as toxins produced by *S. aureus* are associated with various illnesses, affecting cell membranes and host immunological responses. The transmission of infections is evident in the close interaction between animals and humans (Pan et al., 2023). Recently it is revealed that some authors explore the remarkable genomic flexibility exhibited by *S. aureus,* it has dual nature as both a commensal and opportunistic pathogen across multiple mammalian species. It also explores our growing understanding of the roles different bacteria play in affecting *S. aureus* colonization (Howden et al., 2023). Therefore, some E. coli strains has got antimicrobial resistance, which are often transmitted between domestic animals and humans, poses challenges of antimicrobial resistance (AMR) genes that is elusive due to the frequent movement of genes from one plasmid to another (Frei et al., 2023).

According to a study, the One Health idea is greatly impacted by *Escherichia coli*, a serious zoonotic bacteria. Even while warm-blooded animals' gastrointestinal tracts usually include *E. coli*, some serotypes can be harmful to both humans and animals. Furthermore, *E. Coli* grows in water, is resilient in a variety of settings, and may survive without the assistance of a host. In order to determine the prevalence of *E. coli,* this study looked at 171 samples from ten different kinds of poultry hatcheries, including automatic, semiautomated, and manual "traditional" hatcheries. Using primers specific to the 16S rRNA gene, polymerase chain reaction (PCR) was utilized to verify the existence of *E. coli* isolates. 62 isolates of *E. coli* were found in the samples that were gathered, constituting a prevalence of 36.3%. Notably, manual "traditional" hatcheries exhibited the highest prevalence at 57.1%, although this difference was not statistically significant (P = 0.243) across the three types of hatcheries. Additionally, there were notable differences in the prevalence of *E. coli*

amongst various bird species and breeds. In hatcheries for ducks and chickens, the prevalence was 35.7% and 37%, respectively. Notable variations were seen across the breeds of both species (P = 0.024 and 0.001, respectively). The discovery of zoonotic strains of *E. Coli* highlights the significance of cooperative endeavors spanning various domains, such as social, environmental, and governance sectors, in promoting the implementation of the One Health concept in the chicken sector. To reduce the chance of *E. coli* spreading from avian sources to humans, the study suggests strengthening bird immunity, conducting routine surveillance, and improving biosecurity protocols at hatcheries and farms (Yousef et al., 2023).

According to a different study, mobile genetic elements (MGEs) have a direct correlation with the development of multidrug resistance in Enterobacteriaceae that produce extended-spectrum β-lactamase (ESBL). This study explores the function of IS26 components and class 1 integrons in bridging taxonomic gaps. 110 *E. Coli* bacteria were isolated from a total of 300 clinical mastitis milk samples; 98% of these bacteria were found to produce extended-spectrum beta-lactamases. Surprisingly, fluoroquinolone co-resistance was seen in 83% of the isolates. A thorough investigation showed that extended-spectrum beta-lactamase and quinolone resistance determining region mutations were present in 76% of isolates concurrently, and that plasmid-mediated quinolone resistance genes were present in 44% of isolates. Notable MGEs were found in 82% of the isolates and 40% of the isolates had class 1 integrase. Three detected class 1 integron gene cassettes were dfrA7, (dfrA17 + aadA5), and (dfrA1 + aadA1). The investigation revealed two unique variations of the dfrA17 gene and four novel variants of the aadA5 gene, which is intriguing. Furthermore, a version of aadA5 with the E235G mutation was discovered in the Indian subcontinent. This variant was previously only known to exist in a clinical isolate from a human from Belgium. Additionally, 19 isolates have IS26 connected to the integrase gene intI1, which has an internal deletion of 265 bp in the 5~CS of intI1. This occurrence has only been seen in *E. coli* ST131 isolates from wastewater and clinical samples from humans in the past. These results highlight the possibility of mobile genetic components facilitating the transcontinental spread of antibiotic-resistant genes (ARGs) throughout various microbiomes (Behera et al., 2023).

In a different work, Safdar et al. created an easy one-pot synthesis technique that was used to make nanoparticles by employing medications as a capping agent in the reduction process as well as a reducing agent. These nanoparticles were specifically chosen for their antimicrobial and anticancer properties. Using the well diffusion procedure, the zones of inhibition against Gram-positive (*S. aureus* and *S. pyogenes*) and Gram-negative (*E. coli* and *S. typhimurium*) bacteria were determined. Using this technique, it was discovered that while newly made nanoparticles had a strong antibacterial activity, traditional antibiotics have an inhibiting effect. Interestingly, we believe that these nanoparticles' remarkable efficiency, low economy, and reprocessing properties will allow them to be widely thrown off as anti-cancerous, antiviral, anti-arthropod, and antiprotozoal mediators in the future (Safdar et al., 2020).

Therefore, recent research has demonstrated significant advancements in the development of antimicrobial materials and treatments with applications in various fields. The utilization of renewable resources, such as castor oil, has been a focal point in creating materials with long-lasting antibacterial properties and robust mechanical strength (Canaparo et al., 2019). Researchers have employed innovative techniques, including electrostatic self-assembly and grafting processes, to enhance the

performance of materials like waterborne polyurethane and gelatin films for applications in food packaging and wound dressings (Klemm et al., 2018). The exploration of composite films from guanidine and waterborne polyurethane revealed exceptional antibacterial capabilities, with rates exceeding 99.9% against common pathogens like S. aureus and E. coli (C. Wang et al., 2020). Additionally, the incorporation of quaternary ammonium groups into gelatin films significantly improved their mechanical characteristics, thermal stability, and antimicrobial properties, showcasing the potential for eco-friendly alternatives in various industries (Rukmanikrishnan et al., 2020). Wound dressing films have been a focus of research, with an emphasis on achieving antibacterial activity, biocompatibility, and durability (Homaeigohar & Boccaccini, 2020). The development of gelatin-based wound dressing films through an easy-to-use and eco-friendly process demonstrated promising bactericidal action against various bacterial strains, presenting a potential solution for effective wound care. Furthermore, studies have explored the antibacterial and antidiarrheal properties of natural extracts, showing significant inhibitory effects against bacterial strains and suggesting its potential therapeutic use (Dubreuil, 2013). However, it is essential to consider potential challenges, such as cytotoxicity associated with higher concentrations of essential oils. Careful consideration of concentration levels is crucial to balance antimicrobial efficacy with safety in practical applications (Junejo et al., 2019).

In summary, these recent studies underscore the continuous efforts to develop sustainable, effective, and safe antimicrobial materials and treatments with broad applications in diverse fields, ranging from healthcare to food packaging. The findings contribute valuable insights to the ongoing pursuit of innovative solutions to address bacterial infections and enhance overall health and safety.

References

1. Wu G, Qiu H, Liu X, Luo P, Wu Y, Shen Y. Nanomaterials-based fluorescent assays for pathogenic bacteria in food-related matrices. Trends in Food Science & Technology. 2023:104214.
2. Sharma K, Sharma M. Optical biosensors for environmental monitoring: Recent advances and future perspectives in bacterial detection. Environmental Research. 2023:116826.
3. Cabezas-Cruz A, Bermúdez-Humarán LG. Exploring the relationship between Faecalibacterium duncaniae and Escherichia coli in inflammatory bowel disease (IBD): insights and implications. Computational and Structural Biotechnology Journal. 2023.
4. Ilieva Y, Zaharieva MM, Dimitrova L, Kaleva MD, Jordanova J, Dimitrova M, et al. Preliminary Data on Escherichia coli, Yersinia enterocolitica, and Other Bacteria, as Well as Absent African Swine Fever Virus in the Gut Microbiota of Wild Mice and Voles from Bulgaria. Microbiology Research. 2023;14(4):1788-819.
5. Pal M, Shuramo MY, Tewari A, Srivastava JP, HD C. Staphylococcus aureus from a Commensal to Zoonotic Pathogen: A Critical Appraisal. 2023.
6. Pan D, Nazareth J, Sze S, Martin CA, Decker J, Fletcher E, et al. Transmission of monkeypox/mpox virus: A narrative review of environmental, viral, host, and population factors in relation to the 2022 international outbreak. Journal of Medical Virology. 2023;95(2):e28534.

7. Howden BP, Giulieri SG, Wong Fok Lung T, Baines SL, Sharkey LK, Lee JY, et al. Staphylococcus aureus host interactions and adaptation. Nature Reviews Microbiology. 2023:1-16.
8. Frei A, Verderosa AD, Elliott AG, Zuegg J, Blaskovich MA. Metals to combat antimicrobial resistance. Nature Reviews Chemistry. 2023;7(3):202-24.
9. Yousef HM, Hashad ME, Osman KM, Alatfeehy NM, Hassan WM, Elebeedy LA, et al. Surveillance of Escherichia coli in different types of chicken and duck hatcheries: one health outlook. Poultry science. 2023;102(12):103108.
10. Behera M, Parmanand, Roshan M, Rajput S, Gautam D, Vats A, et al. Novel aadA5 and dfrA17 variants of class 1 integron in multidrug-resistant Escherichia coli causing bovine mastitis. Applied Microbiology and Biotechnology. 2023;107(1):433-46.
11. Safdar M, Ozaslan M, Khailany RA, Latif S, Junejo Y, Saeed M, et al. Synthesis, characterization and applications of a novel platinum-based nanoparticles: catalytic, antibacterial and cytotoxic studies. Journal of Inorganic and Organometallic Polymers and Materials. 2020;30:2430-9.
12. Canaparo R, Foglietta F, Giuntini F, Della Pepa C, Dosio F, Serpe L. Recent developments in antibacterial therapy: Focus on stimuli-responsive drug-delivery systems and therapeutic nanoparticles. Molecules. 2019;24(10):1991.
13. Klemm D, Cranston ED, Fischer D, Gama M, Kedzior SA, Kralisch D, et al. Nanocellulose as a natural source for groundbreaking applications in materials science: Today's state. Materials Today. 2018;21(7):720-48.
14. Wang C, Mu C, Lin W, Xiao H. Functional-modified polyurethanes for rendering surfaces antimicrobial: An overview. Advances in Colloid and Interface Science. 2020;283:102235.
15. Rukmanikrishnan B, Jo C, Choi S, Ramalingam S, Lee J. Flexible ternary combination of gellan gum, sodium carboxymethyl cellulose, and silicon dioxide nanocomposites fabricated by quaternary ammonium silane: Rheological, thermal, and antimicrobial properties. ACS omega. 2020;5(44):28767-75.
16. Homaeigohar S, Boccaccini AR. Antibacterial biohybrid nanofibers for wound dressings. Acta biomaterialia. 2020;107:25-49.
17. Dubreuil JD. Antibacterial and antidiarrheal activities of plant products against enterotoxinogenic Escherichia coli. Toxins. 2013;5(11):2009-41.
18. Junejo Y, Safdar M, Akhtar MA, Saravanan M, Anwar H, Babar M, et al. Synthesis of tobramycin stabilized silver nanoparticles and its catalytic and antibacterial activity against pathogenic bacteria. Journal of Inorganic and Organometallic Polymers and Materials. 2019;29:111-20.

About The Authors

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkiye. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 68 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.
E-mail: msafdar@cuvas.edu.pk , ORCID: 0000 0002 3720 2090

Yasmeen JUNEJO, PhD, is an Assistant Professor of Physiology and Biochemistry at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. He holds a PhD in Analytical Chemistry from Sindh University, Jamshoro, Pakistan. His main areas of interest are analytical chemistry, nanomedicine, nanostructures, nanobiotechnology and their applications.
E-mail: yasmeen@cuvas.edu.pk, ORCID: 0000 0002 3720 3214

Shahnaz graduated from the Islamia University, Bahawalpur. With a background in Zoology, she brings a wealth of expertise to the scientific community. She has done her postgraduate study in Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur. She worked on nanotechnology and bacteriology field, having research interests in public health related diseases, influenza, waterborne diseases, virology and animal related diseases.
Email: shehnazch205@gmail.com , ORCID: 0009-0003-5448-7567

To Cite This Chapter :
Shahnaz, M, et al. (2023). **Drug based synthesized silver nanoparticles against pathogenic bacteria**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 117-122). ISRES Publishing

REVOLUTIONIZING HEALTHCARE INTEGRATING GENOMICS TO TRANSFORM PRECISION MEDICINE

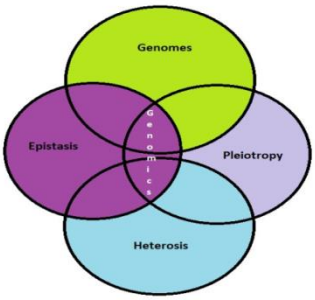
Munaza RASHEED
Muhammad YASİN
Muhammad Moazzam Bin RASHEED
Saqib JAVEED

1. Introduction

1.1. Genomics and Precision Medicine:

Precision medicine, commonly referred to as personalized medicine, is one of the most effective methods for treating illnesses for which a workable medication or cure is not yet available. The study of an organism's entire genome is known as genomic science, which incorporates concepts from genetics. Genomic techniques include recombinant DNA, DNA sequencing techniques, and bioinformatics to sequence, assemble, and study genome structure and function. It considers all of an organism's genetic composition, as opposed to only as opposed to "classical genetics," which only takes into account one gene or one gene product at a time. Moreover, genomics focuses on interactions other than those between loci and alleles within the genome, such as epistasis, pleiotropy, and heterosis (Figure 1). Since Fred Sanger's groundbreaking work and more recent next-generation sequencing technology made full DNA sequences for whole organisms available, genomic science has profited from this (Bentley et al., 2008; Wishart, 2016).

In the 1970s and 1980s, Fred Sanger's group developed techniques for genome mapping, sequencing, data storage, and bioinformatics assessments. The human genome project, a very successful global cooperation that saw the publishing of the whole human genome sequence in 2003, was made possible by the work completed in the 1980s. The speed, volume, and affordability of genome sequencing have all significantly improved as a result of the development of next-generation sequence technology. Additionally, hundreds of life-science databases and projects are now able to help scientific study because to advancements in bioinformatics. These databases include well-organized and stored data that is easy to search, compare, and assess (Bentley et al., 2008; Gauthier et al., 2019).



Fiure 1. Genomics studies the genomes of whole organisms and other intragenomic interactions (Bentley et al., 2008)

Precision medicine, often known as precision health, represents an innovative strategy for comprehending health and illness through individual patient data. This data encompasses medical diagnoses, clinical characteristics (such as disease severity and functional limitations), biological examinations.
The term "precision medicine" has changed over time, therefore its origins are not totally recognized (Phillips, 2020). But Blood transfusions were revolutionized by the early 1900s discovery of blood types, which made it possible to match donor and recipient blood types and avoided complications resulting from incompatible donor and recipient blood (Dance, 2016; Giangrande et al., 2000; R. J. N. Hodson, 2016). One of the earliest medical specialties to use precision medicine to human disease treatment was transfusion medicine. Since then, the field of precision medicine has evolved to include state-of-the-art techniques for therapy, intervention, and diagnosis that are revolutionizing medical practice. Gene therapy is one example of how precision medicine therapies are improving patient quality of life and prolonging life for many deadly diseases. Before the age of two, spinal muscular atrophy (SMA) type I was invariably fatal in neonates. The fact that children with SMA type I treated with gene therapy are living longer and needing far less invasive respiratory assistance has transformed the lives of these patients and their families(Singh & Gupta, 2020). Precision medicine is gaining a lot of support from government agencies, therapeutic financing companies research funding companies and laypeople, which includes politicians and private donors, due to its current success and future promise.

1.2.Genetics, genomics, and precision medicine

The study of genetics and genomics has advanced exponentially since the discovery of DNA by Friedrich Miescher in 1869, and the first characterizations of genetic material by Watson, Crick, and Franklin in 1953 (Dahm, 2008; Watson & Crick, 1953). It has also advanced since the identification of specific genetic mutations for diseases like cystic fibrosis and color vision in the late 1980s, and today we have an incredible understanding of the genetic basis of health and disease. Genes and their roles in inheritance were studied, and the fields of genetics and genomics were born out of the investigation of an individual's genome and the interactions between that genome and the outside world. The precision medicine revolution was significantly aided by these investigations as well. Consequently, the majority of precision medicine's initial focus has been on genetics and genomics. As Grainger (2016) points out, genetics and genomics account for the majority of the precision medicine data that is now available (Grainger et al., 2016). These days, a genome may be read for about US$1000 in less than a day (R. J. N. Hodson, 2016). After more than ten years and an estimated US$3 billion in expenses, the first genome was sequenced in 2001. It's also feasible now that the cost and turnaround time for genetic testing have drastically decreased.

1.3.The “omics” revolution

But the area of precision medicine is quickly expanding to incorporate information not just from genetics and genomes (Peck 2018). Advances in techniques have made it possible to include data from a wide range of other omics sources, including microbiome studies (also known as "microbiomics"), protein ("proteomics"), radiology ("radiomics"), metabolism ("metabolomics"), pharmacology ("pharmacomics"), and others. For this reason, merging data from many domains is often called "multi-omics (Peck & toxicology, 2018)."

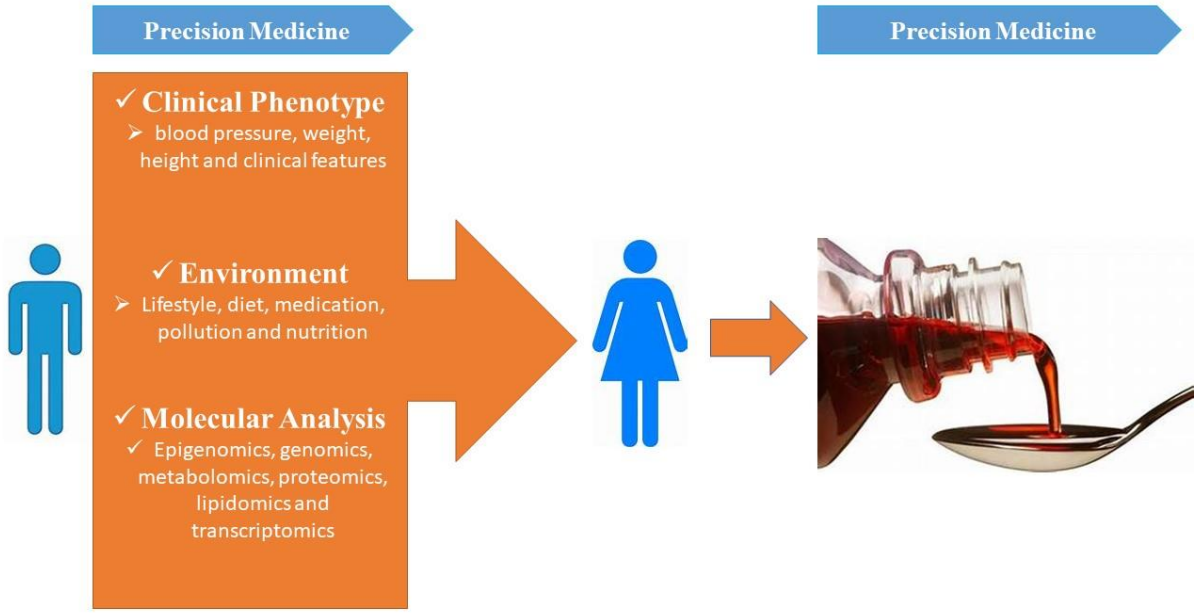


Figure 2. An ambitious challenge for medicine is to guarantee targeted care paths, starting with more tailored strategies. A multilevel approach to treating patients is required to accomplish this goal. The multi-omics approach (transcriptomic, metabolomic, genomic, proteomic, and epigonomics) offers a greater understanding of patient situations at the molecular level, from the underlying causes of diseases to the functional effects. The study of the "exposome," which is defined as the sum of all exposures a person has during their life and the effects those exposures have on their health (Wild CP.), should incorporate thisinformation.The major issue of environmental exposure quantification in molecular epidemiology is to "exposome" the genome. 2005; (Cancer Epidemiol Biomarkers Prev). Doctors can develop a personalised therapy that is catered to the specific patient by studying the clinical characteristics of the patient (Wild & Prevention, 2005).

1.4.Evolution of health care: the need for transformation

At the beginning of twenty century, the growing fields of medical changed their thought due to experimental work. The germ theory's creation, which blamed microbes for many then-common ailments, was particularly influential. But until a few decades later, when the establishment of academic medical centres made it possible for a scientific-based strategy and the first significant revolution of medical practise, the therapeutic occupation did not easily integrate science into practice.
Medicine has been significantly impacted by science. The advantages of the reductionist approach, which condenses the idea of pathogenesis to the fewest feasible causative components, are illustrated by the developing molecular understanding of human diseases. There are advance techniques available for disease treatment due to modern research. However, our healthcare system also demonstrates the drawbacks of the reductionist scientific approach, with complicated chronic illnesses accounting for the majority of health care costs. We have created a strategy that prioritizes urgent care over preventing chronic illnesses (Danchin, 2022).

As we've observed, the learning of basic living processes is strongly tied to development and, more specifically, to the very beginning of life, which is the starting point of evolution. Along with the things, genes, and proteins related to life, we have

talked about the universal metabolic, information-transfer, and compartmentalization processes. Also alluded to are the abstract functions necessary for life. For these abstract processes to be included in physics and chemistry, we require a comparable, real-world framework. This hypothetical situation updates and gathers previous concepts (Danchin, 2022).

According to Berwick, Whittington, and Nolan (2008) Blumenthal and, Buntin, Jain (2010), Better treatment, improved health, and cost savings are the three goals that health information technology, or health IT, is meant to help achieve. Electronic health records, or EHRs, have not gained widespread adoption despite the potential advantages of health information technology (Berwick et al., 2008) . Only 8% of hospitals and 13% of doctors practicing in ambulatory settings in 2008 have at least a basic HER in place. Many are worried about a "digital divide" in health IT access among underprivileged and rural populations since, according to Mostashari, Tripathi, and Kendall (2009), EHR adoption rates have historically been lower in small practices and critical access hospitals (CAHs) (Mostashari et al., 2009).

The HITECH Act of 2009 encouraged the use of health IT by providing financial incentives and technical help for HER adoption and meaningful use (MU) as well as the exchange of health information (Blumenthal 2010). The Secretary of Health and Human Services gave the Office of the National Coordinator (ONC) $2 billion under HITECH and authorized ONC to launch a health IT initiative at the Regional Extension Center (REC). In order to increase the standard and effectiveness of the healthcare delivery system, the $720 million REC program was created to offer support and advice on best practices to accelerate the deployment of HER technology and maximize its usage. The primary objective of the REC program was to help at least 100,000 providers overcome the organizational and technological challenges they faced in order to maximize the use of health IT. As per Maxson et al. (2010), the principal responsibilities of the implementation were requirements analysis, product selection, and support for project management about the HER system's deployment. In small, underfunded medical practices in particular, significant outreach could be required in addition to ongoing technical support, practice coaching, and practice facilitation. various practices have various needs, particularly when it comes to employing health information technology for quality improvement, as demonstrated by two previous extension projects that promoted the use of HER in ambulatory care (Blumenthal, 2010; Maxson et al., 2010; Mostashari et al., 2009).

Practices that had already implemented EHRs were also eligible for REC help with optimization, since many practices lack the resources to manage the business and practice adjustments required to enhance care through health IT (Crabtree et al. 2011, p. Practice coaching attempts to "train the trainer" and assist practices and CAHs in increasing their capacity for learning and change management, hence reducing disruption to and fatigue from practices (Crabtree et al., 2011). Engage in coaching and facilitating activities, according to Nagykaldi, Mould, and Aspy (2008) assist practitioners in increasing patient care and implementing evidence-based recommendations (Mold et al., 2008).

Table 1. The traditional medical record compared with the prospective approach

Traditional Medical Evaluation and Record	Prospective Evaluation and Record
• Chief Complaint • History of illness • Past Medical History • Family History • Medical History • Physical Exam • Diagnostic Test • Assessment and Plan	• Health Profile Summary • Current Health Status • Health Risk Analysis (Genetic, Environmantal and Lifestyle) • Disease Susceptibilities • 1 year Health plan • 5 year Health plan

Traditional Medical Evaluation and Record	Prospective Evaluation and Record
• Chief Complaint	• Health Profile Summary
• History of illness	• Current Health Status
• Past Medical History	• Health Risk Analysis (Genetic, Environmantal and Lifestyle)
• Family History	
• Medical History	• Disease Susceptibilities
• Physical Exam	• Disease Susceptibilities
• Diagnostic Test	• 5 year Health plan
• Assessment and Plan	• 1-year Health plan

1.5.Genomics unveiling the blueprint of life

Nearly two decades ago, the first map of the human genome was published by scientists, and it was heralded as a breakthrough. But it was lacking because only 8% of human DNA had been sequenced. All of an organism's genetic material is collectively referred to as its genome. The majority of the human genome is shared by all people, although some of the DNA differs from person to person. In order to better

understand the differences that might be the cause of diseases, scientists would benefit from building a full human genome. Between 1990 and 2003, an international partnership, In 2003, the genetic sequence from the Human Genome Project that contained data from the euchromatin region of the human genome—where the DNA encodes for protein and the chromosome is dense with genes—became publicly available.

Mineral matter and living stuff have traditionally been seen as distinct from one another. Stones were immovable, In contrast to mobile life, which was linked to some sort of cyclical development and decay, much like the cycles of day and night (Burge, 1898) permanent life was sluggish to weather. Fast silver serves as a demonstration of the mobility of gases and liquids, which exist in the region between these two states. Living beings fall between solids and gasses, comprising all four "elements," in accordance with a cosmogony similar to that of Anaximenes, which is based on the reciprocal modification of the elements (Earth, Water, Air, and Fire) (Guthrie, 1978). Thus life has always been understood to have a source of animation that demonstrated it was more than simply a simple form of stuff. The theory that life originated from the combination of the four elements in specific proportions based on local forms was held by early physicists and atomists. Lavoisier, who was executed by guillotine, was among those who coined the term "biology," but Lamarck ignored his contributions in favor of concentrating on the inner fire. Schwann, Schleiden, and Virchow to look for the atom of life, which they found in the cell. The atom of life, which was discovered in the cell, attracted the attention of Schwann, Schleiden, and Virchow as a result of the rebirth of atomism. Partitioning must thus be the very earliest, most essential, and universal process of life. To be more precise, there are two basic methods in which living things have evolved to deal with compartmentalization: either they have adapted to live as single cells with a more or less complicated envelope, or they have replicated membranes and skins in more complex animals. The advancement of chemistry allowed for the understanding that the inner fire that gave organisms life—and even made some of them warm—was caused. Lamarck (1809) spoke of an active force that propelled life's evolution. Lavoisier (1789) had noted that respiration produced a specified amount of air fixe (our carbon dioxide) and required air vital (oxygen). Studies and experiments with microbes and plants, particularly those involving fermentation, have shown that a second process, metabolism, is essential for the formation of life. Without metabolism, no living thing could exist. It was possible to see dormancy, an intermediate stage present in seeds and spores, but germination was necessary to show that life had begun. But life is not all there is. In actuality, qualities are passed on from parents to offspring of living organisms, at least those that have perpetuated their species (Burkhardt Jr, 2013). Additionally, the person develops into an adult form from some kind of egg or cell as though a function of the adult has already been carried out in one of the parent. Many explanations have been offered for this fact, but it was not until recently—with the emergence of the concept of information and the model of the genetic program that goes along with it—that it became clear that what is passed down through the generations is not a preformed organism but rather a sequence of operations that allow the organism to be constructed. For information transfer to be effective, it must be well structured. It has frequently been observed that these processes are so difficult to reconcile with one another that physicist Freeman Dyson (1988) even claimed that life began twice! Because molecular biology solely takes into account information transfer involving molecules, it is understandable why metabolism and compartmentalization are major issues that all models of life should address yet are frequently ignored by molecular

biology (Dyson, 1988). In summary, moving forward, we will consider living organisms to be analogous to a computer powered by metabolic processes that can understand and execute a genetic program (which may be inherited from previous generations). The genetic code is contained, at least in part, in the genome. The cell may be compared to a Turing machine, and Archaea, Bacteria, and Eukarya are comparable to different operating systems (Danchin, 2004–2005). Since Turing computers, by nature, cannot create other Turing machines, we need to find additional principles in order to expand the metaphor. If such a machine were to reproduce itself, according to John von Neumann's 1966 idea, there would be an internal picture of the equipment that was likewise connected (Danchin, 2008).

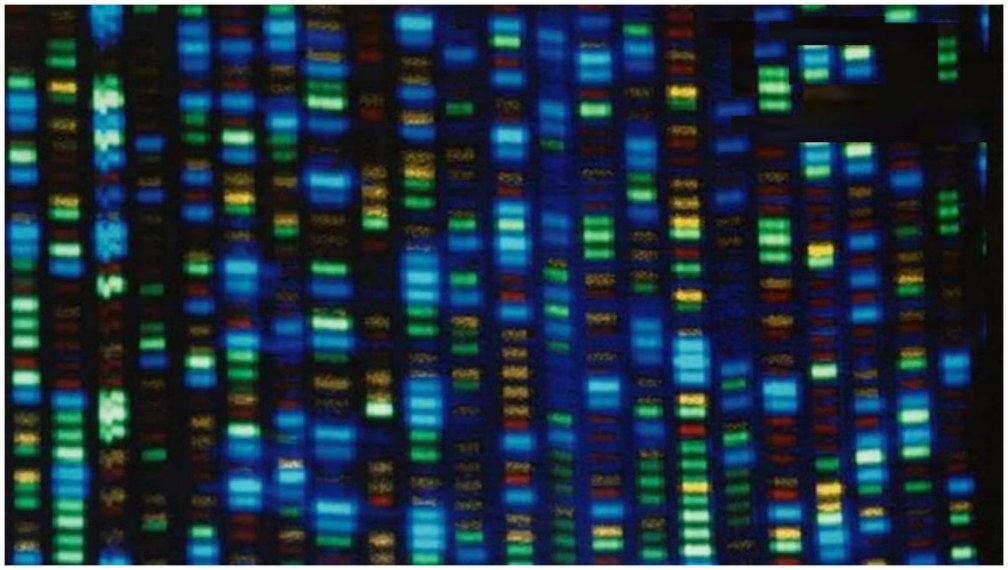


Figure 3. Human life genetic blue print (Andrade et al., 2003)

The Telomere-2-Telomere (T2T) project, which used novel DNA sequencing techniques and computational analysis to finish reading 8% of the genome, is responsible for producing the fully sequenced genome.

The many colours in the new pangenome reference illustrate the numerous directions a sequence could go in: The colour yellow represents a repetition variant, the colour pink an inversion variant, the colours green and blue a deletion variant, and the colour light blue an insertion.

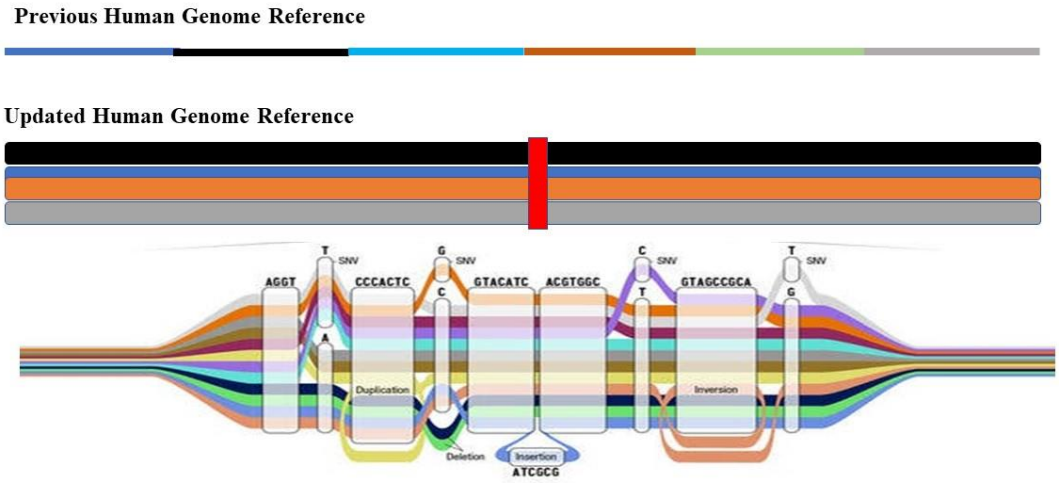


Figure 4. The new pangenome reference shows many possible routes for a sequence to take, represented by the different colors (Liao et al., 2023)

Pink is an inversion variant, green and blue are deletion variants, light blue is an insertion variant, and yellow is a duplication variant.

1.6.Precision medicine tailoring treatments to individual genomes

The twenty-first century medical field will be significantly impacted by the genome project, both in terms of diagnosis and treatment. Finding the genes that predispose people to disease will likely be the most crucial aspect of DNA diagnostics. Nevertheless, some of these conditions, including neurological, cardiovascular, and autoimmune disorders, are polygenic, indicating that two or more genes play a role in their development. Human genetic mapping will enable the identification of specific predisposing genes, and DNA diagnostics will provide the examination of these genes in a broad population. Twenty years from now, we may be able to take a baby's DNA and test fifty or more genes for allelic variations that might predispose the kid to a host of common illnesses [notice that he made this statement in 1992]. For every broken gene, there will be a therapy strategy that works around the gene's limitations. As a result, the approach to medical practice will change from being reactive to preventative. Thanks to preventative medicine, the majority of individuals ought should be able to live normal, disease-free lives without experiencing mental impairment. comprises over 20,000 coding sequences. is optimistic despite this: "I believe we'll see a shift from reactive to proactive treatment." P4 medicine is very participatory, preventive, and individualized. Individual genomes will be standard for medical records in 10 years or so, and we will be able to make inferences [about an individual's health] when paired with phenotypic data. Then, in ways we have never done before, we may begin to create personalized health care programs. We will get substantial and fundamentally new insights into predictive medicine when we compare patient data with individual genotype phenotype correlations in 10 years, when we have billions of data points on each patient. Medical research will evolve into a data-driven science (Wishart, 2016).

1.7.Personal genomics

Genetic testing are offered to certain groups by a multitude of governments and health agencies. These examinations include heel-prick testing for young children and tests for couples who are very likely to have children who have serious genetic diseases. It is the responsibility of medical professionals and public health officials to choose appropriate groups for the testing, get access to the tests, and interpret the results.

However, companies began to commercially sell genetic tests to individuals directly approximately 10 years ago. People can learn about their risk factors for a number of complicated diseases, such as diabetes, cancer, and even neurodegenerative disorders like Parkinson's disease, with these tests, which are usually based on a genetic analysis of a saliva sample mailed to the patient. For a number of reasons, these firms have come under fire; one of the main ones being that people believe they are offering medical advice by sharing information that is relevant to health. In the fine print on their websites, all of the firms state that using the information to make medical decisions is not advised. This is probably partly because of this, as well as the firms' encouragement to avoid lawsuits. However, this is not true.The following instances are provided in the Nuffield Report: 23 and find out whether you have Tay-Sachs, cystic fibrosis, or breast cancer as inheritable indicators. Find out which diseases, such as type 2 diabetes and Parkinson's disease, you are more likely to develop genetically. Predictability is frequently poor, and variations in an individual's risk level above or below the population's are negligible and have little bearing on health.However, people can mistakenly think that their degree of risk is higher or more definite than it actually is because it could be so difficult to explain and understand risk projections. Another indication of inaccuracy is the large variation in the test providers' results. Some consumers have seen fairly startling differences in the risk assessments provided after sending samples to numerous firms. Third, there is a lack of utility: even in cases where the risk assessment is reliable and correct, possible modes of action are nearly never accessible. Thus, the best counsel that can be offered to the concerned person is usually useless and scarcely "empowering": keep up a good diet, live a healthy lifestyle, refrain from smoking and binge drinking, etc (Singh & Gupta, 2020).

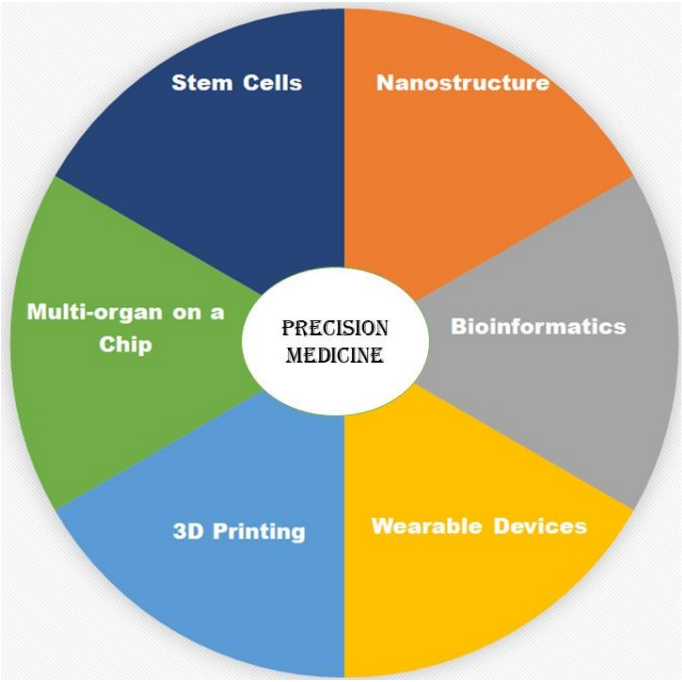


Figure 5. Biomaterials engineering, cell engineering, organs-on-chips, customized implants, and personalized devices, as well as genomics-based methods, enable precision medicine(Moysidou et al., 2021).

1.8.Genomics technologies: Empowering precision medicine

One such industry that gains from AI is healthcare. Medical professionals have always had oversight over this area. The potential for individualized care has increased due

to developments in machine learning and artificial intelligence, cloud computing's large data storage capacity, and the integration of health data. A Syrjala (2018). Using natural language processing (NLP), data may be automatically collected and condensed from paper-based or digital medical records (Syrjala et al., 2018; J. Wang et al., 2017). In the identification of melanoma, several ocular abnormalities, and metastatic breast cancer, deep learning has outperformed experienced pathologists and dermatologists (De Fauw et al., 2018). Additionally, according to Cohen et al. (2018), artificial intelligence is helping to progress pharmacogenomics and liquid biopsies, which will transform cancer screening and monitoring and enhance the ability to forecast unfavorable occurrences and patient outcomes. Significant progress has been made in the use of AI in areas such as CRISPR gene editing and drug development (Cohen et al., 2018). AI-powered services, such as those that track a person's health and offer suggestions to improve wellbeing, are starting to appear in the market. These services make use of mobile devices and the internet of things (IoT). The availability of genome-wide association study (GWAS) genotype-phenotype data and multi-omics data and cancer genomics literature mining have all aided in the creation of state-of-the-art AI methods and tools that enable healthcare providers to deliver personalized treatment through precision medicine (J. C. Wu et al., 2019).

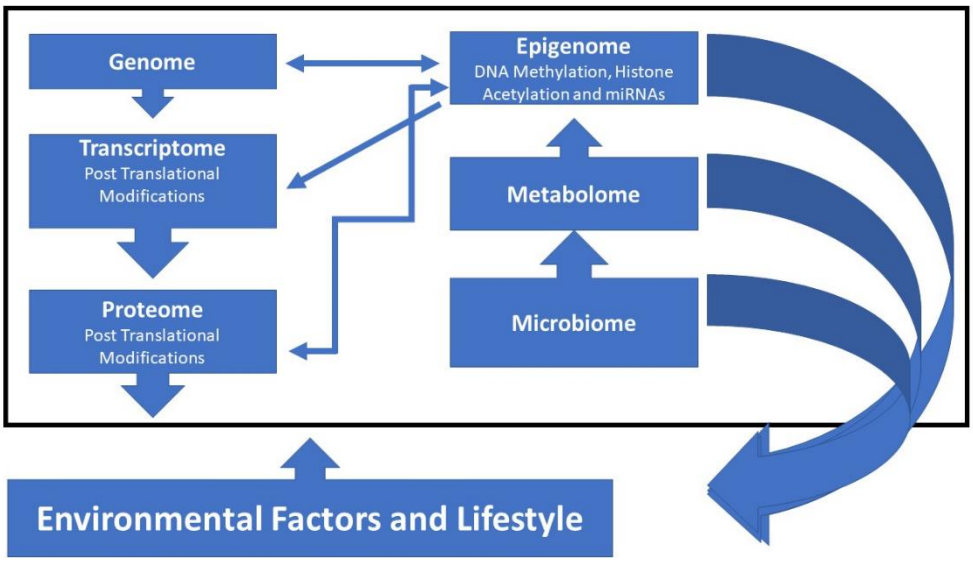
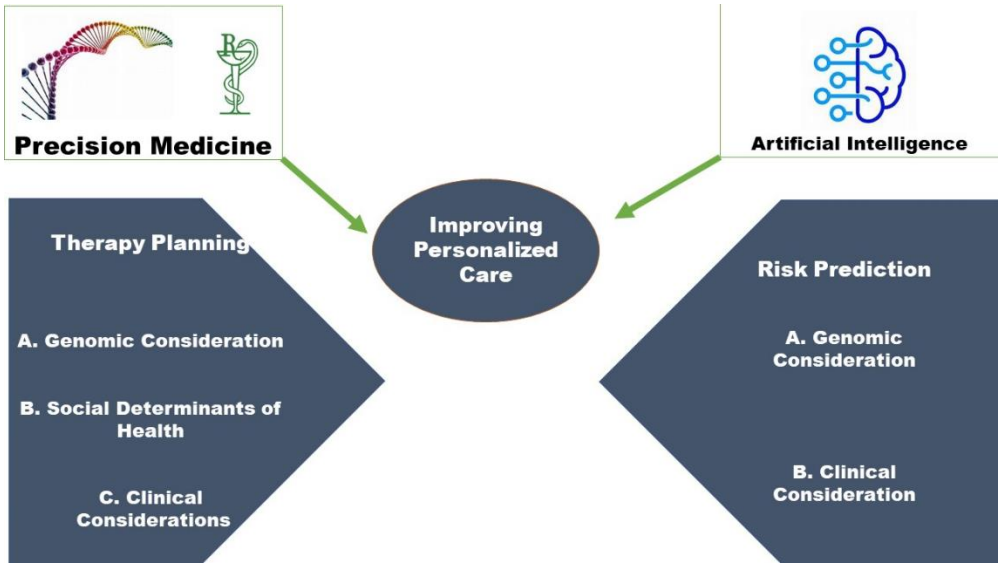


Figure 6. Factors affecting Genomics signature (Katrib et al., 2016).

"Precision medicine" is a revolutionary approach to sickness prevention and treatment that focuses on each patient's unique genetic makeup, environment, and lifestyle choices. AI systems are advancing the disciplines of personalised medicine, especially cancer genomics(Kalinin et al., 2018). Individual drug-response variability may be identified by AI systems, and they can also suggest treatments based on patterns found in vast quantities of public and private data. This study will concentrate on the ways in which AI-based techniques and applications, which are now being employed in the field of cancer genomics, are influencing precision oncology.

The development of new biomarkers, such as mutational marks and tumor mutational problem (TMB), is aided by NGS. Through statistical analysis of the millions of mutations found by NGS, patterns are found.



Figüre 7. Dimensions of AI and precision medicine's interaction (Johnson, Wei, Weeraratne, Frisse, Misulis, Rhee, Zhao, Snowdon, et al., 2021).

Five factors have an impact on the goal of individualised treatment, including the planning of therapy using clinical, genomic, social and behavioral, as well as genetic or other factors, risk prediction or diagnosis.

1.9.Integrating genomics to into clinical practice

The particular disease susceptibilities and treatment reactions of each person depend on their own genetic makeup. This idea serves as the foundation for the emerging field of genomic medicine. In this field, pre-symptomatic health risks are identified and managed, existing disorders are more easily diagnosed, prognostic predictions are improved, and treatment options are guided by the knowledge gained from studying an individual's genomic variants. Rapid developments in DNA sequencing and bioinformatics have made it possible to use genomic medicine in clinical settings.

Genomic data is being used more frequently in therapeutic treatment. Genomic information is durable, and since we know more now than ever before, it can be applied over the course of a patient's life. As a result, Clinicians and patients must embrace genetics for healthcare to improve and recognize when the information might be helpful. Clinicians without a background in genetics frequently claim they are ill-equipped to treat patients using genomic data (Mikat-Stevens et al., 2015).

1.10. Storage of Genomic Data

The ability of modern HER systems to store and process genetic data in clinical practise will be put to the test. Current HER systems cannot handle the potential volume of a complete genetic sequence, as opposed to a small number of genotypes. The auxiliary genetic system is one remedy for this issue (Starren et al., 2013). An auxiliary genomic system can provide federated storage choices, much like an image archiving system that are suited for the diversity and volume of genetic data and conclusions. Extensible Markup Language (XML) files comprising recognized variations as part of a custom panel, star alleles for results of pharmacogenetic testing, and other data from several laboratories may be sent to an institution for the same patient. or even files that contain enhanced sequencing data in Variant Call Format

(VCF). To enable quick retrieval of these data, which range in size from bytes to gigabytes, specific indexing techniques must be used. These data may be utilized to give synthesis deeper perspectives into the outcomes of genomic tests and the ancillary data using an auxiliary genomic system that carries out specific processing and is connected to the HER. At three eMERGE facilities, a genomic auxiliary system has been developed and put to use. At Northwestern University, a prototype auxiliary genomic system for pharmacogenomic testing and reporting was built. The Mayo Clinic also followed similar procedures to create a genomic data warehouse. A distributed system was developed by Partners HealthCare to manage genetic testing for certain objectives (Horton et al., 2017).

1.11. Genomic data analysis: extracting insights for precision medicine

Precision medicine begins with data mining. High-throughput screening biotechnologies generate the data by exploring all facets of complex biological systems in an attempt to discover new information. The extraction of relevant data still presents a significant challenge, despite the discipline's integration of several scientific fields, including medicine, biology, biochemistry, computer science, mathematics, physics, and engineering. This is because the underlying problems we are attempting to address are complex, and the volume, heterogeneity, and many temporal and geographical aspects of the data make it difficult to extract relevant information. Because of this, a significant obstacle facing precision medicine in the future is determining how to transform enormous amounts of data into fresh knowledge that can be specifically applied to each individual (Starren et al., 2013).

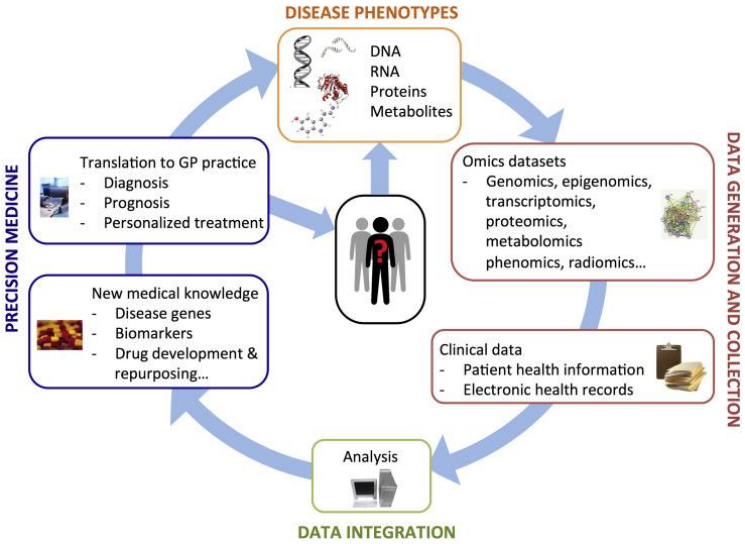


Figure 8. The generation and collection of omics data, which includes information about DNA (genomics), mRNA (expression and coexpression), proteins (proteomics), metabolites (metabolomics), among many other things, can reveal the underlying molecular mechanisms of a person's disease state (disease phenotypes) (Hedayat & Valeri, 2020).

It is possible to provide individualized therapies and treatments based on each person's particular biology. A paradigm shift towards a new medical model called "Precision Medicine" has recently occurred in the healthcare industry as an enhancement of personalised medicine. The new clinical system model focuses on a patient's genes, microbiota, environment, family history, and lifestyle to select accurate diagnostic and therapeutic approaches for each patient.

1.12. Ethical considerations in genomics and precision medicine

The existing public health paradigm of post-diagnostic management is unsustainable despite the best efforts of medical professionals to treat human ailments like cancer or chronic diseases. The majority of clinical units already struggle to provide the best treatment possible to treat patients who have recently been diagnosed.

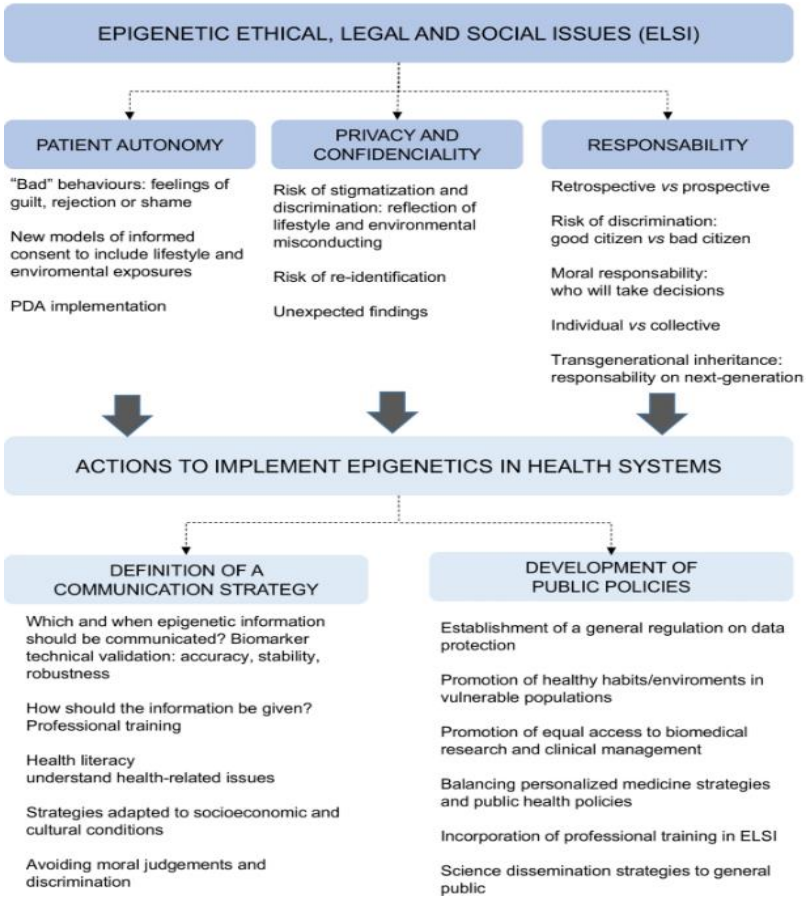


Figure 9. Epigenetic-based biomarkers' usage in the treatment of human illnesses is fraught with ethical, legal, and social problems (ELSI) (Santaló & Berdasco, 2022).

Nine programming areas, including PM, have been highlighted by the World Economic Forum's Center for the Fourth Industrial Revolution. The National Academies of Sciences, Engineering, and Medicine (2018) reports that over 40 nations have contributed to PM projects. For instance, the All of US program, launched by the National Institutes of Health (NIH) in the US, intends to register one million individuals to contribute genetic samples and data for scientific purposes. According to Cyranoski (2016), the China Precision Medicine Initiative will receive $9.2 billion USD in funding over a 15-year period (Cyranoski, 2016).

Precision medicine (PM) is now being studied in the multiethnic setting of Singapore through the SG10k program and the SingHealth Duke-NUS Institute of PRecISion Medicine (PRISM) (J. C. Wu et al., 2019). This work will be extended beginning in 2021 as part of the National Precision Medicine project. Because Asian genomes are underrepresented in global databases (Bentley et al., 2008), this effort will contribute to increasing genetic diversity and may lead to the development of more specialized treatment techniques appropriate for Asian populations. According to Popejoy and Fullerton (2016), just 14% of the 2511 genome-wide association studies that have

been carried out as of 2016 included people of Asian ancestry. UN projections for 2019 indicate, however, that 60% of the the globe (Popejoy & Fullerton, 2016).
While "personalised medicine" was first used by the Human Genome Project, the phrase "precision medicine" has gained traction over time. The term "personalised medicine" was changed to address concerns that it did not adequately describe the novel approaches driven by genome data and that it could lead to unrealistic expectations for personalised treatments and drugs instead of focusing on developing therapies for disease subtypes largely identified by genomics. Still, in certain systems (like those in the European Union), the phrase "personalised medicine" is used. In contrast, some programs refer to the same idea generally under either label. According to Erikainen and Chan (2019), these phrases reflect overlapping future visions of healthcare and medicine that are influenced by data-intensive biomedical research in different ways. Some authors claim that varied language indicates a preference for distinct goals, values, and conceptual frameworks. Through thorough understanding of the social, cultural, and ethical ramifications of these terms, researchers and decision-makers may build reliable connections with participants that are built on open dialogue and transparency. If the general public and study participants have access to science-related materials that will support the formation of critical understandings, they will be able to engage with significant topics more effectively. The "deficit model," which views public communications as a one-way flow of information from scientists to the general public, should not, however, be automatically adopted by researchers. This is because ethical norms have a complex role in both policy-making and scientific communication (Erikainen et al., 2019).

1.13. Challenges and opportunities in the integration of genomic

From $100 million in 2001 to $1,000 in 2014, the price of sequencing has decreased thanks to the introduction of NGS. The medical community now has greater access to sequencing for diagnostic support because to the lower cost. Sequencing has an advantage over other methods for characterizing nucleic acids, such as PCR and microarrays, in that it can produce a wide range of data kinds. For DNA, RNA, and bisulfite sequencing, conventional NGS platforms like the Illumina HiSeq sequencer are frequently utilized. These platforms' short reads have alternatives thanks to recent developments in sequencing techniques. Combining patient genome, transcriptome, and DNA methylome analyses can help with prognostic classifications and diagnosis.

One of the trickiest computational issues to date is short read alignment (SRA), which is essential to the modern bioinformatics pipeline in the study of genomics (Lightbody et al., 2019). Read alignment, which is essentially a string matching issue, is quite complex due to the substantial amount of raw genomic data. The vastness of the human genome is demonstrated by the fact that short read lengths often span between 100 and 300 characters (Sboner, 2011). According to McVicar et al. (2016), a search frequently encompasses the whole reference genome for every read, leading to billions of searches.Many-core processing (McVicar et al., 2016) pre-alignment filters, they have all been used in previous attempts to boost read alignment throughput.Short read alignment's computational difficulties. They states that downstream analysis and experimental design are now seen to be the main areas of concern in computing, as opposed to fifteen years ago when the main bottlenecks were associated with gene sequencing.

1.14. Alignment challenges

Muir et al. (2018) state that the prevailing alignment paradigm, paired sequence alignment, compromises speed and power in order to generate flawless exhaustive alignments via global or local alignment. While both alignments are correct, quadratic complexity—a phenomenon in which processing time grows exponentially as data input rises linearly—means that pragmatic short read alignment cannot be used to map sequences to large reference genomes (Muir & Vander Heiden, 2018).

Banerjee et al. (2021) state that between 50% and 70% of the total time is usually needed to compute the edit distance during short read alignment. To more clearly show this, have a look at the 51 edit distance calls per read of the SRA algorithm SNAP. Considerable investigation into index techniques has contributed to reducing the quantity of candidate sites requiring computation. This has led to the development of many alignment algorithms to pre-filter alignment candidate sites before performing Levenshtein distance computations, in an effort to reduce the search space (Banerjee et al., 2021).

Defining stakeholder roles at any or all phases of policy or guideline creation is essential for accurately evaluating and understanding the policy-making process. There is no one-size-fits-all, ideal method for doing this. A number of frameworks, some of which expressly target important stakeholder consultation, have been established in a number of sectors to help with the planning, development, and analysis of policy.

1.15.Case studies: success stories in genomics driven precision medicine

Conversational chatbots to scale genetic counseling (GC) services, digital delivery of complete genetic services, and digital genomics decision support systems are three use cases that demonstrate the immense potential of these converging technologies. The use cases presented here highlight state-of-the-art technological innovations created to address clinical needs and challenges that occur throughout the genomics continuum of care. These include digital decision aids, chatbots, and digital portals, as well as pre- and post-test counseling and the full service delivery of genomics.

Offering patient-centered choice support for genetic testing is one of the primary responsibilities of genomic counseling, and decision support tools help to do this.One of the main strategic aims and objectives of the next ten years for many pharmaceutical companies, biotech companies, university medical institutions, and the National Institutes of Health is personalized medicine, or more recently, precision medicine (Khoury et al., 2012).
Human genetics has benefited greatly from pharmacogenetic and pharmacogenomics research. The promise of translational research to move genetic linkages from "bench to bedside" for several drugs and drug-related features has been realized (detailed in this article). It is obvious that pharmacogenomics has advanced with astounding efficacy in the GWAS era, when thousands of genetic links for hundreds of phenotypes are discovered.

1.16.*Future Directions: Advancements and Genomics in Healthcare*

The cost of genome sequencing has dropped dramatically, and the number of firms offering genetic diagnostics has sharply increased, which has hastened the development of personalized medicine.Direct-to-consumer companies, according to

Khan and Mittelman (2018), are quickly providing people with access to genomics, which is changing how doctors and patients see genetic testing. The genetic testing industry is expected to reach $22 billion globally by 2024 (https://www.gminsights.com/pressrelease/ genetic-testing-market). Future developments in genomic technology are predicted to have a big influence on the field of genetic testing (Khan & Mittelman, 2018). There are more and more pilot studies using whole-genome sequencing (WGS), even though panels or exomes are still used in the majority of clinical sequencing today, It might allow for the comprehensive examination of all genomic variations. 100,000 individuals with rare diseases or cancer and their families have had their genomes sequenced by Genomics England and the National Health Service (NHS) England, demonstrating the effective large-scale deployment of WGS in direct healthcare. Fast WGS has been demonstrated to reduce morbidity and hospitalization expenses for critically unwell babies admitted as inpatients in the US. These are but a handful of the issues that still need to be resolved, including cost, clinical interpretation, and data storage in health systems, despite the fact that there is much more to learn about the therapeutic potential of WGS in clinical settings. The cost of a number of long-read sequencing methods is currently declining (e.g., PacBio, 10X Genomics), and these methods promise to help us better understand the structure and order of genomic rearrangement—a structure that is challenging to detect with next-generation technology. The diagnostic yield for genetic illnesses may rise if these technologies are able to make a successful transition into the therapeutic market. There will be more technical advancements as the scientific community strives to build expansive, open-access databases of population-scale variations, multiethnic reference genomes, and expanding clinical genetic databases (S. J. Aronson & H. L. J. N. Rehm, 2015).We still anticipate difficulties with genetic testing's interpretation, comprehension, and communication, despite the fact that it is becoming increasingly accurate and dependable. It is possible that genetics will lead to the discovery of new medical pathways, such as the ability to predict lifetime risk for particular diseases. However, predicting the genomic profiles of complex illnesses is more difficult and likely less beneficial therapeutically because these diseases may have multiple underlying genetic and environmental factors (Manolio et al., 2009). However, advances from very large GWASs have made polygenic risk scores (PRSs) increasingly accurate, incorporating a large number of genetic variants into a single test for prediction. Recent studies (Khera et al., 2018) have shown that PRSs can predict several complex diseases, like coronary artery disease and breast cancer, with an accuracy comparable to testing for those diseases' u. It is expected that these assessments will become more accurate as GWASs advance. PRSs not only provide information about lifestyle and family history, but they may also be used to identify high-risk persons in the general population for a variety of ailments. Even though it's unclear how effectively PRSs would function in real therapeutic settings, there are a number of obstacles to overcome before these results may be used to clinical therapy. Many clinical trials are currently being conducted to determine how PRSs affect the treatment of some complicated conditions, including as breast cancer (https://clinicaltrials.gov/ct2/show/NCT03688204). However, mounting data indicates that PRS might not be very effective at dispersing throughout communities (Martin & Wang, 2019). European folks fare better than other ethnicities in the early PRS applications. European populations do better than other groups in early PRS applications in terms of prediction accuracy (Martin & Wang, 2019). The existing health inequalities may worsen as a result of this discrimination. The development of predictive analytics for complex illnesses that offers fair clinical value for all populations is necessary to advance personalized medicine in an ethical manner.

Because they can store enormous volumes of genetic data and effectively incorporate pertinent genomic information into clinical treatment, electronic health records will play a more significant role in the adoption of personalized medicine. However, the IT and healthcare infrastructure required for the broad adoption of EHRs is lacking in many developing nations. Patients with the least access to genomics and customized medicine are those who receive treatment in small and rural hospitals that have not yet implemented electronic health records. This division makes it more difficult to apply customized therapy and ensure that a diverse range of ethnicities are represented in genetic research. Through mobile enrollment and education centers, AoURP is relentlessly working to interact with poor communities in order to enhance health for all various groups. The Genomic Medicine Alliance is an international consortium of educational establishments that facilitates cooperation and management of genomic research initiatives in both developed and developing nations. The fact that transnational, coordinated efforts are required to supplement the existing one-off, local implementation efforts of personalised medicine is becoming more and more evident, given the goal of clinical care having a global effect on personalized medicine. The International 100K Cohort Consortium (IHCC) was established in 2018 with the goal of combining genetic data from global, substantial, unselected population cohorts. This consortium is an alliance between the Global Alliance for Genomics and Health (GA4GH), which seeks to promote and facilitate data sharing in genomic research, and the Global Genomic Medicine Collaborative (G2MC), which seeks to develop and share best practices for the application of global genomic medicine. These projects offer broadly applicable frameworks to comprehend the viability of effectively integrating complicated genomic data into health systems. Powerful information engines with great promise to help patients have been created during the past few decades by fundamental discoveries uncovering the genetic pathways of illness. It is predicted that the enormous expenditures incurred over the next ten years to incorporate this state-of-the-art information into standard clinical care would revolutionize medicine as it exists now. Patients' and physicians' interactions with healthcare data may alter as the usage of EHRs in healthcare delivery grows. The future of individualized therapy will be made possible by new paradigms for integrating genetic data with other biological data, artificial intelligence, and robotics in health systems.

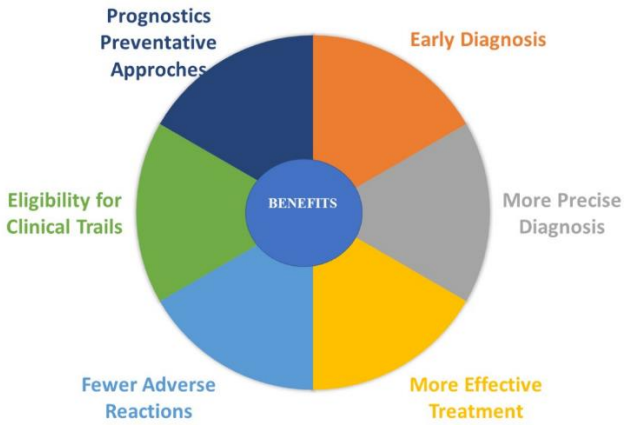


Figure 10. The advantages of genetic medicine are illustrated in this pie chart, which also includes prognostic/preventative methods, early diagnosis, more accurate diagnosis, more effective treatments, less side responses, and eligibility for clinical trials.

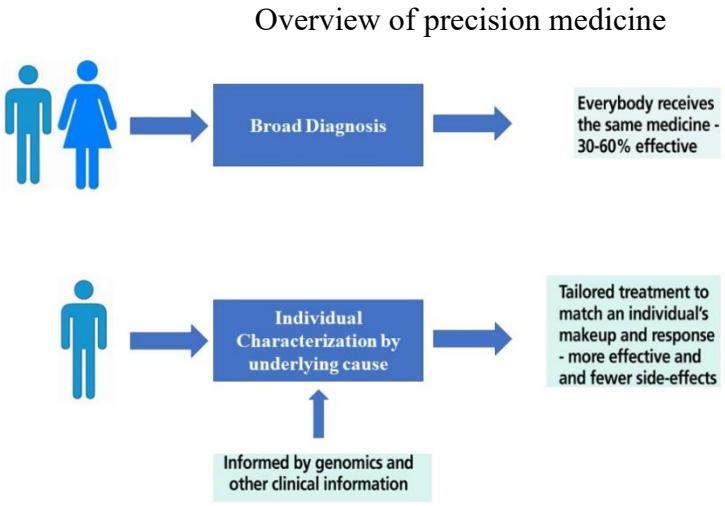


Figure 11. How individual treatments can be tailored by precision medicine to be more efficient and have fewer negative effects.

It is essential that equitable access to genetic research promotes advancements in diagnostic discoveries, translational research, and the creation of novel precision therapies for all in order to enhance the health of future generations. This is especially important for cancer as well as unusual and genetic disorders as well as increasingly common diseases.

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

References

2. Andrade, L., Manolakos, E. S. J. J. o. V. s. p. s. f. s., image, & technology, v. (2003). Signal background estimation and baseline correction algorithms for accurate DNA sequencing. *35*, 229-243.
3. Aronson, S. J., & Rehm, H. L. J. N. (2015). Building the foundation for genomics in precision medicine. *526*(7573), 336-342.
4. Banerjee, S., Prabhu Basrur, N., & Rai, P. S. J. P. M. (2021). Omics technologies in personalized combination therapy for cardiovascular diseases: Challenges and opportunities. *18*(6), 595-611.
5. Bentley, D. R., Balasubramanian, S., Swerdlow, H. P., Smith, G. P., Milton, J., Brown, C. G., . . . Bignell, H. R. J. n. (2008). Accurate whole human genome sequencing using reversible terminator chemistry. *456*(7218), 53-59.
6. Berwick, D. M., Nolan, T. W., & Whittington, J. J. H. a. (2008). The triple aim: care, health, and cost. *27*(3), 759-769.
7. Blumenthal, D. J. N. E. J. o. M. (2010). Launching hitech. *362*(5), 382-385.
8. Burge, C. O. (1898). *Surveys and other Preliminaries to Railway Construction in New South Wales.* Paper presented at the Minutes of the Proceedings of the Institution of Civil Engineers.
9. Burkhardt Jr, R. W. J. G. (2013). Lamarck, evolution, and the inheritance of acquired characters. *194*(4), 793-805.

10. Cohen, H. P., Blauvelt, A., Rifkin, R. M., Danese, S., Gokhale, S. B., & Woollett, G. J. D. (2018). Switching reference medicines to biosimilars: a systematic literature review of clinical outcomes. *78*(4), 463-478.
11. Crabtree, B. F., Chase, S. M., Wise, C. G., Schiff, G. D., Schmidt, L. A., Goyzueta, J. R., . . . Nutting, P. A. J. M. c. (2011). Evaluation of patient centered medical home practice transformation initiatives. *49*(1), 10.
12. Cyranoski, D. J. N. (2016). China embraces precision medicine on a massive scale. *529*(7584), 9-10.
13. Dahm, R. J. H. g. (2008). Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. *122*, 565-581.
14. Dance, A. J. N. (2016). Medical histories. *537*(7619), S52-S53.
15. Danchin, A. J. F. m. r. (2008). Bacteria as computers making computers. *33*(1), 3-26.
16. Danchin, E. (2022). More than Fifty Shades of Epigenetics for the Study of Early in Life Effects in Medicine, Ecology, and Evolution. In *Development Strategies and Biodiversity: Darwinian Fitness and Evolution in the Anthropocene* (pp. 3-35): Springer.
17. De Fauw, J., Ledsam, J. R., Romera-Paredes, B., Nikolov, S., Tomasev, N., Blackwell, S., . . . Visentin, D. J. N. m. (2018). Clinically applicable deep learning for diagnosis and referral in retinal disease. *24*(9), 1342-1350.
18. Dyson, F. J. (1988). Infinite in all directions: Gifford lectures given at Aberdeen, Scotland, April-November 1985.
19. Erikainen, S., Chan, S. J. N. G., & Society. (2019). Contested futures: envisioning "Personalized,""Stratified," and "Precision" medicine. *38*(3), 308-330.
20. Gauthier, J., Vincent, A. T., Charette, S. J., & Derome, N. J. B. i. b. (2019). A brief history of bioinformatics. *20*(6), 1981-1996.
21. Giangrande, P. H., Kimbrel, E. A., Edwards, D. P., McDonnell, D. P. J. M., & biology, c. (2000). The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding.
22. Grainger, J., Dufau, S., & Ziegler, J. C. J. T. i. C. S. (2016). A vision of reading. *20*(3), 171-179.
23. Guthrie, J. P. J. C. J. o. C. (1978). Hydrolysis of esters of oxy acids: p K a values for strong acids; Brønsted relationship for attack of water at methyl; free energies of hydrolysis of esters of oxy acids; and a linear relationship between free energy of hydrolysis and p K a holding over a range of 20 p K units. *56*(17), 2342-2354.
24. Hedayat, S., & Valeri, N. J. C. O. (2020). Patient-derived organoids: promises, hurdles and potential clinical applications. *32*(4), 213-216.
25. Hodson, R. J. N. (2016). Precision medicine. *537*(7619), S49-S49.
26. Horton, I., Lin, Y., Reed, G., Wiepert, M., & Hart, S. J. J. o. p. m. (2017). Empowering Mayo Clinic individualized medicine with genomic data warehousing. *7*(3), 7.
27. Johnson, K. B., Wei, W. Q., Weeraratne, D., Frisse, M. E., Misulis, K., Rhee, K., . . . science, t. (2021). Precision medicine, AI, and the future of personalized health care. *14*(1), 86-93.
28. Kalinin, A. A., Higgins, G. A., Reamaroon, N., Soroushmehr, S., Allyn-Feuer, A., Dinov, I. D., . . . Athey, B. D. J. P. (2018). Deep learning in pharmacogenomics: from gene regulation to patient stratification. *19*(7), 629-650.
29. Katrib, A., Hsu, W., Bui, A., & Xing, Y. J. Q. B. (2016). "RADIOTRANSCRIPTOMICS": A synergy of imaging and transcriptomics in clinical assessment. *4*, 1-12.

30. Khan, R., & Mittelman, D. J. G. b. (2018). Consumer genomics will change your life, whether you get tested or not. *19*(1), 1-4.
31. Khera, A. V., Chaffin, M., Aragam, K. G., Haas, M. E., Roselli, C., Choi, S. H., . . . Ellinor, P. T. J. N. g. (2018). Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *50*(9), 1219-1224.
32. Khoury, M. J., Gwinn, M. L., Glasgow, R. E., & Kramer, B. S. J. A. j. o. p. m. (2012). A population approach to precision medicine. *42*(6), 639-645.
33. Liao, W.-W., Asri, M., Ebler, J., Doerr, D., Haukness, M., Hickey, G., . . . Abel, H. J. J. N. (2023). A draft human pangenome reference. *617*(7960), 312-324.
34. Lightbody, G., Haberland, V., Browne, F., Taggart, L., Zheng, H., Parkes, E., & Blayney, J. K. J. B. i. b. (2019). Review of applications of high-throughput sequencing in personalized medicine: barriers and facilitators of future progress in research and clinical application. *20*(5), 1795-1811.
35. Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., . . . Chakravarti, A. J. N. (2009). Finding the missing heritability of complex diseases. *461*(7265), 747-753.
36. Martin, S. P., & Wang, X. W. J. H. O. (2019). The evolving landscape of precision medicine in primary liver cancer. *6*(2), HEP12.
37. Maxson, E., Jain, S., Kendall, M., Mostashari, F., & Blumenthal, D. J. A. o. I. M. (2010). The regional extension center program: helping physicians meaningfully use health information technology. *153*(10), 666-670.
38. McVicar, M., Sach, B., Mesnage, C., Lijffijt, J., Spyropoulou, E., & De Bie, T. J. P. R. L. (2016). SuMoTED: An intuitive edit distance between rooted unordered uniquely-labelled trees. *79*, 52-59.
39. Mikat-Stevens, N. A., Larson, I. A., & Tarini, B. A. J. G. i. M. (2015). Primary-care providers' perceived barriers to integration of genetics services: a systematic review of the literature. *17*(3), 169-176.
40. Mold, J. W., Aspy, C. A., & Nagykaldi, Z. J. T. J. o. t. A. B. o. F. M. (2008). Implementation of evidence-based preventive services delivery processes in primary care: an Oklahoma Physicians Resource/Research Network (OKPRN) study. *21*(4), 334-344.
41. Mostashari, F., Tripathi, M., & Kendall, M. J. H. A. (2009). A tale of two large community electronic health record extension projects. *28*(2), 345-356.
42. Moysidou, C.-M., Barberio, C., Owens, R. M. J. F. i. b., & biotechnology. (2021). Advances in engineering human tissue models. *8*, 620962.
43. Muir, A., & Vander Heiden, M. G. J. S. (2018). The nutrient environment affects therapy. *360*(6392), 962-963.
44. Ortega, M. A., Poirion, O., Zhu, X., Huang, S., Wolfgruber, T. K., Sebra, R., . . . medicine, t. (2017). Using single-cell multiple omics approaches to resolve tumor heterogeneity. *6*(1), 1-16.
45. Peck, R. W. J. A. r. o. p., & toxicology. (2018). Precision medicine is not just genomics: the right dose for every patient. *58*, 105-122.
46. Phillips, K. A. J. V. i. H. (2020). Methods for moving the evaluation of precision medicine into practice and policy. *23*(5), 527-528.
47. Popejoy, A. B., & Fullerton, S. M. J. N. (2016). Genomics is failing on diversity. *538*(7624), 161-164.
48. Sankowska, P.-J. J. I. J. T. (2018). Smart government: An European approach toward building sustainable and secure cities of tomorrow. *9*.
49. Santaló, J., & Berdasco, M. J. C. E. (2022). Ethical implications of epigenetics in the era of personalized medicine. *14*(1), 1-14.

50. Singh, R. S., & Gupta, B. P. J. N. g. m. (2020). Genes and genomes and unnecessary complexity in precision medicine. *5*(1), 21.
51. Starren, J., Williams, M. S., & Bottinger, E. P. J. J. (2013). Crossing the omic chasm: a time for omic ancillary systems. *309*(12), 1237-1238.
52. Syrjala, K. L., Yi, J. C., Artherholt, S. B., Romano, J. M., Crouch, M.-L., Fiscalini, A. S., . . . Leisenring, W. M. J. J. o. C. S. (2018). An online randomized controlled trial, with or without problem-solving treatment, for long-term cancer survivors after hematopoietic cell transplantation. *12*, 560-570.
53. Wang, J., Chang, S., Li, G., & Sun, Y. J. F. o. m. (2017). Application of liquid biopsy in precision medicine: opportunities and challenges. *11*, 522-527.
54. Watson, J. D., & Crick, F. (1953). A structure for deoxyribose nucleic acid.
55. Wild, C. P. J. C. E. B., & Prevention. (2005). Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *14*(8), 1847-1850.
56. Wishart, D. S. J. N. r. D. d. (2016). Emerging applications of metabolomics in drug discovery and precision medicine. *15*(7), 473-484.
57. Wu, J. C., Garg, P., Yoshida, Y., Yamanaka, S., Gepstein, L., Hulot, J.-S., . . . Schwartz, P. J. J. C. r. (2019). Towards precision medicine with human iPSCs for cardiac channelopathies. *125*(6), 653-658.

About The Authors

Ms. Munaza Rasheed is Master final year **Scholar** in Microbiology from Cholistan University of Veterinary and Animal Scones Bahawalpur, Pakistan. She completed her Graduation in Applied Microbiology from Cholistan University of Veterinary and Animal Scones Bahawalpur, Pakistan. Her research interest is in genetic, medical microbiology, molecular biology, biosafety and biosecurity, environmental microbiology, bacteriology, virology and food science. She has research articles published with great impact factor in national and international journals.
E-mail: munazarasheed2930@gmail.com , ORCID: 0009-0002-5153-2085

Mr. Muhammad Yasin is final year PhD Scholar in Health and Medical Biotechnology from Kohat University of Science and Technology (KUST). He is a visiting lecturer of molecular biology and genetics. His research interests include epilepsy genetics, molecular biology, molecular genetics, microbiology, genetic mutations, medical genetics, virology, infectious diseases, biotechnology and bioinformatics. He has published more than 13 research articles in international well-reputed journals. He has also written book chapters. He is an editor and editor-in-chief of many well-reputed national and international journals.
E-mail: yasinkhattakk@gmail.com, ORCID: 0000-0001-6889-6753

Mr. Muhammad Moazzam Bin Rasheed earned Mphil (Master of philosophy degree in Biotechnology) from the University of Agriculture Faisalabad, Pakistan. Currently he is doing PhD in Biotechnology from University of Agriculture Faisalabad Pakistan (UAF). His research interest includes; Molecular Biology, genetics, meta-genomics, drug discovery, molecular docking and simulation, protein biochemistry, biotechnology and bioinformatics. He has published 3 research articles in international journals.
E-mail: ranamoazzamuaf@gmail.com, ORCID: 0009-0000-4744-9831

Mr. Saqib Javeed graduated in Applied Microbiology Cholistan University of Veterinary and Animal Scones Bahawalpur, Pakistan. His interest is in Medical Microbiology, Environmental Microbiology, Biosafety and Biosecurity, Molecular Biology.
E-mail: saqibbwr789@gmail.com, ORCID: 0009-0007-5644-075X

To Cite This Chapter:
Rasheed, M., Yasin, M., Rasheed, B.M.M., & Javeed, S**.** (2023). **Recent Advances in the Application of CRISPR-Cas9**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 123-144). ISRES Publishing.

NEAR EAST'S MEDICINAL HERBS FOR CANCER TREATMENT

Salih MUVAKIT
Mehmet OZASLAN

1. Introduction

Cancer is a group of diseases caused by abnormal cells that proliferate rapidly compared to normal cells and can spread to other normal tissues or organs in the body. This group of diseases that cause mortality is also known as neoplasia or malignant tumor (Klug et al., 2014). Today, cancer treatments are based on surgical methods, radiation or the use of chemotherapeutic agents (Huang et al., 2017). Continuous proliferation of cancerous cells may cause spread metastasis to other tissues or organs. (Mrsny, 2013).

Apoptosis, defined as programmed cell death, is an important biological event, which is regulated by genes and which ensures the maintenance or maintenance of homeostasis, in which abnormal cells destroy themselves without inflammation and require energy from many molecules (Lowe & Lin, 2000; Brown et al., 2002). Damages (more or less) occurring in apoptosis can cause autoimmune disorders, neurodegenerative diseases or cancer. Therefore, studies are currently being conducted on the inhibition of anti-apoptotics or activation of pro-apoptotics (Matsuura et al., 2016).

The side effects that cancer patients encounter during or after their treatment, and the cost of anti-neoplastic drugs and their toxic effects on healthy cells have led scientists to conduct research on herbal medicines (Kooti et al., 2017; Thapliyal et al., 2018). The fact that an ideal anti-cancer agent candidate does little or no damage to normal cells and has an inhibitory effect on cancerous cells has formed the basis of the studies. Medicinal plants have been used in the treatment of various diseases since ancient times. Both developed and developing countries frequently apply to medicinal plants, which are seen as alternative treatment methods (Jiao et al., 2018; Greenwell & Rahman, 2015; Yin et al., 2013; Kaefer & Milner, 2008). It has been observed that the National Cancer Institute (NCI) has benefited from plant extracts in anti-tumor activity studies from the 1960s to the present. Today, the best known herbal anti-cancer agents are taxol, topotecan, vicrystine, vinblastine, pomiferin, sulforaphane and epipodophyllotoxin (Safarzadeh et al., 2014). According to the data of the Food and Drug Administration (FDA), they stated that 40% of the approved molecules in anti-cancer treatments are natural compounds and 74% of them are used (Seca & Pinto, 2018).

2.Complementary and Alternative Treatment in Cancer

It has been reported in studies that cancer has become one of the biggest diseases that threaten people's lives, leaving heart diseases behind in the 21st century. The resistance of cancer cells to antineoplastic agents complicates the fight against cancer disease (Mbaveng et al., 2019). Since the First World War, one of the most important treatments for cancer has been considered as chemotherapy. However, although it can cure cancer, it has been found to have serious side effects such as fertility, anemia, thyroid, psychological disorders, gastrointestinal disorders and kidney disease. That's

why there has been a constant demand for new treatment research. Herbal sources have attracted interest from past to present, compared to current treatments such as chemotherapy. At the same time, reliable herbal sources have been an important focus of researchers for effective anti-cancer treatments in many cancer types (Khan et al., 2020; Hassan 2015; Kooti et al., 2017).

The use of medicinal plants is as old as human history. Information about medicinal plants in history comes from the remaining inscriptions and archaeological materials (URL-2). Herbal medicines have been used for the treatment of cancer patients in the Middle East and various European countries, and have survived since the 1950s. It is known that medicinal plants are used as the source of drug discovery in the fight against cancer and that more than 70% of anti-cancer drugs have natural origins (Jacobo-Herrera et al., 2016). The literature review indicated that some compounds in the plant have anti-proliferative effects on cancer cells and showed that they were chosen as the raw material of drugs. As shown in **Table 1**, the chemotherapy drug Taxol was one of the first cancer drugs approved by the FDA in 1992 and had a major impact in cancer treatment. It has been shown to be used in the treatment of non-small cell lung cancer, breast cancer, ovarian cancer, sarcoma and cervical cancers (Che et al., 2015; Prota et al., 2013; Newman and Cragg, 2005). Vincristine, another best-known cancer drug, was the first cancer drug to be isolated in 1961. It was approved by the FDA in 1963 and reported to be used in the treatment of lymphoma and sarcoma cancers (Solowey et al., 2014; Unnati et al., 2013; Pezzuto 1997). It has been reported that the plants mentioned in the studies and called as potential anti-neoplastic are used not only in cancer patients, but also in skin diseases, infections, parasitic and viral diseases (Salehi et al., 2019; Saeed et al., 2016; Chen et al., 2013; Xiong et al., 1992). Thus, the biological activities of medicinal plants have been the subject of curiosity of researchers.Although many developed countries accept herbal treatment for cancer, it has been reported by WHO that only 5-15% of these plants are investigated to detect their bioactive compounds, ie anti-cancer compounds (Shabani, 2016; Ahmad et al., 2016; Zaid et al., 2012). The fact that medicinal plants have antitumor activity has been associated with having natural compounds called secondary metabolites. These compounds are alkaloids, flavonoids, terpenoids or glycosides (Khan et al., 2020; Seca and Pinto, 2018; Devi & Ganjela, 2009). It has been stated in literature studies that the anti-cancer effects of plants may be related to the presence of antioxidants in the compounds mentioned, inhibition of DNA damage, mitotic disruptors, apoptosis inducers or angiogenesis inhibitors in tumor cells (Greenwell & Rahman, 2015).

Another important problem with chemotherapeutic agents is multi-drug resistance (MDR), which is used to treat cancer cells and occurs when they become insensitive to chemotherapy treatment (Wu et al., 2011). Multi-drug resistance to anti-cancer drugs hinders successful cancer treatments. Many anti-cancer agents have been developed to prevent MDR, but most of them have been reported to fail in clinical trials due to their serious side effects (Hamed et al., 2019; Ye et al., 2019).

It is thought that multidrug resistance is an important problem in effective cancer treatment, and new strategies are needed to overcome this obstacle. The fact that plants, which are shown as natural resources, show low toxicity and prevent MDR, directs successful cancer treatments. In recent studies, the pharmacological activities of plant components against MDR have been investigated and reported. It has also been reported that the use of candidate molecules in combination with anti-cancer

agents may result in successful treatment of chemotherapy-resistant cancer (Tinoush et al., 2020).

Table 1. Plant-derived drugs in research and clinical trials

Plant Name	Family	Used Drugs	References
Echinops setifer	Asteraceae	Echinopsine	Pandey & Madhuri, 2009.
Ocimum sanctum	Lamiaceae	Eugenol, orientin, vicentin	Govind, 2011.
Scrophularia nodosa	Scrophulariaceae	Iridoid	Ardeshiry et al., 2010.
Taxus brevifolia	Taxaceae	Taxanes, taxol, cepholomannine	Prota et al., 2013.
Alo ferox	Liliaceae	Aloe-emodin, emodin	Wasserman et al., 2002; Pecere et al., 2000.
Cholchicum luteum	Liliaceae	Colchicines,demecolcine	Bruneton, 1993.
Rehmanni aglutinosa	Orobanchaceae	Catapol	Zhu et al., 2017.
Catharanthus roseus	Apocynaceae	Vincristine	Solowey et al., 2014.

3.Medicinal herbs in the Near East

In the Near East region, medicinal herbs are widely used as an ethnopharmacological approach by cancer patients. Recent data shed light on the most commonly utilized medicinal plants in the area for treating cancer, highlighting their significance as natural anticancer agents. Notable among these plants are various species of *Arum* and *Artemisia, Calotropis procera, Citrullus colocynthis*, *Nigella sativa*, *Pulicaria crispa*, various species of *Urtica*, *Withania somnifera*, and others. These herbs are frequently employed by cancer patients in the Near East countries.

Furthermore, research has explored the molecular mechanisms of action of plant extracts and isolated compounds from the Near East. Some of the investigated modes of action include cell cycle arrest and induction of apoptosis. Key players involved in these processes include proteins like p53, p21, Bcl-2, Bax, cytochrome c release, poly (ADP-ribose) polymerase cleavage, and activation of caspases, among others. Overall, the use of medicinal herbs in the Near East region for cancer treatment is significant, and ongoing studies continue to explore their potential as natural anticancer agents and their molecular mechanisms of action. These regions; The Near East region encompasses several countries, including the Arabian Peninsula, Egypt, Iraq, Iran, Israel, Jordan, Lebanon, Palestinian territories, Syria, and Turkiye, as defined by the National Geographic Society world map (Frodin, 2001; National Geographic Society (US), 2009). This region has a rich history and cultural heritage. The Arabian Peninsula, which is part of the Near East, includes countries such as Saudi Arabia, Yemen, Oman, United Arab Emirates, Qatar, Bahrain, and Kuwait. It is

surrounded by the Arabian Sea to the east and the Red Sea to the west. It shares borders with Syria, Jordan, and Iraq to the north and is bordered by the Indian Ocean to the south (Ash, 2005).

In terms of biodiversity, the Near East region is home to a diverse range of flora. Worldwide, approximately 50,000 to 80,000 flowering plant species are used for medicinal and therapeutic purposes. The Near East region itself has a significant number of plant species, with approximately 23,000 vascular plant species documented. Among these, around 6,700 species are endemic to the region. This botanical diversity contributes to the potential availability of medicinal plants and their traditional use in the Near East (Boulos et al., 1994; Heywood 2004).

The Near East region, with its unique geography and diverse plant life, offers a fascinating landscape for exploring the traditional use of medicinal plants and their therapeutic properties. The Arabian Peninsula, including countries such as Saudi Arabia, Bahrain, Kuwait, Oman, Qatar, United Arab Emirates (UAE), and Yemen (including Socotra), is home to a diverse flora. The Arabian Peninsula has a total of 7,801 vascular plant species, with 509 species being endemic to the region. In Saudi Arabia alone, there are 2,250 recorded plant species belonging to 142 families, including 242 endemic species and 600 rare and endangered species (Collenette, 1998).

Each country within the Arabian Peninsula has its own unique flora. Bahrain has around 248 vascular plant species, Kuwait has approximately 282 species, Oman has 1,200 species, Qatar has 306 species, UAE has 340 species, and Yemen (including Socotra) has a remarkable 3,640 vascular plant species (Abbas et al., 1992; Saganuwan, 2010; Hall & Miller, 2011; Sakkir et al., 2012) (**Table2**). Approximately 11% of the plant species in the region are considered endemic or regionally endemic (Boulos et al.,1994; Saganuwan, 2010)

Table 2. Plant species with medicinal use in countries of Arabian Peninsula (Sakkir et al., 2012)

Country	**Number of Medicinal Plants**
Bahrain	52
Oman	485
Qatar	184
Saudi Arabia	1200
UAE	20% of all species in UAE are used in folk medicine

In terms of medicinal plants, the data varies for each country. However, a significant number of plant species in the Arabian Peninsula have medicinal properties. Israel and the Palestinian territories have 2,600 plant species with medicinal value, particularly in the traditional medicines of various communities. Jordan has recorded 2,543 plant species, with 363 species having documented uses in traditional medicine (Zohary, 1983; Saganuwan, 2010; Al-Eisawi, 2013).

Overall, the Arabian Peninsula and the countries within it have a rich and diverse flora, with numerous plant species that have medicinal properties. These plants play a significant role in traditional medicine practices and highlight the importance of

conserving and studying the region's botanical resources (Oran & Al-Eisawi, 1998; Abu-Darwish et al., 2014).

Lebanon and Syria are known for having the richest flora in the Bilad all-Sham region. In Lebanon, there are 2,607 plant species, with 783 genera, including 78 endemic species. Syria, on the other hand, has recorded 3,500 vascular plant species belonging to 865 genera and 131 families. Lebanon specifically has a significant number of plant species, with over 130 species being used in folk medicine (Abu Chaar, 2004; Deeb et al., 2013; Baydoun et al., 2015).

Egypt, including Sinai, and Iraq have the highest plant diversity in the region, with 3,005 and 3,000 vascular plant species, respectively. Iran and Turkiye are the Near East countries with the richest biodiversity overall. Iran has approximately 8,000 plant species, while Turkiye boasts 8,650 species. Iran is known for having around 1,100 species used in Iranian folk medicine, while traditional Turkish medicine relies on 500-1,000 plant taxa (Boulos et al., 1994).
The rich plant diversity in these countries highlights the potential for medicinal plant resources and the importance of traditional medicine practices in the region (Ozturk et al.,2012).

3.1 Anticancer Ethnopharmacology of The Near East

Native herbal medicine forms the basis of the Materia Medica in the entire Near East region. The nations in the Near East have shared cultural systems, and their ancient traditional medicines have influenced each other through shared experiences. The traditional medicines of Persia, Mesopotamia, India, Greece, and Rome were greatly influenced by the medicine of ancient Arabia (Abu-Rabia,2015).

With the spread of Islam throughout the Near East, many medical books were translated by Muslim scientists from Persian and Sanskrit into Arabic. Muslim physicians and scholars played a significant role in establishing the foundation of herbal medicine in the Near East. One of the most famous ancient medical references is the "Qanoon f'il tibb" or "Canon of Medicine" authored by the renowned Persian physician Avicenna. This work included detailed descriptions of numerous medicinal plants commonly used in the region.

During the period between the 7th and 14th centuries, Muslim physicians such as Rhazes, Abulcasis, Avicenna, and Ibn al-Baitar not only diagnosed cancer but also realized that early identification of cancer was crucial for a potential cure. They recognized the importance of detecting cancer at its earliest stage for effective treatment.

Overall, the ancient herbal medicine traditions of the Near East, influenced by various cultures and the contributions of Muslim physicians, have played a significant role in shaping the medicinal practices and knowledge in the region. Avicenna, a prominent Persian physician, suggested various approaches to treat cancer in his work. He proposed that when cancer is in its early stages, it may be possible to keep it stable and prevent its progression or ulceration. However, once the cancer is advanced, a cure is unlikely. Avicenna described four methods of cancer treatment: total arrest (difficult to achieve), preventing progress, preventing ulceration, and treating existing ulceration Avicenna emphasized the importance of using medications that are not

overly potent, as strong medications can have carcinogenic effects on their own. He recommended the use of pure minerals such as washed pure tutty mixed with oils like rose oil and the oil of yellow gillyflower. Ibn al-Baitar, an Arab pharmacist and botanist, identified the anticancer properties of *Cichorium intybus* and recognized its potential for treating neoplastic disorders (Zaid et al., 2011).

Al-Kindi, a Muslim physician from the 10th century, described several medicinal plants for cancer treatment, including *Commiphora myrrha, Curcuma longa, Moringa peregrina, Physalis alkekengi, Polypodium vulgare,* and *Vicia ervilia* (Ben-Arye et al., 2012a).

These historical figures and their contributions highlight the early recognition of cancer as a complex disease and the use of herbal remedies in the Near East for its treatment. Their observations and recommendations laid the groundwork for the development of traditional cancer treatments in the region. Muslim physicians in the past also recognized the potential of surgery as a treatment for cancer. Avicenna, in his book "Qanoon f'il tibb," described early surgical interventions for cancer. He emphasized the importance of radical excision, ensuring the complete removal of diseased tissue, even suggesting the amputation of affected areas or removal of veins running towards the tumor. He also mentioned the use of cauterization when necessary. In the Near East region, herbal-based medicines are widely used as a part of complementary and alternative medicine (CAM). A significant number of cancer patients in the region have used CAM, with herbal-based medicines being the most commonly utilized form. Traditional medicinal herbs play a vital role in the indigenous ethnopharmacological systems of traditional medicine in the Near East (Molassiotis et al., 2005; Olaku & White, 2011; Ahmad et al., 2016).

A variety of medicinal plants are frequently reported and used by cancer patients in the Near East countries. These plants are employed in different forms and application methods for prevention and treatment. The interest in comprehensive screenings of traditional medicinal plants and their chemical constituents for cancer prevention and treatment has grown worldwide (**Table 3**).

Table 3. The most commonly used medicinal plants among Near East cancer patients.

Scientific name	**Family**	**Part used**	**Methods Use**	**Location**	**Reference**
Allium cepa L.	Amaryllidaceae	BU	Decoction, juice	PA, IS	Said et al., 2002; Jaradat et al., 2016
Arbutus andrachne L.	Ericaceae	FR	Decoction, syrup	PA, IS	Said et al., 2002; Jaradat et al., 2016
Arum dioscoridis Sibth et Sm.	Araceae	LE	Decoction	JO, PA	Hudaib et al., 2008; Jaradat et al., 2016
Arum maculatum L.	Araceae	LE, BU	Decoction	SY	Alachkar et al., 2011
Arum palaestinum Boiss.	Araceae	LE	Decoction	JO, PA, IS	Said et al., 2002; Hudaib et al., 2008, Jaradat et al., 2016

Bongardia chrysogonum (L.) Spach	Berberidaceae	TU	Decoction	JO	Assaf et al., 2013
Calotropis procera (Aiton)	Apocynaceae	AP	Decoction	AR, PA	Norton et al., 2009; Said et al., 2014, Jaradat et al., 2016
Capparis spinosa L.	Capparaceae	FR, L	Cooked, decoction	PA, SY, AR	Alachkar et al., 2011; Jaradat et al., 2016
Centaurea hyalolepis Boiss.	Compositae	FL, BA	Decoction	LE	Baydoun et al., 2015
Clematis flammula L.	Ranunculaceae	FL	Decoction	LE	Baydoun et al., 2015
Colchicum autumnale L.	Colchicaceae	WP	Powder	SY	Alachkar et al., 2011
Crataegus azarolus L.	Rosaceae	SE, FL, FR	Infusion, decoction	LE, IS	Baydoun et al., 2015
Crocus sativus L.	Iridaceae	FL	Infusion	IS	Said et al., 2002
Cyclamen coum	Primulaceae	FL, FR	Decoction	LE	Baydoun et al., 2015
Ecballium elaterium L.	Cucurbitaceae	WP or TU	Juice	PA, SY	Alachkar et al., 2011; Jaradat et al., 2016
Ephedra alata Decne.	Ephedraceae	WP	Decoction	PA	Saganuwan, 2010
Euphorbia peplus L.	Euphorbiaceae	Milky juice	Milky juice	AR	Rizk and El-Ghazaly, 1995
Fagonia indica L.	Zygophyllaceae	AP	Decoction	AR	Said et al., 2014
Inula viscosa (L.) Aiton	Asteraceae	FL	Decoction	JO, PA	Afifi-Yazar et al., 2011; Jaradat et al., 2016
Nigella sativa L.	Ranunculaceae	SE	Oil	AR	Alachkar et al., 2011
Nerium oleander L.	Apocynaceae	WP	Cream	PA	Jaradat et al., 2016
Ononis viscosa ssp. *sicula* (Guss.)	Leguminosae	WP	Decoction	PA	Jaradat et al., 2016
Onopordum cynarocephalum Boiss.	Compositae	FR	Prepared as tea	SY	Alachkar et al., 2011
Phoenix dactylifera L.	Arecaceae	FR	Decoction, juice	IR	Sadeghi et al., 2014
Plantago lanceolata L.	Plantaginaceae	LE, FL	Boiled	SY	Alachkar et al., 2011

Pulicaria crispa Forssk.	Asteraceae	AP	–	AR	Graham et al., 2000
Punica granatum L.	Lythraceae	FR	Syrup	JO, PA	Said et al., 2002
Quercus calliprinos Decne.	Fagaceae	FR, BA	Decoction	JO, PA,	Afifi-Yazar et al., 2011; Jaradat et al., 2016
Raphanus raphanistrum L.	Brassicaceae	LE, FR	Juice	SY	Afifi-Yazar et al., 2011; Jaradat et al., 2016
Rhazya stricta Decne.	Apocynaceae	AP, seeds	Decoction	AR	Phondani et al., 2016
Ricinus communis L.	Euphorbiaceae	SE	Oil	AR	Saganuwan, 2010
Ruta graveolens L.	Rutaceae	LE	Oil	AR	Saganuwan, 2010
Sarcopoterium spinosum L.	Rosaceae	RO	Decoction	JO	Hudaib et al., 2008
Sinapis arvensis L.	Cruciferae	LE, SE, RO	Sprouted seeds	SY	Alachkar et al., 2011
Teucrium polium L.	Lamiaceae	SE	Prepared as tea	SY	Alachkar et al., 2011
Trifolium stellatum L.	Leguminosae	AP	Prepared as tea	SY	Alachkar et al., 2011
Urtica pilulifera L.	Urticaceae	LE	Decoction	IS	Said et al., 2002
Urtica dioica L.	Urticaceae	LE, SE	Decoction	TU, IR	Malak et al., 2009; Yildiz et al., 2013
Urtica urens L.	Urticaceae	LE, SE	Decoction	JO, PA	Hudaib et al., 2008; Jaradat et al., 2016
Varthemia iphionoides Boiss.	Compositae	LE, FL	Cooked, decocted	JO	Assaf et al., 2013
Viscum cruciatum Sieber ex Boiss.	Santalaceae	LE	Decoction	JO, PA	Assaf et al., 2013; Jaradat et al., 2016
Vitex agnus-castus L.	Verbenaceae	FR, LE, FL, SE	Decoction, syrup	IS	Bachrach, 2012
Withania somnifera L.	Solanaceae	LE, FL	Decoction	JO, PA	Al-Qura'n, 2009; Jaradat et al., 2016
Zhumeria majdae Rech.	Lamiaceae	WP	Applied topically	IR	Zargari, 1992; Graham et al., 2000
Ziziphus spina-christi L.	Rhamnaceae	LE, ST	Infusion	JO, PA, IS	Said et al., 2002; Hudaib et al., 2008, Jaradat et al., 2016

Keyword: AP, aerial parts; BA, barks; BU, bulbs; FL, flowers; FR, fruits; LE, leaves; RO, roots; SE, seeds; ST, stems; TU, tubers; WP, whole plant. AR, Arabian Peninsula; JO, Jordan; PA, Palestinian Authority; LE, Lebanon; IR, Iran; IS, Israel; SY, Syria; TU, Turkiye.

Traditional herbal medicine is a prominent component of CAM in the Near East countries. Studies have shown a high preference for CAM and herbal medicine among both pediatric and adult cancer patients in countries such as Israel, the Palestinian Authority, Egypt, Jordan, Morocco, and Turkiye. CAM-based recipes in these countries may include the use of honey for mucositis prevention, wheatgrass juice for advanced breast cancer, kefir and yogurt for colorectal cancer, and tomato lycopene supplementation for colon cancer, among others (Uysal et al., 2016; Zarshenas et al., 2016; Sobeh et al., 2017).

HESA is a mixture of plant and marine materials that includes ingredients like *Penaeus latisculatus* (King Prawn), *Carum carvi* (caraway), and *Apium graveolens* (celery). It is used to improve the quality of life in patients with colon and breast cancer. The specific benefits and mechanisms of action of HESA in cancer patients may vary and would require further research to fully understand its effects. However, it is suggested as a complementary approach to support the well-being of individuals undergoing treatment for colon and breast cancer (Ben-Arye et al., 2012a).

4. Medicinal Plants of the Arabian Peninsula

Numerous studies have shed light on the potential of medicinal plants from the Arabian Peninsula region for anticancer drug development. For instance, cucurbitacin E glucoside and cucurbitacin I glucoside, isolated from the fruits of *Citrullus colocynthis* L., commonly used in folk medicine across Saudi Arabia and other Near East countries for cancer treatment, displayed cytotoxic effects against HepG2 hepatoma cells, with IC_{50} values of 3.5 and 2.8 nmol/mL, respectively. Researchers have also conducted extensive screening of 40 medicinal plants used in Saudi Arabian folk medicine against various cancer cell lines. Among them, the methanol extract of *Hypoestes forskaoli*i exhibited significant activity against HFB4 normal melanocytes, while the extract of *Adenium obesum*, native to parts of Africa and found in Oman, Saudi Arabia, and Yemen, showed cytotoxicity against HeLa cells. Additionally, the methanol extract of *Capparis tomentosa Lam*. demonstrated potency against MCF7 breast cancer cells. Another study focusing on Yemeni traditional medicine evaluated the cytotoxic activity of methanol and water extracts from 33 medicinal plants against epithelial 5637 and breast cancer MCF7 cell lines. Notably, the methanol extract of *H. forskaolii* showed the highest cytotoxicity against both cell lines. Overall, the findings underscore the higher cytotoxic potential of methanol extracts compared to water extracts and the varying sensitivity of different cancer cell lines to these plant extracts.

These findings highlight the cytotoxic potential of these medicinal plants and their potential as sources for developing anticancer drugs. Further research and investigations are necessary to fully understand the mechanisms of action and evaluate the safety and efficacy of these plants for cancer treatment.

Leptadenia pyrotechnica (Forssk.) Decne. is a native plant of the Gulf countries and is used in traditional medicine in the Arabian Peninsula for various diseases, including

cancer. Khasawneh et al. (2015) evaluated the cytotoxicity of the 80% ethanolic extract of *Leptadenia pyrotechnica* and its fractions (n-hexane, ethyl acetate, n-butanol, and water) against colon cancer cell lines. The hexane fraction of *Leptadenia pyrotechnica* exhibited the highest cytotoxic activity against both cell lines, leading to a dose- and time-dependent decrease in cell viability.

Rhazya stricta Decne. is another medicinal plant used in traditional medicine in the Arabian Peninsula, particularly in the form of water decoction, for the treatment of cancer. El-Awady et al. (2015) studied the cytotoxic activity of *Rhazya stricta* against hepatocellular carcinoma (HepG2) and colon cancer cells (CaCo) in vitro. The ethanol extract of *Rhazya stricta* demonstrated cytotoxic activity against HepG2 and CaCo cells, with IC_{50} values of 25 and 35 mg/mL, respectively. Other studies have also shown the cytotoxic activity of *Rhazya stricta* and its active component, rhazinilam, against various cell lines, similar to the control drug paclitaxel (Gu & Zakarian, 2010; Baeshen et al., 2015).

These findings indicate the potential of *Leptadenia pyrotechnica* and *Rhazya stricta* as sources of cytotoxic compounds for cancer treatment. However, further research is needed to identify the active compounds responsible for the cytotoxic effects and to evaluate their efficacy and safety for clinical use (El-Awady et al., 2015).

The chloroform and ethyl acetate extracts obtained from the aerial parts of *Rhazya stricta* grown in Oman were evaluated for their in vivo cytotoxicity using a brine shrimp assay. The results showed significant cytotoxic activity, with LC_{50} values of 18.1 and 13.9 mg/mL for the chloroform and ethyl acetate extracts, respectively. This suggests that these extracts contain compounds with potential cytotoxic properties (Ghazanfar, 2011; Norton et al., 2009).

Similarly, extracts obtained from the latex of *Calotropis procera*, another plant used in Omani and Arabian Peninsula folk medicine for various purposes including cancer treatment, were also tested using the brine shrimp assay. The chloroform and ethyl acetate extracts of *Calotropis procera* exhibited cytotoxic activity, with LC_{50} values of 3.0 and 8.2 mg/mL, respectively. These findings indicate the presence of bioactive compounds in the extracts that may have cytotoxic effects (Said et al., 2014).

It's important to note that the brine shrimp assay is a preliminary screening method used to evaluate the cytotoxic potential of plant extracts. Further studies are necessary to identify and characterize the specific compounds responsible for the observed cytotoxic activity and to assess their efficacy and safety in more advanced preclinical and clinical studies (Fawzy et al., 2013; Chhetri, 2015).

In a study conducted on the Yemeni flora, methanol extracts of 24 medicinal plants were screened in vitro for their cytotoxic activity against various cancer cell lines, including lung cancer, urinary bladder carcinoma, and breast cancer. The extracts from *Dendrosicyos socotrana, Withanina aduensis, Withania riebeckii, Dracaena cinnabari,* and *Buxus hildebrandtii* exhibited the highest toxicities against all tumor cell lines, with IC_{50} values ranging between 0.40 and 1.47, 0.30 and 4.30, 0.29 and 3.78, 2.59 and 5.54, and 0.32 and 15.1 mg/mL, respectively (Mothana et al., 2007).

Furthermore, some aromatic medicinal plants from Yemen and their essential oils were also screened for cytotoxic activity. The essential oil of *Pulicaria jauberti*, which

is found in southern Saudi Arabia and northern Yemen, showed significant cytotoxic activity in vitro against MCF7 and HepG2 cells, with IC_{50} values of 3.8 and 5.1 mg/mL, respectively (Fawzy et al., 2013; Chhetri, 2015). Another species from Yemen, *Pulicaria undulata*, which is also native to other countries in the Arabian Peninsula and used in traditional medicine, exhibited moderate in vitro cytotoxicity against MCF7 cells, with an IC_{50} value of 64.6 mg/mL (Ali et al., 2012; Norton et al., 2009).

4.1 Medicinal Plants of Egypt, Israel, Jordan, Lebanon, Palestinian Territory, and Syria

Over the past decade, numerous studies have reported the in vitro cytotoxic activity of medicinal plants from Egypt, Israel, Jordan, Lebanon, the Palestinian territory, and Syria. For example, El-Seedi et al. (2013) conducted a study on 61 Egyptian medicinal plants originating from the Sinai Desert. They found that the methanol extracts of *Nerium oleander*, traditionally used in cream form for treating skin cancer, and *Pulicaria undulata* exhibited cytotoxic effects against human U-937 GTB lymphoma cells.

Arum palaestinum, which is traditionally used for cancer treatment in several countries of the Middle East, was identified among the medicinal plants in the Egyptian flora with superior in vitro cytotoxic activity. In fact, it demonstrated a remarkable 97.29% inhibition of the growth of Ehrlich ascites carcinoma cells (EACC) (Aboul-Enein et al., 2012).

These findings highlight the potential of medicinal plants from the region as sources of cytotoxic compounds. Further research is needed to identify the active constituents responsible for the observed cytotoxic effects and to explore their potential for the development of novel cancer therapies.

The 95% ethanol extract of *Diplotaxis harra*, originating from South and North Sinai in Egypt, demonstrated cytotoxic activity against HCT116, HepG2, and MCF7 cell lines with IC_{50} values of 4.65, 12.60, and 17.90 mg/mL, respectively. Furthermore, flavonoids isolated from this extract, including quercetin, quercetin 3-O-b-glucoside, isorhamnetin 7-O-b-glucoside, apigenin 3-O-b-rhamnoside, and kaempferol 3-O-b-glucoside, exhibited in vitro cytotoxic activity against the same cell lines with IC_{50} values of 20.1, 24.3, 22.8, 23.4, and 41.9 mg/mL, respectively (Mohammed et al., 2011).

In another study involving 16 medicinal plants originating from Egypt, the dichloromethane crude extract of *Ferula hermonis* demonstrated remarkable in vitro cytotoxic activity against human MIAPaCa-2 pancreatic cancer cells, MCF7 breast cancer cells, CCRF-CEM leukemia cells, and their multidrug-resistant subline CEM/ADR5000, with IC_{50} values below 20 mg/mL (Kuete et al., 2012).

These findings highlight the potential of *Diplotaxis harra* and *Ferula hermonis* as sources of cytotoxic compounds for potential use in cancer treatment. Further research is necessary to identify the active constituents responsible for the observed cytotoxic effects and to explore their mechanisms of action. The essential oil derived from the leaves and berries of *Juniperus phoenicea*, grown in Sinai, Egypt, demonstrated high

cytotoxic activities against brain and lung, liver, and breast human cell lines, with IC_{50} values of 0.6, 0.7, and 0.8 mg/mL, respectively (El-Sawi et al., 2007).

In a study involving 17 plants from Israel, their 50% ethanol extracts were tested for cytotoxic activity against various human cancer cell lines. The extracts of *Urtica membranacea*, *Artemisia monosperma*, and *Origanum dayi* exhibited superior time- and concentration-dependent cytotoxic activity against all cancer cell lines tested, including LNCaP prostate adenocarcinoma, Colo 205 colon carcinoma, Hec-1A endometrial adenocarcinoma, OVCAR-3 ovarian carcinoma, HepG2 hepatocellular carcinoma, MCF7 breast carcinoma, 293 embryonic kidney adenocarcinoma, Karpas 299 T-cell non-Hodgkin's lymphoma, A494 alveolar basal epithelial adenocarcinoma, SU-DHL-1 anaplastic large cell lymphoma, YC and OSTRA normal EBV-transformed lymphoblasts, HUT-102T-cell lymphoma, and T24P urinary bladder carcinoma. Importantly, these extracts did not exhibit cytotoxicity against normal human cells (Solowey et al., 2014).

Furthermore, the ethanol extract of a chemotype of *Varthemia iphionoides*, originating from Israel, showed remarkable cytotoxic activity against HL-60 leukemia cells, surpassing its activity on other cell lines such as SKOV3 ovarian carcinoma cells, BG melanoma cells, and A549 lung cancer cells (Yarmolinsky et al., 2015).

These findings suggest the potential of *Juniperus phoenicea*, *Urtica membranacea*, *Artemisia monosperma*, *Origanum dayi*, and *Varthemia iphionoides* as sources of cytotoxic compounds for potential use in cancer treatment. Further research is necessary to identify the specific bioactive constituents responsible for their cytotoxic effects and to investigate their mechanisms of action.
Asphodelus microcarpus, Ecballium elaterium, Eryngium creticum, Mercurialis annua, Pistacia lentiscus, Rhamnus alaternus, Teucrium polium, and *Urtica pilulifera* are traditionally used in Arab medicine in Israel and the Palestinian territory. The water extracts of these plants were investigated for their effects on mitochondrial respiration and cell membrane integrity in PC12 and HepG2 cells. Among these extracts, only the water extract of *Ecballium elaterium* showed a concentration-dependent inhibition of mitochondrial respiration in HepG2 cells, in a concentration range of 0.1–1 mg/mL. However, the other extracts did not significantly inhibit mitochondrial respiration in these cells (Ljubuncic et al., 2005). It is worth noting that the fruit juice of *Ecballium elaterium* is used in Palestinian and Syrian folk medicine to treat liver and throat cancer (Alachkar et al., 2011; Jaradat et al., 2016).

In terms of Jordanian medicinal plants, a study by Assaf et al. (2013) focused on the cytotoxic activity of methanol extracts from *Mercurialis annua*, *Bongardia chrysogonum*, and *Viscum cruciat*um against Burkitt's lymphoma and U266-IgE producing myeloma cells. Among these extracts, only the *Viscum cruciatum* extract exhibited selective cytotoxic activity against Burkitt's lymphoma, with an IC_{50} value of 14.21 mg/mL. The decoction of *Viscum cruciatum* is used in Jordanian and Palestinian folk medicine for esophageal cancer (Assaf et al., 2013; Jaradat et al., 2016). These findings provide insights into the cytotoxic potential of certain plants used in traditional Arab medicine. Further research is needed to identify the active components responsible for their cytotoxic effects and to explore their potential applications in cancer treatment.

The aqueous extracts of *Nigella sativa (*black seed) seeds and *Allium sativum* (garlic) bulbs, which are commonly used in Jordanian folk medicine for cancer treatment, were found to enhance the cytotoxicity of splenic natural killer (NK) cells in an in vivo study. The administration of *Nigella sativa* extract resulted in 62.3 ± 6.4% cytotoxicity, while *Allium sativum* extract led to 52.6 ± 5.4% cytotoxicity. This suggests that these extracts have immunomodulatory effects that may be beneficial in cancer treatment (Abuharfeil et al., 2001).
In the context of Palestinian and Jordanian folk medicine for cancer treatment, several medicinal plants are used, including *Withania somnifera*, *Psidium guajava*, *Laurus nobilis* (by leaf), and *Salvia fruticosa* (sage). However, when screened for their cytotoxic activity against murine L929sA fibrosarcoma cells and two human breast cancer cell lines (MDA-MB231 and MCF7), only weak cytotoxic activity was observed. *W. somnifera* extract exhibited IC_{50} values of 150 mg/mL on L929sA cells and 60 mg/mL on MCF7 cells, while *P. guajava* extract showed an IC_{50} value of 55 mg/mL on MCF7 cells Further studies are needed to explore the potential mechanisms and efficacy of these plant extracts in cancer treatment (Kaileh et al., 2007).

The leaves and stems of *Trigonella berythea*, a plant grown in south Lebanon, were found to have significant cytotoxic activity against MCF7 and U937 cell lines. Both ethanol and aqueous extracts inhibited the growth of these cell lines by more than 60%, with IC_{50} values ranging from 29.46 to 61.54 mg/mL (Farhan et al., 2013).

Essential oils derived from *Cedrus libani* (cedar)*, Pinus pinea* (stone pine)*, Juniperus oxycedrus,* and *Juniperus excelsa*, also grown in Lebanon, exhibited remarkable cytotoxic activity against drug-sensitive human CCRF-CEM leukemia cells and their multidrug-resistant subline CEM/ADR5000. The multidrug-resistant cells did not show significant cross-resistance to these essential oils, indicating their potential as alternative treatments. The resistance degree was less than twofold compared to the control drug doxorubicin (Saab et al., 2012).

Two Salvia species, *S. bracteata* and *S. rubifolia,* traditionally used in Lebanese medicine, demonstrated in vitro cytotoxic activity against human M14 melanoma cells. The essential oil of *S. rubifolia* was particularly more active than that of *S. bracteata*, showing significant efficacy against the melanoma cells (Cardile et al., 2009).

The essential oils extracted from the peel of *Citrus limon* (lemon) collected from different locations in Syria exhibited cytotoxic effects against the doxorubicin-resistant colorectal cancer cell line LIM1863. The IC_{50} values of these essential oils ranged from 5.75 to 7.92 mg/mL. The cytotoxic activity of the Syrian lemon peel essential oils has been attributed to the presence of the monoterpene limonene, which is known to induce apoptosis and enhance the activity of phase 1 and 2 carcinogen-metabolizing enzymes. These mechanisms help prevent the interaction of chemical carcinogens with DNA, thus exerting potential anticancer effects (Jomaa et al., 2012).

4.2 Medicinal Plants of Iran and Turkiye

In a study conducted by Naghibi et al. (2014b), the cytotoxicity of methanol extracts from 19 plant species was investigated in Iran. The study utilized MCF7, HepG2, A-549, and HT29 cancer cells to evaluate the cytotoxic activity. Although these plants are traditionally used in Iranian traditional medicine (ITM) for managing cancers,

most of them showed no or weak cytotoxic activity, with IC_{50} values below 100 mg/mL. However, the methanol extract of *Tanacetum polycephalum* exhibited relatively higher cytotoxicity, with IC_{50} values of 28.3, 53.0, and 43.3 mg/mL against MCF7, A-549, and HT-29 cell lines, respectively. It suggests that *Tanacetum polycephalum* may hold promise as a potential cytotoxic agent (Naghibi et al., 2014b).

C. procera, also known as *Euphorbia tirucalli*, is a plant whose milky latex and leaves are used in Iranian traditional medicine to treat various diseases, including cancer. In a study involving 27 medicinal plants from the southern provinces of Iran, the methanol extract of the aerial parts of *C. procera* exhibited the highest cytotoxicity among the tested plant extracts. The extract showed cytotoxic activity against a panel of cell lines including MCF7, HepG2, A-549, and MDBK cells, with IC_{50} values ranging from 1.9 to 12.16 mg/mL (Esmaeili et al., 2014).

U. dioica, commonly known as stinging nettle, is also used in traditional medicine in the Near East for cancer treatment. However, among the ethanol extracts of Iranian medicinal plants tested against HT-29, Caco-2, T47D cancer cell lines, and Swiss mouse embryo fibroblasts (3T3), only the extract of the aerial parts of U. dioica demonstrated cytotoxicity, with an IC_{50} value of 46.14 mg/mL in T47D cells (Kashani et al., 2014).

These studies indicate that both *C. procera* and *U. dioica* possess cytotoxic properties and hold potential for further exploration as anticancer agents in the context of traditional medicine in Iran and the Near East.

In an in vivo brine shrimp lethality assay conducted in Iran, methanol extracts of 23 plant species belonging to the Leguminosae family were screened for their cytotoxic activity. Among the tested plants, *Taverniera spartea* and the endemic plant *Tephrosia persica* demonstrated high cytotoxic activity, with IC_{50} values of 0.34 and 2.43 mg/mL, respectively (Khalighi-Sigaroodi et al., 2012).

Additionally, the essential oils extracted from the peel of Citrus limon, C. medica, and Camellia sinensis collected in Iran were found to exhibit cytotoxic activity against MCF7 and HeLa cells. The IC_{50} values ranged from 0.5 to 17 mg/mL (Monajemi et al., 2010). These findings are consistent with a study by Jomaa et al. (2012), which showed that the essential oil of *C.limon* collected in Syria displayed cytotoxic effects against human LIM1863 colorectal carcinoma cells.

According to Ozkan et al. (2016), several native and endemic Turkish plants have been identified with cytotoxic activity against various types of cancer cell lines. Artun et al. (2015) conducted a study screening the methanol extracts of native and endemic Turkish plants for their cytotoxicity against Vero and HeLa cells. Among the plants tested, six showed cytotoxicity against Vero cells and eleven showed cytotoxicity against HeLa cells. The methanol extract of the cormus of *Colchicum sanguicolle* exhibited the highest cytotoxic activity against HeLa cells, with an IC_{50} value of 2 ± 0.02 mg/mL.

Despite *Bellis perennis* L. (English daisy) and *Convolvulus galaticus* Rostan ex Choisy (Grizzle bindweed), an endemic plant of the Turkish flora, being used in Turkish folk medicine for the treatment of various ailments, including cancer, their cytotoxic activity was found to be weak. The methanol extract of the aerial parts of *B.*

perennis showed weak cytotoxicity against MCF7 cells (IC_{50}: 71.6 mg/mL), while the dichloromethane extract of the aerial parts of *C. galaticus* exhibited some activity against HepG2/C3A cells (IC_{50}: 57.3 mg/mL) (Karakas et al., 2015).

KL-21 is a commercial product from Turkiye that contains extracts from various plant species, including *Achillea millefolium*, *Acorus calamus, Cichorium endivia, Curcuma longa, Equisetum arvense*, *Fumaria officinalis, Juniperus communis, Hypericum perforatum, Lavandula stoechas, Melissa officinalis, Nigella sativa*, *Peganum harmala, Rosmarinus officinalis, Silybum marianum, Solidago virgaurea, Taraxacum officinale, Thymus vulgaris, Urtica dioica, Valeriana officinalis, Viscum album*, and *Zingiber officinale*.

In a study by Gokbulut et al. (2015), it was found that KL-21 reduced the viability of 232B4 chronic lymphocytic leukemia cells in a dose- and time-dependent manner. However, it did not affect the viability of normal BEAS-2B epithelial cells at concentrations up to 100 mg/mL. This indicates that KL-21 selectively targeted the leukemia cells while sparing the normal epithelial cells.

5. Future Perspectives

The search for effective treatment options continues. In recent years, there has been growing interest in exploring the potential of medicinal herbs as a complementary or alternative approach to cancer treatment. As we look to the future, several promising perspectives emerge regarding the use of medicinal herbs for cancer treatment:

Enhanced Understanding of Mechanisms:
Advancements in scientific research and technology are contributing to a better understanding of the mechanisms by which medicinal herbs exert anticancer bodies. Future studies may unveil specific bioactive compounds within these herbs, their interaction with cancer cells, and the underlying molecular pathways involved. This knowledge can lead to the development of targeted therapies that harness the full potential of medicinal herbs.

Synergistic Combinations:
Combining medicinal herbs with conventional cancer treatments, such as chemotherapy or radiation therapy, holds great promises. Synergistic effects have been observed when herbs are used alongside standard treatments, resulting in improved therapeutic outcomes, reduced side effects, and increased treatment tolerability. Future research may focus on identifying optimal combinations and dosage regimens, paving the way for personalized treatment approaches.

Standardization and Quality Control:
One of the challenges in utilizing medicinal herbs for cancer treatment lies in ensuring standardized and high-quality products. Future perspectives involve establishing rigorous quality control measures, including authentication of herb species, identification and quantification of active compounds, and adherence to good manufacturing practices. This will provide healthcare professionals and patients with reliable and consistent herbal products for cancer treatment.

Individualized Treatment Approaches:
The future of cancer treatment lies in personalized medicine, and medicinal herbs have the potential to play a vital role in this paradigm. Advances in genomics, metabolomics, and other omics technologies may enable the identification of specific genetic and molecular profiles that determine an individual's response to herbal treatments. Tailoring treatment plans based on these profiles can optimize therapeutic efficacy and minimize adverse reactions.

Clinical Trials and Evidence-Based Practice:
To establish the efficacy and safety of medicinal herbs for cancer treatment, well-designed clinical trials are crucial. Future perspectives involve conducting rigorous studies to generate robust evidence supporting the use of specific herbs or herbal formulations. This will enable healthcare professionals to make informed decisions and incorporate evidence-based herbal treatments into cancer care protocols.

9. Summary

One of the most important factors limiting cancer treatment is that the chemotherapeutic agents used for treatment are not specific to cancerous tissue. For this reason, new studies on the detection of new molecules specific to cancer cells are increasing day by day. Future perspectives on medicinal herbs for cancer treatment hold great promise in the fight against this devastating disease. With advancements in scientific research, technology, and evidence-based practice, we can harness the potential of medicinal herbs to complement existing cancer treatments, improve patient recovery, and enhance the quality of life for individuals affected by cancer. However, it is essential to maintain a cautious and evidence-driven approach to ensure the safe and effective integration of medicinal herbs into mainstream cancer care.

References

Aboul-Enein, A. M., El-Ela, F. A., Shalaby, E. A., & El-Shemy, H. A. (2012). Traditional medicinal plants research in Egypt: studies of antioxidant and anticancer activities. J. Med. Plants Res. 6, 689–703.

Abu Chaar, C. I. (2004). Medicinal plants of Lebanon. Archaeol. Hist. Leban. 19,70–85.

Abu-Darwish, M. S., Wang, M., Zulfiqar, F., Ali, Z., and Khan, I. A. (2016). Chemical composition of the essential oil of Salvia ceratophylla L. from Jordan. Planta Med. 82:PC1. doi: 10.1055/s-0036-1578703.

Abu-Darwish, M. S., Cabral, C., & Salgueiro, L. (2014). “Medicinal and aromatic plants of the Middle East,” in Juniperus phoenicea L. from Jordan, Vol. 5, eds Z. Yaniv, and N. Dudai (Dordrecht: Springer), 241–252. doi: 10.1007/978-94-017-9276-9_13

Abuharfeil, N. M., Salim, M., & von Kleist, S. (2001). Augmentation of natural killer cell activity in vivo against tumour cells by some wild plants from Jordan. Phytother. Res. 15, 109–113. doi: 10.1002/ptr.692

Abu-Rabia, A. (2015). Key plants in fighting cancer in the Middle East. Chin. Med. 6, 124–135. doi: 10.4236/cm.2015.62014

Ahmad, R., Ahmad, N., Naqvi, A. A., Shehzad, A., & Al-Ghamdi, M. S. (2016). Role of traditional Islamic and Arabic plants in cancer therapy. J. Tradit. Complement. Med. 7, 195–204 doi: 10.1016/j.jtcme.2016.05.002

Ahmadi, A., Mohagheghi, M., Karimi, M., Golestanha, S. A., Naseri, M., Faghihzadeh, S., et al. (2010). Therapeutic effects of HESA-A in patients with end-stage metastatic cancers. Integr. Cancer Ther. 9, 32–35. doi: 10.1177/1534735409357934.

Alachkar, A., Jaddouh, A., Elsheikh, M. S., Bilia, A. R., & Vincieri, F. F. (2011). Traditional medicine in Syria: folk medicine in Aleppo governorate. Nat. Prod. Commun. 6, 79–84.

Al-Eisawi D. M, (2013). Flora of Jordan Checklist, 1st Edn. Amman: The University of Jordan.

Ali N. A., Sharopov F. S., Alhaj M., Hill G. M., Porzel A., Arnold N., et al. (2012). Chemical composition and biological activity of essential oil from Pulicaria undulata from Yemen. *Nat. Prod. Commun.* 7 257–260.

Ardeshiry, L.A., Rezaie-Tavirani, M., Mortazavi, S.A., Barzegar, M., Moghadamnia, S.H., Rezaee, M.B. (2010). Study of anti cancer property of *Scrophularia striata* extract on the human astrocytoma cell line (1321). *Iranian Journal of Pharmaceutical Research*, 9(4), 403-410.

Artun, F. T., Karagoz, A., Ozcan, G., Melikoglu, G., Anil, S., Kultur, S., et al. (2015). In vitro anticancer and cytotoxic activities of some plant extracts on HeLa and Vero cell lines. Mitt. Klosterneuburg 65, 55–64.

Ash, R. (2005). Every Subject on Earth! Whitaker's World of Facts. London: A & C Black Publishers Ltd., 320.

Assaf, A. M., Haddadin, R. N., Aldouri, N. A., Alabbassi, R., Mashallah, S., Mohammad, M., et al. (2013). Anti-cancer, anti-inflammatory and antimicrobial activities of plant extracts used against hematological tumors in traditional medicine of Jordan. J. Ethnopharmacol. 145, 728–736. doi: 10.1016/ j.jep.2012.11.039

Baeshen, M. N., Khan, R., Bora, R. S., &Aeshen, N. A. (2015). Therapeutic potential of the folkloric medicinal plant Rhazya stricta. Biol. Syst. Open Access 5:151. doi: 10.4172/2329-6577.1000151.

Baydoun, S., Chalak, L., Dalleh, H., & Arnold, N. (2015). Ethnopharmacological survey of medicinal plants used in traditional medicine by the communities of Mount Hermon, Lebanon. J. Ethnopharmacol. 173, 139–156. doi: 10.1016/j.jep.2015.06.052

Ben-Arye, E., Ali-Shtayeh, M. S., Nejmi, M., Schiff, E., Hassan, E., Mutafoglu, K.,et al. (2012a). Integrative oncology research in the Middle East: weaving traditional and complementary medicine in supportive care. Support. Care Cancer 20, 557–564. doi: 10.1007/s00520-011-1121-0

Boulos, L., Miller, A. G., Mill, R. R. (1994). "Regional overview: South West Asia and the Middle East Boulos et," in Centres of Plant Diversity. A Guide and Strategy for Their Conservation, Vol. 1, eds S. D. Davis, V. H. Heywood and A.C. Hamilton) 293–308.

Brown, S., Heinisch, I., Ross, E., Shaw, K., Buckley, C.D., Savill, J. (2002). Apoptosis disables CD31 mediated cell detachment from phagocytes promoting binding and engulfment. *Nature*, 418, 200-203

Bruneton, J. (1993). Pharmacognosy, phytochemisty medicinal plants. Lavoisier publisher, Baskı 2, 1136 s., 771-777.

Cardile, V., Russo, A., Formisano, C., Rigano, D., Senatore, F., Arnold, N. A., et al. (2009). Essential oils of Salvia bracteata and Salvia rubifolia from Lebanon:

chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. J. Ethnopharmacol. 126, 265–272. doi: 10.1016/j.jep.2009.

Che, E., Gao, Y., Wan, L., Zhang, Y., Han, N., Bai, J., Li, J., Sha, Z., Wang, S. (2015). Paclitaxel/gelatin coated magnetic mesoporous silica nanoparticles: Preparation and antitumor efficacy in vivo. *Microporous and Mesoporous Materials*, 204, 226-234. https://doi.org/10.1016/j.micromeso.2014.11.013

Chhetri B. K. (2015). *A Gas Chromatographic/Mass Spectral Analysis of Aromatic Medicinal Plants from Yemen.* Doctoral dissertation, The University of Alabama in Huntsville, Huntsville, AL

Collenette, I. S. (1998). A Checklist of Botanical Species in Saudi Arabia. Burgess Hill: International Asclepiad Society, 80.

Deeb, T., Knio, K., Shinwari, Z., Kreydiyyeh, S., & Baydoun, E. (2013). Survey of medicinal plants currently used by herbalists in Lebanon. Pak. J. Bot. 45, 543–555. doi: 10.1016/j.jep.2008.08.024

El-Awady, M. A., Awad, N. S., & El-Tarras, A. E. (2015). Evaluation of the anticancer activities of pomegranate (Punica granatum) and harmal (Rhazya stricta) plants grown in Saudi Arabia. Int. J. Curr. Microbiol. Appl. Sci. 4, 1158–1167.

El-Sawi, S. A., Motawae, H. M., & Ali, A. M. (2007). Chemical composition, cytotoxic activity and antimicrobial activity of essential oils of leaves and berries of Juniperus phoenicea L. grown in Egypt. Afr. J. Tradit. Complement. Altern. Med. 4, 417–426. doi: 10.4314/ajtcam.v4i4.31236

El-Seedi, H. R., Burman, R., Mansour, A., Turki, Z., Boulos, L., Gullbo, J., et al. (2013). The traditional medical uses and cytotoxic activities of sixtyone Egyptian plants: discovery of an active cardiac glycoside from Urginea maritima. J. Ethnopharmacol. 145, 746–757. doi: 10.1016/j.jep.2012.12.007

Esmaeili, S., Hamzeloo-Moghadam, M., Ghaffari, S., & Mosaddegh, M. (2014). Cytotoxic activity screening of some medicinal plants from south of Iran. Res. J. Pharmacogn. 1, 19–25. doi: 10.4103/0973-1296.93327

Farhan, H., Rammal, H., Hijazi, A., Annan, H., Daher, A., Reda, M., et al. (2013). Chemical composition, in vitro cytotoxicity and anti-free radical properties of six extracts from Lebanese Trigonella berythea Boiss. Pak. J. Pharm. Sci. 26,1157–1163.

Fawzy G. A., Al Ati H. Y., El Gamal A. A. (2013). Chemical composition and biological evaluation of essential oils of *Pulicaria jaubertii*. *Pharmacogn. Mag.* 9 28–32. 10.4103/0973-1296.108133

Frodin, D. G. (2001). Guide to the Standard Floras of the World, 2nd Edn. Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511541803

Ghazanfar S. A. (2011). *Medicinal and Aromatic Plants-Arabia and Iran. Ethnopharmacology Section, Biological, Physiological and Health Sciences, Encyclopedia of Life Support Systems (EOLSS).* Oxford: EOLSS Publishers.

Gokbulut, A. A., Yasar, M., & Baran, Y. (2015). A novel natural product, KL-21, inhibits proliferation and induces apoptosis in chronic lymphocytic leukemia cells. Turk. J. Hematol. 32, 118–126. doi: 10.4274/tjh.2013.0381

Govind, P. (2011). Some important anticancer herbs: a review. *International Research Journal of Pharmacy*, 2(7), 45-52.

Greenwell, M., Rahman, P.K.S.M. (2015). Medicinal plants: Their use in anticancer treatment. *International Journal of Pharmaceutical Sciences and Research*, 1;6(10), 4103-4112. https://doi:10.13040/IJPSR.0975-8232.6(10).4103-

Gu, Z., & Zakarian, A. (2010). Total synthesis of rhazinilam: axial to point chirality transfer in an enantiospecific Pd-catalyzed transannular cyclization. Org. Lett. 12, 4224–4227. doi: 10.1021/ol101523z

Hall, M., & Miller, A. G. (2011). Strategic requirements for plant conservation in the Arabian Peninsula. Zool. Middle East 54, 169–182. doi: 10.1080/09397140.2011.10648908

Hamed, A.R., Abdel-Azim, N.S., Shams, K.A., Faiza M. Hammouda, F.M. (2019). Targeting multidrug resistance in cancer by natural chemosensitizers. *Bulletin of the National Research Centre*, 43, 8. https://doi.org/10.1186/s42269-019-0043-8

Hassan, A. A. (2015). Knowledge and attitude of oncology practitioners towards complementary and alternative medicine for cancer care in Qatar. J. Anesthesiol. Clin. Res. 6, 1–7. doi: 10.4172/2155-6148.1000561

Heywood, V. H. (2004). Modern approaches to floristics and their impact on the region of SW Asia. Turk. J. Botany 28, 7–16.

Huang, C.Y., Da-Tong Ju, D.T., Chang, C.F., Reddy, P.M., Bharath Kumar Velmurugan, B.K. (2017). A review on the effects of current chemotherapy drugs and natural agents in treating non–small cell lung cancer. *Biomedicine*, 7(4), 12-23

Jacobo-Herrera, N.J., Jacobo-Herrera, F.E., Zentella-Dehesa, A., Andrade-Cetto, A., Heinrich, M., Pérez-Plasencia, C. (2016). Medicinal plants used in Mexican traditional medicine for the treatment of colorectal cancer. *Journal of Ethnopharmacology*, 179, 391-402

Jaradat, N. A., Al-Ramahi, R., Zaid, A. N., Ayesh, O. I., & Eid, A. M. (2016). Ethnopharmacological survey of herbal remedies used for treatment of various types of cancer and their methods of preparations in the West Bank-Palestine. BMC Complement. Altern. Med. 16:93. doi: 10.1186/s12906-016-1070-8

Jiao, L., Ling Bi, L., Yan Lu, Y., Wang, Q., Yabin Gong, Y., Jun Shi, J., Xu, L. (2018). Cancer chemoprevention and therapy using chinese herbal medicine. *Biological Proced Online*, 20, 1. https://doi: 10.1186/s12575-017-0066-1.

Jomaa, S., Rahmo, A., Alnori, A. S., & Chatty, M. E. (2012). The cytotoxic effect of essential oil of Syrian Citrus limon peel on human colorectal carcinoma cell line (Lim 1863). Middle East J. Cancer 3, 15–21.

Kaefer, C.M., Milner, J.A. (2008). The role of herbs and spices in cancer prevention. *The Journal of Nutritional Biochemistry*, 19, 347-361. https://doi:10.1016/j.jnutbio.2007.11.003.

Kaileh, M., Berghe, W. V., Boone, E., Essawi, T., & Haegeman, G. (2007). Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity. J. Ethnopharmacol. 113, 510–516. doi: 10.1016/j.jep.2007.07.008

Karakas, F. P., Yidirim, A. B., Bayram, R., Yavuz, M. Z., Gepdiremen, A., & Turker, A. U. (2015). Antiproliferative activity of some medicinal plants on human breast and hepatocellular carcinoma cell lines and their phenolic contents. Trop. J. Pharm. Res. 14, 1787–1795. doi: 10.4314/tjpr.v14i10.8

Kashani, L. M. T., Majdzadeh, M., Khanavi, M., Taghizadeh, M., Sadati, N., Kahkeshani, N., et al. (2014). Cytotoxic activity of selected Iranian traditional medicinal plants on colon, colorectal and breast cancer cell lines. Arch. Breast Cancer, 1, 95–98.

Khalighi-Sigaroodi, F., Ahvazi, M., Hadjiakhoondi, A., Taghizadeh, M., Yazdani, D., Khalighi-Sigaroodi, S., et al. (2012). Cytotoxicity and antioxidant activity of 23 plant species of Leguminosae family. Iran. J. Pharm. Res. 11, 295–302.

Khan, T., Ali, M., Khan, A., Nisar, P., Jan, S.A., Afridi, S., Shinwari, Z.K. (2020). Anticancer Plants: A review of the active phytochemicals, applications in animal models, and regulatory aspects. *Biomolecules*, 10(1), 47.

Khasawneh, M. A., Koch, A., Elwy, H. M., Hamza, A. A., and Schneider-Stock, R. (2015). Leptadenia pyrotechnica induces p53-dependent apoptosis in colon cancer cells. Nat. Prod. Chem. Res. 3, 1–8.

Klug, WS., Cummings, M.R., Spencer, C.A., Palladino, M.A. (2014). Concept of genetics. Pearson Education Limited, 10th edition.

Kooti, W., Servatyari, K., Behzadifar, M., Asadi-Samani, M., Sadeghi, F., Nouri, B., Marzouni, H.Z. (2017). Effective medicinal plant in cancer treatment, part 2: review study. *Journal of Evidince Based Complementary Alternative Medicine*, 22(4), 982–995

Ljubuncic, P., Azaizeh, H., Portnaya, I., Cogan, U., Said, O., Saleh, K. A., et al. (2005). Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel. J. Ethnopharmacol. 99, 43–47. doi: 10.1016/j.jep.2005.01.060

Lowe, S.W., Lin, A.W. (2000). Apoptosis in cancer. *Carcinogenesis*, 21(3), 485-495

Matsuura, K., Canfield, K., Feng, W., Kurokawa, M. (2016). Metabolic regulation of apoptosis in cancer. *International Review of Cell and Molecular Biology*, 327, 43-87. https://doi: 10.1016/bs.ircmb.2016.06.006

Mbaveng, A. T., Kuete, V., & Efferth, T. (2017). Potential of central, Eastern and Western Africa medicinal plants for cancer therapy: spotlight on resistant cells and molecular targets. Front. Pharmacol. 8:343. doi: 10.3389/fphar.2017.00343

Mohammed, M. M., El-Sharkawy, E. R., & Matloub, A. A. (2011). Cytotoxic flavonoids fromDiplotaxis harra (Forssk.) Boiss. growing in Sinai. J. Med. Plants Res. 5, 5099–5103.

Molassiotis, A., Fernadez-Ortega, P., Pud, D., Ozden, G., Scott, J. A., Panteli, V., et al. (2005). Use of complementary and alternative medicine in cancer patients: a European survey. Ann. Oncol. 16, 655–663. doi: 10.1093/annonc/mdi110

Monajemi, R., Oryan, S., Haeri-Roohani, A., Ghannadi, A., & Jafarian, A. (2010). Cytotoxic effects of essential oils of some Iranian Citrus peels. Iran. J. Pharm. Res. 4, 183–187.

Mothana R. A. A., Gruenert R., Lindequist U., Bednarski P. J. (2007). Study of the anticancer potential of Yemeni plants used in folk medicine. *Pharmazie* 62 305–307.

Mrsny, R. (2013). Cancer cell biology, In: biometarials for cancer therapeutics, diagnosis, prevention and therapy, K. Park (Eds), *Woodhead Publishing*, Baskı 1, 530 s, 20-30.

Naghibi, F., Khalaj, A., Mosaddegh, M., Malekmohamadi, M., & Hamzeloo-Moghadam, M. (2014b). Cytotoxic activity evaluation of some medicinal plants, selected from Iranian traditional medicine Pharmacopoeia to treat cancer and related disorders. J. Ethnopharmacol. 155, 230–239. doi: 10.1016/j.jep.2014.05.025

National Geographic Society (US) (2009). National Geographic Visual Atlas of the World. Washington, DC: National Geographic Books.

Newman, D. J., & Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 Years. J. Nat. Prod. 70, 461–477. doi: 10.1021/np068054v

Norton J., Majid S. A., Allan D., Al Safran M., Böer B., Richer R. (2009). *An Illustrated Checklist of the Flora of Qatar.* Gosport: Browndown Publications.

Olaku, O., &White, J. D. (2011). Herbal therapy use by cancer patients: a literature review on case reports. Eur. J. Cancer 47, 508–514. doi: 10.1016/j.ejca.2010.11.018

Oran S. A., &Al-Eisawi D. M. (1998). Check-list of medicinal plants in Jordan. Dirasat 25, 84–112. doi: 10.3390/molecules21030257

Ozkan, G., Kamiloglu, S., Ozdal, T., Boyacioglu, D., & Capanoglu, E. (2016). Potential use of turkish medicinal plants in the treatment of various diseases. Molecules 21:257. doi: 10.3390/molecules21030257

Ozturk, M., Altundag, E., & Gücel, S. (2012). Medicinal and Aromatic Plants (Turkiye). Ethnopharmacology, Encyclopedia of Life Support Systems (EOLSS). Available at: http://www.eolss.net/sample-chapters/c03/e6-79-48.pdf

Pandey, G., Madhuri, S. (2009). Some medicinal plants as natural anticancer agents. *Pharmacognosy Review*, 3, 259-263.

Pecere, T., Gazzola, M.V., Micignat, C. (2000). Aloe-emodin is a new type of anticancer agent with selective activity against neuro-ectodermal tumors. *Cancer Research,* 60, 2800-2804.

Prota, A.E., Bargsten, K., Zurwerra, D., Field, J.J., Díaz, J.F., Altmann, K.H. (2013). Molecular mechanism of action of microtubule-stabilizing anticancer agents. *Science*, 339, 587-590.

Saab, A. M., Guerrini, A., Sacchetti, G.,Maietti, S., Zeino, M., Arend, J., et al. (2012). Phytochemical analysis and cytotoxicity towards multidrug-resistant leukemia cells of essential oils derived from Lebanese medicinal plants. Planta Med. 78, 1927–1931. doi: 10.1055/s-0032-1327896

Said S. A., Al-Saadi S. H. A., Al-Abri A. R., Akhtar M. S., Weli A. M., Al-Riyami Q. (2014). Cytotoxic properties of some herbal plants in Oman. *J. Taibah Univ. Sci.* 8 71–74. 10.1016/j.jtusci.2014.01.004

Saeed, M.E.M., Meyer, M., Hussein, A., Efferth, T. (2016). Cytotoxicity of South-African medicinal plants towards sensitive and multidrug-resistant cancer cells. *Journal of Ethnopharmacology*, 186, 209-223

Safarzadeh, E., Shotorbani, S.S., Baradaran, B. (2014). Herbal medicine as inducers of apoptosis in cancer treatment. *Advanced Pharmaceutical Bulletin*, 4(Suppl 1), 421-427. https:// doi: 10.5681/apb.2014.062

Saganuwan, A. (2010). Some medicinal plants of Arabian Peninsula. J. Med. Plants Res. 4, 767–789.

Sakkir, S., Kabshawi, M., & Mehairbi, M. (2012). Medicinal plants diversity and their conservation status in the United Arab Emirates (UAE). J. Med. Plants Res. 6, 1304–1322.

Salehi, B., Venditti, A., Frezza, C., Yücetepe, A., Altunta, Ü., Uluata, S., Butnariu, M., Sarac, I., Shaheen, S., Petropoulos, S.A., Matthews, K.R., Kılıç, C.S., Atanassova, M., Adetunji, C.O., Ademiluyi, A.O., Özçelik, B., Fokou, P.V.T., Martins, N., Cho, W.C., Sharifi-Rad, J. (2019). Apium plants: beyond simple

food and phytopharmacological applications. *Applied Sciences*, 9, 3547. https://doi:10.3390/app9173547.

Seca, A.M.L., Pinto, D.C.G.A. (2018). Plant secondary metabolites as anticancer agents: successes in clinical trials and therapeutic application. *International Journal of Molecular Sciences*, 19(1), 263. https://doi: 10.3390/ijms19010263

Shabani, A. (2016). A review of anticancer properties of herbal medicines. *Journal of Pharmaceutical Care and Health Systems*, 3, 2. https://doi: 10.4172/2376-0419.1000160.

Sobeh, M., Mahmoud, M. F., Abdelfattah, M. A. O., El-Beshbishy, H. A., El-Shazly, A. M., & Wink, M. (2017). Hepatoprotective and hypoglycemic effects of a tannin rich extract from Ximenia americana var. caffra root. Phytomedicine 33, 36–42. doi: 10.1016/j.phymed.2017.07.003.

Solowey, E., Lichtenstein, M., Sallon, S., Paavilainen, H., Solowey, E., & Lorberboum-Galski, H. (2014). Evaluating medicinal plants for anticancer activity. Sci. World J. 2014:721402. doi: 10.1155/2014/721402

Thapliyal, A., Khar, R.K., Chandra, A. (2018). Overview of cancer and medicinal herbs used for cancer therapy. *Asian Journaal of Pharmaceutics*, 12(1), S1-S8

Tinoush, B., Shirdel, I., Wink, M. (2020). Phytochemicals: Potential Lead Molecules for MDR Reversal. *Frontiers in Pharmacology*, 11, 832. https:// doi: 10.3389/fphar.2020.00832.

Unnati, S., Ripal, S., Sanjeev, A., Niyati, A. (2013). Novel anticancer agents from plant sources. *Chinese Journal of Natural Medicines*, 11(1), 0016-0023. https://doi.org/10.1016/S1875-5364(13)60002-3.

Uysal, A., Zengin, G., Mollica, A., Gunes, E., Locatelli, M., Yilmaz, T., et al. (2016). Chemical and biological insights on Cotoneaster integerrimus: a new (-)-epicatechin source for food and medicinal applications. Phytomedicine 23, 979–988. doi: 10.1016/j.phymed.2016.06.011.

Wasserman, L., Avigad, S., Beery, E. (2002). Nordenberg J Fenig E The effect of aloe-emodin on the proliferation of a new merkel carcinoma cell line. *The American Journal of Dermatopathology*, 24(1), 17-22.

Wu, C.P., Ohnuma, S., Ambudkar, S.V. (2011). Discovering natural product modulators to overcome multidrug resistance in cancer chemotherapy. *Current Pharmaceutical Biotechnology*, 12(4), 609-620

Xiong, J., Ma, Y., Xu, Y. (1992). Diterpenoids from Siegesbeckia pubescens. *Phytochemistry*, 31(3), 917-921

Yarmolinsky, L., Bari, G., Hamias, R., Maor, H., Budovsky, A., Wolfson, M., et al. (2015). Preferential anti-proliferative activity of Varthemia iphionoides (Chiliadenus iphinoides). Isr. J. Plant Sci. 62, 229–233. doi: 10.1080/07929978.2015.1027122

Ye, Q., Liu, K., Shen, Q., Li, Q., Hao, J., Han, F., Jiang, R.W. (2019). Reversal of multidrug resistance in cancer by multi-functional flavonoids. *Frontiers in Oncology*, 9, 487. https://doi: 10.3389/fonc.2019.00487.

Yin, S.Y., Wei, W.C., Jian, F.Y., Yang, N.S. (2013). Therapeutic applications of herbal medicines for cancer patients. *Evidince Based Complementary Alternative Medicine*, 2013, 302426. https://doi: 10.1155/2013/302426

Zaid, H., Silbermann, M., Ben-Arye, E., & Saad, B. (2011). Greco-Arab and Islamic herbal-derived anticancer modalities: from tradition to molecular mechanisms.

Evid. Based Complement. Alternat. Med. 2012:349040. doi: 10.1155/2012/349040.

Zaid, H., Silbermann, M., Ben-Arye, E., Saad, B. (2012). Greco-Arab and Islamic herbalderived anticancer modalities: From tradition to molecular mechanisms. *Evidence-based Complementary and Alternative Medicine*, 2012, 1-14

Zarshenas, M. M., Jamshidi, S., & Zargaran, A. (2016). Cardiovascular aspects of geriatric medicines in traditional Persian medicine; a review of phytochemistry and pharmacology. Phytomedicine 23, 1182–1189. doi: 10.1016/j.phymed.2016.01.014

Zhu, P., Wu, Y., Yang, A., Fu, X., Mao, M., Liu, Z. (2017). Catalpol suppressed proliferation, growth and invasion of CT26 colon cancer by inhibiting inflammation and tumor angiogenesis. *Biomedicine and Pharmacotherapy*, 95, 68-76. https://doi: 10.1016/j.biopha.2017.08.049.

Zohary, M. (1983). "Man and vegetation in the Middle East," in Man's Impact on Vegetation (Geobotany), eds W. Holzner, M. J. A. Werger, and I. Ikusima (Berlin:Springer), 187–295

About the Authors

Salih MUVAKIT, PhD student in Molecular Biology and Genetics at Gaziantep University, Turkiye. He graduated MD in Biochemistry at Gaziantep University, Turkiye. He has MSc/ Diploma, Pharmacy at Kharkov National medical Academy, Ukraine. Also, he has Certification / diploma, Licensed Lab Technician at Medical Laboratory Institute, Syria. He worked as a chief of medical lab in Aleppo, Syria. He has a focus and follow-up on the latest scientific findings related to molecular biology, microbiology, clinical biochemistry, hematology, and immunology.
E-mail: salihmukavit@gantep.edu.tr , **ORCID:** 0000 0003 3783 2444

Prof. Dr. Mehmet OZASLAN received his PhD in 1995 Institute of Natural Sciences at Cukurova University, Turkiye. He is a Professor in Molecular Biology and Genetics. His research interests are included Cancer Genetics, Molecular Virology, Molecular Genetics, Microbiology, and Genetic mutations etc. He has published more than 200 research articles in national and international well reputed journals. He also has written many book chapters as well as edited books. He is an editor and editor in chief of many well reputed national and international journals.
E-mail: ozaslanmd@gantep.edu.tr , **ORCID:** 0000 0001 9380 4902

To Cite This Chapter:
Muvakit, S., & Ozaslan, M**.** (2023). **Near east's medicinal herbs for cancer treatment**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023* (pp. 145-167). ISRES Publishing.

Section

II

LIFE SCIENCES

NANO PARTICLE BASED DRUG DELIVERY IN CANCER THERAPY

Khadija NASİR
Saba SABA
Abid HUSSAİN
Kainat RAMZAN
Sobia MUSHTAQ

1. Introduction

The concept of "cancer" first appeared by the Greek physician Hippocrates, refers to abnormal growths that often have finger-like extensions and the term "oncos," by coined by Galen which means inflammation (Koirala et al., 2023; Stathopoulos, 2017). The tumor hallmark traits include unregulated cell growth, a deficit in the cell division, resistant to DNA damage, disruptions in oxidative equilibrium, raised cell stress responses, and metabolic imbalance (Emami Nejad et al., 2021). In 2020, there were 10.3 million cancer-related fatalities, alongside 19.3 million newly reported cases. Research also predicts that this number is projected to increase to 22 million in the future (Li et al., 2017). The population rate, economic and ecological challenges have contributed to an increase in cancer rates and deaths (Li et al., 2017). With varied degrees of effectiveness, many therapies are being tried against cancer; the most common include radiation therapy, chemotherapy, and surgeries (Joshi & Badgwell, 2021). The lack of a target, the severe side effects of prescribed drugs, and the low water solubility are the three main execution-related problems. With a focus on optimizing drug delivery, nanoparticles (NPs) were widely studied for a broad spectrum of therapeutic uses in cancer diagnosis (Afzal et al., 2022; Fu & Yang, 2023). To address these issues, treatment drugs are made from nanomaterials (NPs), which can be divided into inorganic, organic, composite, metallic, polymeric, and nanotube forms (J. Liu et al., 2023). The ability to target certain regions and the use of the permeability and retention (EPR) effect to improve drug retention in tissue targets are just a few of the significant advantages that nanoparticle-based drug delivery offers over traditional approaches. NP-based medications improve the pharmacokinetics and pharmacodynamics of medicine (Tiwari et al., 2023). In 1959, Richard Feynman suggested NPs as drug carriers, and around 12,000 papers have discussed their relevance against tumor treatment (Cheng et al., 2021; Nirmala et al., 2023). Liposomes based drugs were first discovered by a group led by British scientist Alec D. Bangham in 1965 (Li et al., 2017). The US Food & Drug Administration (FDA) has given Doxil (doxorubicin) permission to treat Kaposi sarcoma linked to HIV-AIDS. Breast, non-small cell lung, and pancreatic carcinoma are managed with abraxane (paclitaxel), a nano-formulation based on albumin (Cardoso et al., 2022; Carretta & Cardarelli, 2023).

Several methods are currently being studied to address drug resistance, including the use of solid lipid NPs, mesoporous silicon NPs, nanoparticulated chemosensitizers, polymer nanoparticles, and magnetized nanoparticles (Y. Zhang et al., 2018). Anticancer drugs often exhibit poor water solubility and digestion due to their hydrophobic nature, but this issue can be solved using nanocrystals, polymeric micelles, and chitosan-based formulations (Kapse-Mistry et al., 2014; Zhang et al., 2022). A developing method of treating cancer in the field of nano-oncology that shows efficacy in both laboratory studies and clinical trials on humans is the use of nano-dimensional medicinal drugs as antioxidants (Sato et al., 2021). Nanoparticles are now an innovative method for treating numerous diseases due to their enormous

potential and curative efficacy. Cancer nanomedicine is used to optimize tumor therapy and has potential for targeting, imaging, the use of viral nanoparticles, and improved drug delivery (Kemp & Kwon, 2021). The present study examines how nanoparticles can be used in cancer treatment beyond their conventional use as therapeutic agents. It entails a comprehensive NPs classification, as well as a review of drug delivery methods. We understand the potential advances in this area, particularly in considering the rapid and dynamic progress being made in both nanotechnology and cancer research. In the future, it might open up opportunities for a number of highly effective methods that will greatly enhance cancer therapy. By highlighting their expanding roles, challenges, and promise for ground-breaking advancements in the domain of tumor treatment, this research study offers a comprehensive evaluation of the present scenario and possibilities for nanoparticles in tumor treatment.

2. Emergence of FDA approved drugs

Both the FDA and the European Medicines Agency (EMA) have allowed to several nanomedicines for cancer diagnosis and numerous formulations are currently undergoing testing and evaluation (Martinelli et al., 2019; Tucci et al., 2019). Colloidal gold (Au) nanoparticle use as medication may represent the initial phase of nanomedicine (Bhagyaraj et al., 2018). The pegylated liposome Doxil® was approved for use in Japan to treat cases of hepatic carcinoma, while the polymer-protein combo Zinostatin stimalamer was recently authorized for the cure of ovarian carcinoma (Beltrán-Gracia et al., 2019; Bulbake et al., 2017). Numerous nano-formulations are now in various stages of clinical and preclinical research, illustrating their ongoing evolution. Following the approval of an Investigational New Drug (IND), studies are conducted to evaluate the effectiveness and reliability of the 30 new nanomedicines (Fu & Yang, 2023). A list of the authorized nanomedicines that are contained therein is given in **Table 1** for therapeutic purpose.

Table 1. Nanomedicine licensed for cancer therapy

Product	Drug	Drug Based	Cancer type	Approval	References
Zinostatin stimalamer	Neocarzinostatin -maleic anhydride of styrene	Protein-Polymer Combination	Liver Cellular Cancer	1994 - Japan	(Pereira et al., 2021)
Doxil (Caelyx)	Doxorubicin hydrochloride	Pegylated Liposome	Kaposi's Sarcoma And Ovarian Carcinoma	1995 - FDA	(Yang et al., 2022)
DaunoXome	Daunorubicin	Liposome	HIV-Related Kaposi Sarcoma	1996 - FDA	(Ansari et al., 2020)
Lipo-Dox	Doxorubicin	Liposome	Breast, Ovarian, And Kaposi's Sarcoma	1998 - Taiwan	(Chiang et al., 2021)
Myocet	Doxorubicin	Liposome	Mammary Tumor	2000 - EMA	(Aulic et al., 2020)

Mepact	Muramyl tripeptide phosphatidyl ethanolamine	Liposome	Non-Metastatic Osteosarcoma	2009 - EMA	(Brard et al., 2019)
Lipusu	Paclitaxel	Liposome	Breast Cancer, Non-Small-Cell Lung Cancer	2013 - EMA	(Ghosh et al., 2021)
NanoTherm	Fe_2O_3	Iron Oxide Superparamagnetic Coated With Amino Silane	Pancreatic, Prostate, and Glioblastoma Tumors	2013 - EMA	(Ghosh et al., 2021)
Ameluz	5-Aminolevulinic acid	PG, E211, Soypc, And 5-Aminolevulinic Acidic Gel	Nodular Or Superficial Cancer Of The Basal Cells	2011 - EMA	(Rodríguez et al., 2022)
Depocyt	Cytarabine	Liposome	Meningitis With Lymphomatous Tumor	1999 (FDA)	(Salehi et al., 2020)
Nanoxel	Docetaxel	Micelle of polymeric NPs	NSCLC, Kaposi's Sarcoma Tied To AIDS, Cancers Of The Breast And Ovaries	2006 - India	(Harshita et al., 2019)
Marqibo	Vincristine	Liposome	Leukemia	2012 - FDA	(Fulton & Najahi-Missaoui, 2023)
Onivyde	Irinotecan	Liposome	Pancreatic Cancer	2015 - FDA	(X. Wang et al., 2021)
Vyxeos	Daunorubicin and cytarabine	Liposome	Acute Myeloid Leukemia (AML)	2017 - EMA	(Tzogani et al., 2020)
Oncaspar	l-Asparaginase	Pegylated Conjugate	Acute Lymphoblastic Leukemia	2006 - FDA	(Van Trimpont et al., 2022)
Irinotecan	Onivyde (Merrimack)	Pegylated Liposome	Tumors of The Breast, The Spleen Sarcoma or Brain	2015 - FDA	(Chiang et al., 2021)
DPH107	Paclitaxel	Lipid Nanoparticles	Acute Stomach Malignancy	2016 - Korea	(Nirmala et al., 2023)
NBTXR3 (Hensify)	Hafnium oxide	Hafnium Oxide Nanocrystal	Metastatic Cancinoma Of	2019 - CE Mark	(Anselmo &

			Squamous Cells		Mitragotri, 2019)
Apealea	Paclitaxel	Polymeric Micelles	Fallopian Tube, Peritoneal, And Cancer Of The Ovary	2018 - EMA	(Parodi et al., 2022)
Ontak	Denileukin diftitox	Toxic Peptide Made From Recombinant DNA	Skin-Specific T Cell Malignancy	1999 - FDA	(Yang & Bae, 2023)
Eligard	Leuprolide acetate	Polymeric Nanoparticles	Advanced Prostate Cancer	2002 - FDA	(S. Wang et al., 2022)
Abraxane	Paclitaxel	Protein Carrier	Metastatic And Pancreatic Cancers	2005 - FDA	(Ying et al., 2023)
Kadcyla	DM1	Trastuzumab, Covalently Linked To DM1	HER^{2+} Breast Cancer	2013 - FDA, EMA	(Rodríguez et al., 2022)
Pazenir	Paclitaxel	Albumin-Bound Nanocrystals Of Paclitaxel	Non-Small Cell Lung Cancer, Distant Breast Carcinoma, And Advanced Tumors Of The Pancreas	2019 - EMA	(Rodríguez et al., 2022)

The FDA now permits over 50 drugs for clinical usage, and Lipusu was created in 2013 by combining paclitaxel for the curative use of lung, ovarian, and stomach malignancies (Beltrán-Gracia et al., 2019). In 2017, Vyxeos a liposomal drug containing daunorubicin and cytarabine are tested against acute myeloid leukemia (Krauss et al., 2019). Irinotecan-loaded PEGylated liposome, marketed as Onivyde, has indeed received FDA approval for the treatment of pancreatic adenocarcinoma. For the treatment of lymphomatous meningitis, Cytarabine-loaded liposomes sold under the trade name DepoCyt were authorized (Nirmala et al., 2023). Nearly 250 nanomedicines have obtained market licenses or are in multiple phases of the medical advancement, as indicated in **Table 2**. There are now more nanodrugs in clinical studies than ever before, with many of them in Phase 2 and Phase 3 trials. The variety of nano-formulations with passed clinical trials remains a relatively minor challenge to overcome until cancer nanomedicine can be used to its greatest extent (Bremer-Hoffmann et al., 2018).

Table 2. Clinical studies involving nanomedicine are now underway to combat tumors.

Nanomaterials	Drug trademark	Medication usage	Cancer Subtype	Phase	Identifier	References
Nanoparticles	Nanoxel	Paclitaxel	Advanced breast cancer	I	NCT00915369	(Brard et al., 2019)
	Nav-paclitaxel with S-1	Nav-paclitaxel with S-1	Pancreas Cancinoma	II	NCT02124317	(Ghosh et al., 2021)
	Magnetic nanocrystal	Iron based NPs	Prostate tumor	Initial I (finished)	NCT02033447	(Rodriguez et al., 2022)
	ABI-007	Synthesis of paclitaxel albumin-stabilized nanocrystals	Non-small cell lung carcinoma in stage 4	I,II	NCT00077246	(Salehi et al., 2020)
	Nanotax	Nanoparticulate paclitaxel	Peritoneal cancers	I (completed)	NCT00666991	(Harshita et al., 2019)
	NC-6300	Epirubicin	carcinoma of the connective tissue	I,II	NCT03168061	(Fulton and W. Najahi-Missaoui, 2023)
	Rexin-G	Gene	Sarcoma	I,II	NCT00505713	(Tzogani et al., 2020)

	Lurtotecan liposome	Lurtotecan	ovarian epithelial tumor	II (completed)	NCT00010179	(Van Trimpont et al., 2022)
	Lipodox®	Doxorubicin hydrochloride	Ovary and breast tumors	I	NCT05273944	(Cheng et al., 2021)
	DOTAP:Chol-FUS1	fus1 gene	Pulmonary tumor	I (completed)	NCT00059605	(Anselmo and Mitragotri, 2019)
	Liposomal daunorubicin	Daunorubicin	Breast carcinoma	I	NCT00004207	(Parodi et al., 2022)
	Immunoliposomes using anti-EGFR and doxorubicin	Doxorubicin with anti-EGFR immunoliposomes	Solid tumors	I (completed)	NCT01702129	(Wang et al., 2022)
	Liposomal paclitaxel	Paclitaxel	Cancer	IV	NCT00606515	(Ying et al., 2023)
	E7389 liposomal formulation	Eribulin	Solid tumor	I	NCT03207672	(Krauss et al., 2019)
	FF-10832	Gemcitabine	Solid tumors	I	NCT03440450	(Bremer et al., 2018)
	Liposomes of irinotecan and bevacizumab	Irinotecan sucrosofate	Fallopian tube, primary peritoneal, or ovarian	II	NCT04753216	(Cheng et al., 2021)

Liposome			carcinoma that is resilient to platinum			
	NL CPT-11	CPT-11	Lymphoma	I	NCT00734682	(Li et al., 2022)
	liposomes of mitoxantrone HCL	Hydroxychloride of mitoxantrone	Ovary tumors tolerant to platinum	I	NCT04718376	(Dreaden et al., 2012)
	Irinotecan liposome	Irinotecan	liver tumor	I	NCT04796948	(Barua and S. Mitragotri, 2014)
	LEP-ETU	Paclitaxel	Advanced Tumorigenesis	I	NCT00080418	(Gigliobianco et al., 2018)
	LE-DT	Docetaxel	Persistent Carcinoma	I	NCT01151384	(Kaur et al., 2021)
	Topotecan liposomes	Topotecan	solid tumors, cervical cancer, and small-cell lung carcinoma	I	NCT00765973	(Jhaveri and V.P. Torchilin, 2014)
	Liposome doxorubicin	Doxorubicin	Desmoid growth	III	NCT05561036	(Cheng et al., 2021)

	FF-10850	Topotecan	Merkel cell tumor	I	NCT04047251	(Hussein and N.K. Maraie, 2021)
Polymeric nanoparticles	Cetuximab nanoparticles	A combo of somatostatin analog and cetuximab	Cancer of the gut	I	NCT03774680	(Das et al., 2019)
	Using taxotere and docetaxel-PNP	Docetaxel	Solid tumors	I	NCT02274610	(Nsairat et al., 2022)
Carbon nanoparticles	Carbon NPs	Nanocrystals with carbon and green indocyanine.	Tumor of the colon	II,III	NCT04759820	(Sonju et al., 2022)
	Carbon NPs	Carbon NPs	Stomach cancer	III	NCT02123407	(Yuan et al., 2017)
Paclitaxel albumin-stabilized nanoparticle formulation	Combination of Abraxane and Lapatinib	Abraxane	Breast tumors may be in phase I, II, or III.	I (completed)	NCT00331630	(Vickers, 2017
	Paclitaxel with granulocyte-macrophage colony-stimulating factor (AbraxaneTM)	Synthesis of paclitaxel albumin-stabilized nanocrystals	acute peritoneal cancer, uterine cancer, or severe ovarian cancer	II (completed)	NCT00466960	Cheng et al., 2021)

Lipid nanoparticles	TKM 080301	anti-siRNA for the PLK1 gene	Malignancies of the Colon, Liver, Stomach, Ovarian Cysts, Breast, and Throat that Traveled To The Liver	I(completed)	NCT01437007	(O'Brien et al., 2013)
	INT-1B3	microRNA (miR-193a-3p)	Advanced solid tumors	I	NCT04675996	(Sasaki et al., 2022)
	OTX-2002	Biscistronic mRNA	MYC oncogene and liver cancer	I, II (completed)	NCT05497453	(Wilhelm et al., 2016)
	WGI-0301	Archexin®	massive solid lesions	I	NCT05267899	(Cheng et al., 2021)
Iron oxide nanoparticles (SPION)	AGuIX gadolinium-based nanoparticles	AGuIX	Pancreatic cancer and lung cancer	I, II (completed)	NCT04789486	(Zhao et al., 2023)
Gold nanoparticles	Nano-QUT	Quercetin	Squamous cell carcinoma	II	NCT05456022	(Cheng et al., 2021)

Super paramagnetic nanoparticle	Combining albumin-bound paclitaxel (Abraxane), carboplatin, and herceptin	Abraxane	Advanced breast cancer	II	NCT00093145	(Cheng et al., 2021)
Albumin-bound nanoparticle paclitaxel	Abraxane® contains Mifepristone or not	Mifepristone	Breast cancer being triple-Negative plus hormone receptor positive	II	NCT02788981	(Cheng et al., 2021)
Micellar nanoparticle	Nab-PTX plus S-1 and sintilimab	Nab-PTX	Stage IIIC gastric cancer	I, II (completed)	NCT04781413	(Cheng et al., 2021)
Magnetic nanoparticles	EP0057 in combination with olaparib	EP0057 and olaparib	Advanced intestinal cancer and small cell lung tumors	II	NCT05411679	(Siddique and J.C. Chow, 2020)
CriPec® nanoparticles	CRLX101(NLG207)	CRLX101	Non-small cell lung cancer in late stages	II	NCT01380769	(Cheng et al., 2021)
Iron nanoparticles	NK105	Paclitaxel	Breast cancer	III	NCT01644890	(Sonju et al., 2021)

Sterile nanoparticulate paclitaxel	Magnetic particle-ICG	Indocyanine green (ICG) and magnetic tracers (FerroTrace)	intestinal cancer	I, II (completed)	NCT05092750	(Cheng et al., 2021)
Nanocrystals Of quaternary ammonium polyethylenimine	CPC634 (CriPec® Docetaxel)	Docetaxel	Ovarian tumors with platinum sensitivity (CINOVA)	II	NCT03742713	(Cheng et al., 2021)
Hafnium oxide-nanoparticles	NanoPac	Paclitaxel	Lung cancer	II	NCT04314895	(Farzin et al., 2020)
Silica nanoparticles	ELU001	Exatecans and derivatives of folic acid	Overexpressed folate receptor alpha positive solid cancers	I, II (completed)	NCT05001282	(Cheng et al., 2021, Desmond et al., 2021)
Doxorubicin hydrochloride liposome	Doxorubicin combined with bevacizumab sodium lipid particles	Bevacizumab	Mammary cancer	II	NCT00445406	(Cheng et al., 2021, Li et al., 2019)

3. Classification of Nanoparticles

Most prescribed drugs have difficulties being through the small capillaries, which are typically 5 to 6 microns wide. NP-based drugs often have exact sizes between 10 and 100 nanometers, enabling it to deliver medicines to various body parts while getting beyond the biological hurdles posed by these tiny capillaries (Dreaden et al., 2012; P. Li et al., 2022). The drugs are bound onto the particle surface and incorporated into their matrix due to a large ratio of surface to volume, resulting in a greater dissolving rate, such as Paciltaxel, Amphotericin B, or Cyclosporine when administered as a nano-suspension in the gastrointestinal tract (Barua & Mitragotri, 2014). Nanoparticles can be created from a diverse range of materials, including lipids, polymers, proteins, metals, and semiconductors, and they can take on various shapes, including spheres, tubes, and rods, as shown in supplementary **Figure S1** (Gigliobianco et al., 2018).

3.1. Amphiphilic polymeric micelles (APMs)

A range of administration methods have proved the value of amphiphilic polymeric micelles (APMs). Copolymers such as ethylene oxide, polybenzoyl aspartate, and poly-N-polyacrylamide polystyrene are often employed to make these micelles. Nanoparticles play a crucial role in diagnosing and treating a wide spectrum of diseases. They achieve this by enhancing the permeability of lipophilic drugs, facilitating molecular targeting, exhibiting stimuli-responsive characteristics, improving therapeutic effectiveness, and reducing dose-related toxicities (Kaur et al., 2021). Micelles with dimensions of less than 100nm and a hydrophobic center covered by a hydrophilic shell make excellent drug carriers. The PEG-poly (D, L lactic acid) and gridlock polymers such as pluronic have been widely used in the micelles fabrication and This improves cancer treatment efficacy and minimizes the side effects associated with standard chemotherapy (Hussein & Maraie, 2021; Jhaveri & Torchilin, 2014).

3.2. Liposome

Liposomes are formed up of cholesterol and organic or synthetic phospholipids and range in size from 1 to 150nm and can be delivered orally or through injection. For several drugs, FDA-approved intravenous injection remains a highly effective method of administration (Marasini et al., 2017) and based on drug accumulation, uptake, and release through active or passive targeting (Das et al., 2019). Liposomes can flow easily through blood vessels while conveying water-soluble or repellent drugs, and they can be defined relying on their charge, such as cationic, anionic, or neutral liposomes, to optimize pharmaceutical administration for a number of medical uses (Nsairat et al., 2022). Liposomes are modified for delivering drugs to tumor sites using antibodies, small molecules, amino acids, and polysaccharides (Sonju et al., 2021). Antibodies have the capability to bind to cancer-specific antigenic surfaces, including MCAM, CD44, VEGFR, and EGFR receptors. Moreover, small molecules like folate, estrone, and anisamide can serve as targets on tumor surfaces. Researchers have also investigated carbohydrates and proteins that can bind to specific receptors on cell surfaces, such as mannose and β-FGF, as potential surface-active molecules in cancer therapy (Nirmala et al., 2023).

In 2013 Doxorubicin (DoxilTM), a PEGylated liposome initially licensed for late ovarian cancers, myeloma, and HIV-combined Kaposi's sarcoma, was a pioneering

small drug for tumor clinical trials (Vickers, 2017; Yuan et al., 2017). Patients with Philadelphia chromosome-negative (Ph-) lymphoblastic leukemia may find potential benefits in the administration of vincristine sulfate liposome, also known as MarqiboTM. OnivydeTM, which is an irinotecan liposome-based formulation, can be employed to treat patients with persistent pancreatic cancer. In the context of ultrasonic hyperthermia, temperature-sensitive liposomes can be loaded with gemcitabine and a copper complex, facilitating the controlled release of these drugs into the tumor vasculature (Farzin et al., 2020; Nirmala et al., 2023; Siddique & Chow, 2020; Zhao et al., 2023). CPX-351 combining with cytarabine and daunorubicin, received expedited status as a future therapy for acute myeloid leukemia. This offers high flexibility in clinical drug coupling, making them a significant asset in drug delivery and tandem therapeutic techniques. Bevacizumab is now certified for the medicinal use of patients with platinum-resistant, recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer when paired with paclitaxel, pegylated liposomal doxorubicin, or topotecan (Knežević et al., 2019). Even so, research is required to address the current drawbacks of liposomal treatment with regard to sustained constancy, accurate dosing, and effective selection.

3.3. Metallic nanoparticles

Nanoparticles made from gold (Au) and silver (Ag) have the ability to provide a wide spectrum of therapeutic compounds to cancer areas. Gold NPs have proven effective as radio sensitizers in various medical applications, including drug delivery and cancer therapy. The primary mechanism for drug targeting involving these NPs often relies on the EPR effect, which allows them to accumulate preferentially in tumor tissues. They may enhance drug selection by utilizing tumor microenvironmental features such as altered reactive oxygen species and acidity. These can also be used in hyperthermic therapy, which involves the use of a natural thermal source (microwaves) to raise the temperature, particularly at the site of cancer, and so improve the therapeutic efficacy of certain medications. However, there are drawbacks to using metal NPs, such as the possibility of aggregation and increased cytotoxicity (Desmond et al., 2021; Li et al., 2019).

3.4. Magnetic nanoparticles

The administration of tiny magnets into the target site, usually via catheters or hypodermic needles, allows for accurate tumor selection and precise ablation. The magnetic NPs show promise as a valuable tool in cancer therapy, providing an amalgam of targeted drug delivery and localized hyperthermic treatment to improve treatment outcomes while reducing negative effects on healthy tissues (Wagner et al., 2019). Microwave-induced hyperthermia can indeed be employed to raise the temperature at a specific target, typically to around 42° degree. This elevated temperature can selectively damage cancer cells, as they tend to have leaky vascular structures, while having a minimal impact on healthy cells. Superparamagnetic materials are useful for a variety of purposes, such as theranostics (integrating therapy and diagnostics), photodynamic therapy, photothermal laser therapy, biological sensors, drug administration, and Magnetic scanning, frequently in conjunction with hyperthermia. Because of their superparamagnetic nature, these applications allow for precise control and targeting, making them ideal tools in cancer diagnosis and treatment (Walling et al., 2009).

3.5. Quantum dots

Quantum dots (QDs) are colloidal fluorescent semiconductors with nanocrystal structures that are often formed of elements from groups 2 to 4 or 3 to 5 of the periodic table, such as Cadmium Selenide, Indium phosphide, and Indium arsenide, and have sizes ranging from 2 to 10nm. Carbon quantum dots (CDQs) have applications in tumor imaging and administering drugs, making them ideal for bioimaging and contributing to their overall theranostic effectiveness (Saifuddin et al., 2013). Because of their low toxicity, excellent biocompatibility, simplicity of targeting, and diagnostic capabilities, QDs are emerging as an exciting avenue for nano-drug carriers. They have lengthy circulation duration in the bloodstream and can generate fluorescence in living creatures for extended periods of time, making them highly useful agents for in vivo cancer diagnosis and therapy. Overall, CQDs and QDs are at the forefront of cancer diagnostics and treatment research (He et al., 2013; Sharma et al., 2016; Zhang et al., 2011).

3.6. Carbon Nanotubes (CNTs)

Carbon atoms are grouped in a variety of configurations, including globes and cylindrical shapes, to form the extraordinary structures known as carbon nanotubes (CNTs). They have a distinctive structure made up of a tubular arrangement of condensed benzene rings, which frequently include fullerenes, carbon nanoparticles, graphene, and nanodiamonds. Single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) are the two primary subtypes of CNTs. In the realm of treating malignant tumors, these nanotubes have shown to have substantial potential (Mittal et al., 2021; Nirmala et al., 2023). SWCNTs have the ability to bind to p-glycoprotein antibodies and serve as carriers for the anticancer drug doxorubicin. This combination showed a significant boost in cytotoxicity, with 2-4 times greater efficacy against K562R leukemia cells than free doxorubicin alone. This intriguing discovery shows SWCNTs' potential as useful components in improving cancer therapy effects (Chiang et al., 2021; Nicoud et al., 2023).

3.7. Dendrimer

Dendrimers are synthetic NPs distinguished by their radially symmetric monomer groups, which give them a tree or branch-like form. Their distinctive structure provides remarkable surface functionality and targeting ability to target specific molecules or cells, making them one of the most versatile nanocarriers with advanced surface functioning attributes (Chiang et al., 2021). These can also be hybridized into a variety of shapes, such as enclosing them in various polymeric shells, resulting in a diverse drug delivery platform for cancer therapy (Jabir et al., 2012). In 2013, Abraxane (ABI-007) made from organic polymeric albumin paired with paclitaxel, proved to be highly effective in the nanomedicine (Zhu et al., 2012). Several paclitaxel (PTX)-polymer designs exist at various phases of clinical experiments, including CT-2103, which uses polyglutamate-conjugated PTX and is being tested for ovarian cancer. Additionally, OP-101 (dendrimer N-Acetyl-Cysteine) and ImDendrim are in the early clinical stage, primarily focusing on safety assessments in phase I trials (Wahren-Herlenius & Dörner, 2013; Wang et al., 2018). Several ways were used to improve the efficacy of dendrimers-mediated drug administration. These tactics often involve changes to the quantity of end groups, adding degradable spacers, and adding linkages that are pH-dependent. Additionally, several intrinsic drawbacks of

dendrimeric-based drug administration have been addressed by surface functionalization approaches using molecules including nutrients, antigens, and polypeptide. (Liu et al., 2018).

3.8. Nanodiamonds and Nanoshells

Nanodiamonds (NDs) are carbon-based gemstones distinguished by their truncated octahedral architecture and diameters between 2 and 8 nanometers, and they exhibit characteristics similar to those of diamond-like materials (Punu et al., 2023). They are made of a silica center encased in a thin gold coating, usually have the unique ability to scatter light, making them useful for cancer imaging applications (Nirmala et al., 2023). One significant application is photothermal treatment (PTT), where metallic nanoshells are being shown to kill localized malignancies. This procedure not only removes tumors but also releases endogenous immunostimulatory chemicals, which activate dendritic cells. As a result, this holds potential as a tool for cancer therapy and immune response augmentation against cancer cells (Akanda et al., 2021; Giri et al., 2023).

3.9. Solid lipid nanoparticles (SLN)

The SLNs typically consist of three main components such as solid lipid, an emulsifier, and water. The solid liquid component can encompass various types of lipids, that offers versatility in terms of administration routes and can be delivered via parenteral (injection), oral (by mouth), rectal (via the rectum), ocular (eye), and topical (applied to the skin) methods. Within the realm of SLNs, the solid solution model and the core-shell model are commonly employed (Du et al., 2019; Mundekkad & Cho, 2022). SLNs showed enhanced drug diffusion rates due to their large surface area, the drug's high mobility and the crystalline nature of lipid carriers may result in simpler release kinetics. Passive, active, or co-delivery approaches can be used to deliver SLNs to their intended target (Dai et al., 2016). Linalool drugs are formulated with Pluronic F68 serving as a surfactant, and the active component comprises cetyl esters, cetyl palmitate, or myristyl myristate. Additionally, a pH-sensitive Solid Lipid Nanoparticle (SLN) formulation of doxorubicin was developed through the modification of an arginine-glycine-aspartic acid (RGD) peptide. This modification aimed to combat drug resistance in breast tumors by harnessing the adaptability of SLNs in drug delivery and leveraging their capability to address therapeutic challenges in cancer therapy (Nam et al., 2019).

Nano-Drug Delivery Targeting mechanism

Drug distribution can vary depending on the medium and is designed to carry drug components straight into blood arteries and to the site of action. They may cause DNA damage by producing an excessive amount of reactive oxygen species (ROS) once they have reached their target. This could ultimately result in cellular death, or programmed cell death, which kills the targeted cells (Ejigah et al., 2022; Kvistborg & Yewdell, 2018). **Figure 2** depicts how nanoparticles (NPs) deliver medications in passive or active targeted modes (Tran et al., 2017; Wu, 2021). These methods are intended to improve the accuracy and efficiency of administering drugs to the tumor site (Alsaggar & Liu, 2018).

4.1. Passive targeting

The formation of a drug at a specific place due to pathophysiological factors that allow the NPs to thrive in the tumor site is referred to as passive targeting and is mainly due to a diffusion method. Factors such as hypoxia (low oxygen levels) or pH imbalances can cause the formation of new blood vessels in fast-developing cancers, allowing for the specific increased penetration of biomolecules into the tumor cells (Scott et al., 2012). Cancer cells exhibit irregular neovascularization, or the disorderly creation of new arteries and veins, and discharge large amounts of pro-inflammatory markers, creating the inflammatory environment seen in lesions Furthermore, tumors often lack an efficient lymphatic flow system, which may hinder the removal of excess fluid and debris from the tumor site (M Rabanel et al., 2012). NPs are not promptly eliminated from the tumor site because to poor lymphatic activity. The EPR effect is regulated by factors such as interstitial escape, dispersion, tumor vasculature, tumor extravascular environment, and physiochemical conditions (Akakuru et al., 2018; Das et al., 2019; Navya et al., 2019; Sebastian, 2017a). Physical properties such as form, size, charged surface, and chemical composition are principally responsible for the NPs deposition (Ejigah et al., 2022; Wu, 2021). Certain NPs can pass through blood vessel walls and penetrate tissue due to their size and surface properties. Passive targeting can be used to treat diseases such as tumors and inflammatory tissues (Pei et al., 2020; H. Wang et al., 2022). The improved plasma half-lives, longer circulation times, and higher drug concentrations are all effects of the particles with charges (neutral or negative). Adjuvants such as nitric oxide donors can also be employed to enhance the EPR effects (Attia et al., 2019). When NPs enter the body, they undergo a series of steps, including circulation, endocytosis, and aggregation inside target tissues. The opsonization mechanism, which identifies NPs for biological clearance, is especially prone to NPs. By limiting opsonin absorption onto the NPs, PEGylation polymers can be used to achieve nanoscale stealth-like properties. To escape immune clearance, another method is to quiet or diminish Kupffer cells, which are specialist macrophages that regulate foreign material intake in the liver's reticuloendothelial system (Nam et al., 2019). Recently, PEGylated Prussian blue particles were used to reduce tumor hypoxia and modulate polyethyleneimine cytotoxicity in breast cancer cells (Chiang et al., 2021). A study revealed tumor cell deaths and necrosis administered with PEGylated nanographene oxide, indicating a prime instance of combined therapy (Attia et al., 2019). Liposomes are designed to be stable at pH 7.4 but disintegrate to release molecules at acidic condition. As a result, glycolysis is used to obtain more energy in an acidic environment. Also, tumor cells express and secrete enzymes that are involved in their mobility and survival, such as matrix metalloproteinase. However, passive target methods have a few drawbacks, including that certain drugs fail to diffuse properly and the process is difficult to manage. The passive technique is further limited since some malignancies do not exhibit the EPR effect and blood flow may fluctuate within a single tumor (Drago et al., 2021; Tran et al., 2017).

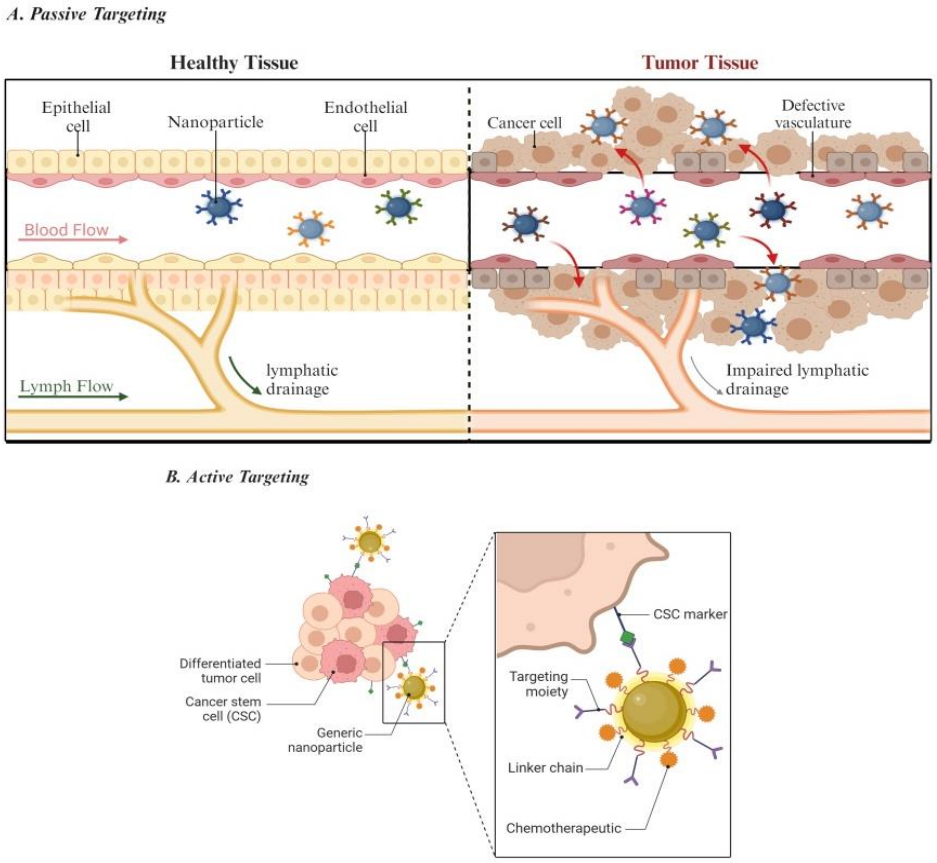


Figure 2. Cancer targeted by nanocarriers. A) Passive drug targeting, B) Active drug targeting

4.2. Active Targeting

The presence of specific ligands on target cells is essential for active targeting, also known as ligand-mediated targeting, which has gained a lot of attention because cancer cells often unregulated certain receptors. Transferrin receptors (TfRs) and folate receptors (FRs) are two types of receptors that exist naturally on many normal cells but are detected in various cancer types due to the increased metabolic activity of cancer cells. For instance, TfRs are being used for administering drugs in the context of circulating leukemic cells. Overexpressed folate receptors have been reported in ovarian, breast, lung, colon, kidney, and brain malignancies, among other cancer types. By focusing on these receptors, drug delivery can be made more selective and effective while simultaneously having less of an influence on healthy, normal cells (Awad et al., 2023). In order to increase the specificity of medication delivery to particular cell types, the idea of actively targeting cell surfaces with ligands such as antibodies, protein peptides nucleic acids, and aptamers initially surfaced in 1980. Micelles were used in studies to target nicotinic acetylcholine receptors (nAChRs) in the CNS and glioblastoma, and it highlights the versatility of ligand-mediated targeting by allowing for the exact targeting of certain receptors or molecules associated with specific disorders (L. Chen et al., 2022). The density of NPs must be constantly managed to optimize their circulation time in the bloodstream and prevent their quick detection by the RES. This entails adjusting the surface design and characteristics of the particles to limit interactions with serum proteins and lymphocytes (Herbst et al., 2013). For great specificity in targeted activity, the receptors must be prevalent in cancer cells and uniformly expressed. The ability of targeted conjugates to be ingested is a basic prerequisite for effective ligand targeting,

this is accomplished through receptor-mediated endocytosis, in which targeting conjugates bind to their receptors on the cell membrane, resulting in the formation of an endosome, which then travels to specific organelles within the cell, where drugs are released, which can be caused by acidic pH or enzymatic activity. The combined use of active and passive targeting is utilized to lessen drug exposure to healthy tissues, improve nanoparticle interface with cancer cell surfaces, and increase drug penetration into tumor tissue (Powles et al., 2014; Rani et al., 2023).

5. Benefits of nanomaterial applications

5.1. Nanomaterial in cancer therapy

Although tumors are diverse and intricate, NPs are often designed as multifunctional nanoplatforms to hold substantial promise for improving cancer treatment, with the possibility for more effective and targeted cancer therapies (Afzal et al., 2022). In 2010, FDA licensed sipuleucel-T drug to treat castration-resistant prostate cancer, and an anti-CTLA-4 antibody (2011) was approved for treating melanoma patients. Moreover, the MPDL3280A showed promising results in the lung and prosthetic melanoma, and the study explores the possible use of antibodies targeting PD-1 and PD-L1 against malignancy (Yuan et al., 2017; Zanganeh et al., 2016). Doxorubicin (DOX), paclitaxel, tamoxifen, trastuzumab, docetaxel, and cisplatin are well-known medications that are being proven to be effective in breast cancer treatment (Smith et al., 2013). Also, Au NPs based drugs that are often used in chemotherapy include EpCAM-RPAnN and DOX-BLM-PEG-Au NPs (Irvine et al., 2015).

An emerging field of study focuses on finding nanoconstructs able to influence certain phases of the inflammatory cascade. For instance, Ferumoxytol an FDA-registered iron based NPs form that was developed to treat anemia. Recent studies indicate that it may alter tumor-associated macrophages into the pro-inflammatory M1 phenotype, boosting tumor cell elimination through reactive oxygen species (ROS) mediation (Singh, 2019). It is now possible to induce the targeted eradication of HER2-positive breast cancer cells by macrophages using a bispecific multivalent nanoengager design. This process is aided by pro-phagocytic signaling, which is mediated by calreticulin (Wan et al., 2016). NPs are prospective platforms for the development of innovative cancer therapies due to their superior physical and chemical characteristics, superior aqueous stability, low toxicity, strong biocompatibility and ability to simultaneously load adjuvants and antigens (Garrigue et al., 2018; Jin et al., 2020). Although many challenges must be solved in the use of NPs as a nanomedicine, the benefits exceed the drawbacks, making NPs a highly promising technology, as illustrated in supplementary **Table S1.**

5.2. Nanoparticles aids in tumor imaging

To identify highly precise tumor areas, cancer treatments and macromolecules including amino acids, immunoglobulins, and different molecules are coupled with NPs, that are suitable for tumor cell identification and monitoring (Kim et al., 2018). Super paramagnetic iron oxide containing NPs (SPIONs) capable of MRI imaging can be used to identify metastases in lung cancer, with tumor cell lines serving as the specific target for these SPIONs (Mubarak et al., 2021). Magnetic powder scanning is also employed in tomographic imaging methods, and showed unusual resolution and sensitivity to tumor tissues (Zeng et al., 2022). In animal studies, researchers were able to safely deliver MNPs to the lungs by nebulization. These were designed to target the EGFR, a protein widely expressed in lung cancer. Also, in vitro studies were executed by nanosystems developed for positron emission tomography, which

consists of self-assembled amphiphilic dendritic molecules that spontaneously produce homogenous supramolecular nanoparticles. The dendritic nanoscale system rapidly accumulates within tumors by leveraging dendritic multivalence and the EPR effect, resulting in extremely selective imaging of distinct tumor types while decreasing treatment-related toxicities (J. Yang et al., 2023). As MRI contrast agents, paramagnetic complexes of gadolinium (Gd^{3+}) chelates are frequently used, For example, Gd-DTPA has been widely used to improve the contrast of MRI scans (Garrigue et al., 2018). The $NiFe_2O_4$, $MnFeO_4$, and $CoFe_2O_4$ NPs have proved their efficacy as T2 contrast molecules, and Gd-based NPs such as Gd_2O_3, $GdPO_4$, and GdF_3 are known to improve the signal in T1-weighted, providing valuable options for tailoring MRI contrast to specific imaging applications (Chu et al., 2022). Furthermore, ultra small-sized iron oxide nanoparticles (ESIONs) measuring less than 4nm have shown promise as possible candidates for T1-weighted imaging in MRI. However, it is vital to highlight that these unique nanoparticles are now in the early stages of in vivo animal investigations for MRI applications. Before they may be considered for human application, significant research is required to address concerns about their biocompatibility and pharmacokinetics. The path from preclinical studies to clinical applications entails intensive testing and evaluation to assure the safety and effectiveness of these nanoparticles (Bhadula et al., 2023; Bhurbhure et al., 2022).

5.3. Cancer diagnosis using nanomaterials

Nanotechnology research has the potential to significantly improve tumor diagnosis by allowing evidence at various levels, including tissue, cellular, and molecular (Jin et al., 2020). Because pH-responsive fluorescent nanoprobes may be designed to target specific molecular markers, such as tumor-associated fibroblast activating protein-alpha, they hold great potential for molecular cancer surveillance. As a result of the peculiar pH situation around these cells, fluorescent signals are created, allowing for precise identification and imaging of such biological markers (Y. Zhang et al., 2020). The debut of QDs with near-IR fluorescence (700-1000nm) makes it easier the diagnose colon, liver, and pancreatic cancers (Bhadula et al., 2023; N. Fu et al., 2018; Jin et al., 2020). The emergence of a second near-IR window, known as NIR-II (900-1700nm), has notably revolutionized tumor imaging by offering greater tissue penetration as well as improved spatial and temporal resolution. The development of Ag_2Te with a sulfur has been cited as a significant advancement in cancer imaging (F. Fu et al., 2018). Nanoshells coated with Au NPs shows unique qualities, including controlled optical properties based on core and shell dimensions, and are valuable for cancer treatment and diagnosis (Jin et al., 2020; Shrivas et al., 2018). Due to its significant atomic mass, AuNPs are an ideal contrasting agent to enhance imaging due to the EPR effect (Song et al., 2023). When AuNPs are combined with EGFR monoclonal antibodies, they actively target cancer cells (Graumann et al., 2019). Rand et al. identified AuNPs with liver cancer cells to produced significantly stronger signals in X-ray imaging when trying to find aggregates of hepatic tumor cells. These results have important impacts on earlier cancer diagnosis, as the technology showed the potential to detect tumors as small as a few millimeters in width inside the body (Qazi & Fayaz; Righetti & Boschetti, 2023).

5.4.Screening of tumor biomarker

Tumor biomarkers ought to have high sensitivity, ensuring they can reliably detect individuals with the disease, and high specificity, limiting the possibility of false-positive results. These features are critical for the dependability and therapeutic value of tumor biomarkers, derived from urine, blood, or oral fluid samples (Steinberg et

al., 2021). However, such biomarkers are still not proven to be useful for cancer detection (Steinberg et al., 2021). As proteomic studies evolved, protein indicators for various cancers were identified. Antibody profiling assays would typically exclude high-MW proteins like albumin and immunoglobulins (Caputo & Caracciolo, 2020). The study led by Geho and Luchini focused on the detection of low-MW proteins with NPs, making substantial progress in the field of biomarker identification and screening. It enables more exact and sensitive marker extraction from body fluids such as urine and blood (S. M. Batool et al., 2023; Grechnikov et al., 2022). Because of surface attributes such as electric charges or active biomolecules, NPs compete with protein carriers, which are currently held by mesoporous silicon, polymeric, and carbon nanotubes (Christodoulides et al., 2019; Khajavinia & El-Aneed, 2023; Mikelez-Alonso et al., 2020). The modified sensitivity of mass spectrometry (MS) achieved by the use of nanocarriers including carbon nanotubes is undoubtedly helpful in analytical chemistry and diagnostic research (Giri et al., 2023; Nayak et al., 2023). Using a lab-on-a-chip system with cadmium selenide (CdSe) center small particles and a zinc sulfide exterior, along with antibodies directed against particular proteins such as carcinoembryonic antigen, cancer antigen 125, and Her-2/Neu, is a novel strategy for enhancing multiplexed protein detection. By improving the simultaneous detection of many cancer-related indicators, this ground-breaking technique has the potential to aid in the early detection and surveillance of cancer (Giri et al., 2023). As shown in **Figure 3**, the use of nanoparticles in medicine has resulted in a wide range of diagnostic tools, contrast agents, analytical tools, therapeutic applications, drug delivery vehicles, and many other products.

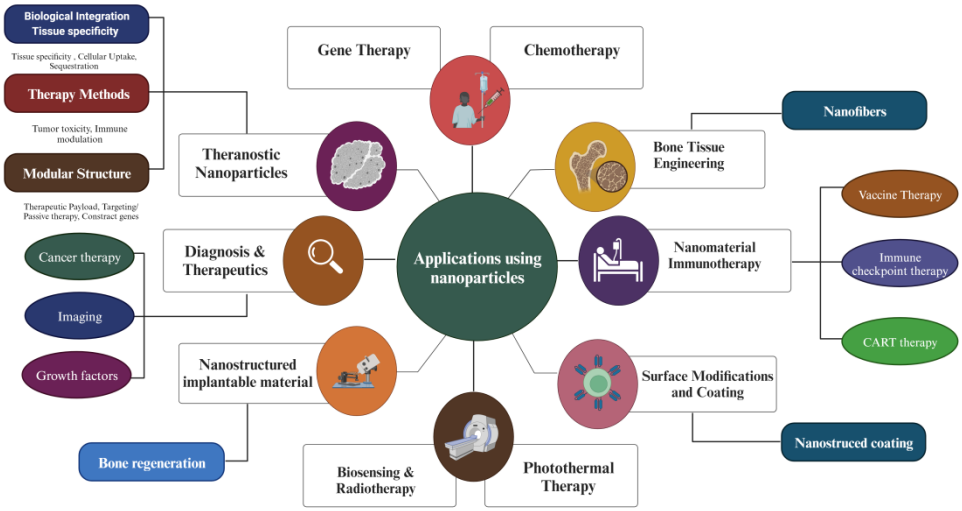


Figure 3. Potential applications of nanomaterials

6. Challenges and limitations

The precise buildup of nanoparticles (NP) in target tissues and coping with the development of a protein corona (PC) surrounding NPs, which can happen as a result of interactions with biological secretions, are just a couple of the difficulties that must be solved (Mirkin et al., 2015). **Figure 4** depicts the significant difficulties in using NPs in the field of nanotechnology, but it's crucial to recognize that there are real barriers to be addressed in this field. Moreover, patient-specific NP surface changes and possible unintended effects of PC formation, such as immune system interactions and immunotoxicity, pose significant challenges. Most cancer nanomedicines on the

market initially relied mainly on the question were raised about the heterogeneity of the EPR effect, which may affect the drug efficacy. Most nanodrugs are now being explored in clinical studies to increase cancer therapy efficacy by active targeting, such as BIND-014. Furthermore, stimuli-responsive drug release systems have gained popularity, a lyso-thermosensitive liposomal doxorubicin (ThermoDox) developed to release its payload in response to specific conditions. Traditional toxicity research, which is typical for bulk chemicals, may not be precise enough to quantify or analyze the potential toxicity of nanocarriers. A crucial issue is the growing buildup of NPs in certain body parts, most notably the hepatic system, gastrointestinal tract, and kidneys. Knowing the effect of this accumulation on organ function and overall health is still a key research priority. The rigorous study of sterility and endotoxin levels in nanoparticles is crucial, especially with the extensive use of nanoparticles in intravenous cancer treatments. Regrettably, these crucial features are usually regretted during the early stages of research, which might pose problems in clinical trials (Đorđević et al., 2022; Giri et al., 2023).

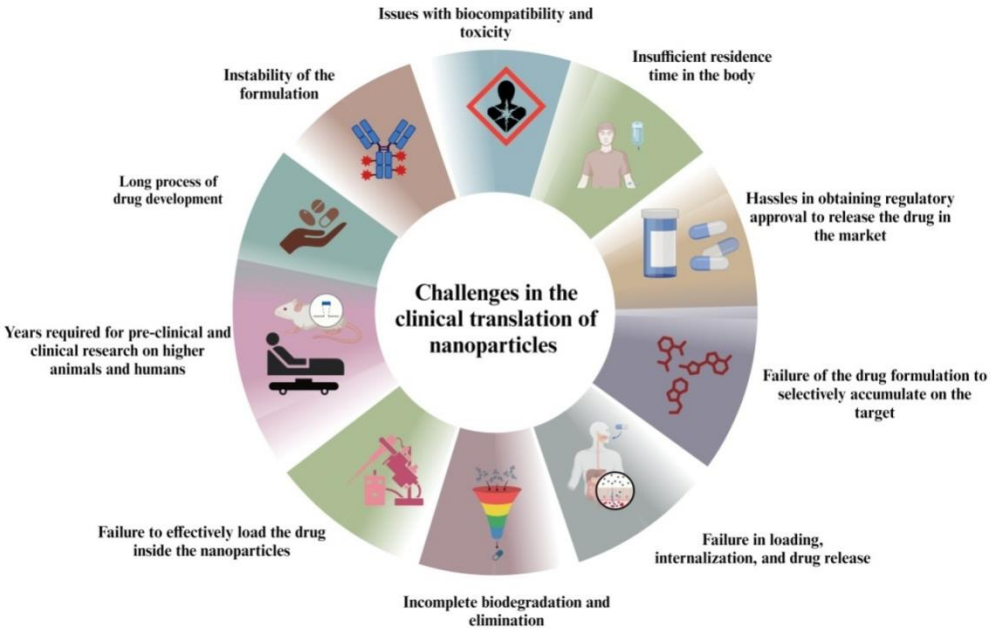


Figure 4. Most Significant Limitations in nanomedicine Innovation.

Moreover, only a small percentage of NPs used in medicine that have promising laboratory findings actually enter clinical trials. Greish et al. (2017) examined over 20,000 scholarly papers on nanomedicine and just 15 NPs-based anti-tumor medicines were on the marketplace demand (Rasool et al., 2022). Because particle size signifies strength, it has significant drawbacks for a variety of nanomedicines. Certain clinical trials have been approved; however, logistical issues such as distribution, uniformity, and reproducibility of challenging nanotechnology systems pose considerable obstacles. Moreover, hundreds of nanomedicine have been rejected at various phases of clinical testing, and several have been pulled off the market even after clearance (Chow, 2022). Moreover, more innovation is needed in terms of drug-loading abilities and consistency. The inability of laboratory tumor models to closely resemble human clinical malignancies is a hurdle to therapeutic application. Because of their proximity to human malignancies, their experimental models for assessing nanomedicine metabolism and safety must be carefully established (Zhao et al., 2018). To study the biological challenges facing nanomedicine in cancer while designing a novel pharmacological entity, a preclinical study should include NP detection and analysis

of appropriate model animals and humans. While addressing logistical concerns, an extensive cost-benefit assessment can be executed early in the nanotechnology advancement (Katragadda et al., 2013; Liu et al., 2017). However, researchers are trying hard to overcome such obstacles since the distinctive attributes of NPs hold great potential to enhance pharmaceutical delivery, therapeutic efficacy, and the field of customized medicine.

7. Recent Advances and case study

Nanomaterials are employed in a range of applications, including drug carriers, chemotherapeutic agents, photoacoustic agents, molecular synthesis, photothermal agents, radiation dose enhancers, materials reactivity, biomarker recognition chemicals, molecular target therapy, and biological imaging (R. Liu et al., 2023). Several therapeutic studies focusing on combination therapy were made possible by the benefits of functionalized nanocarriers (Y.-x. Chen et al., 2020). Katragadda et al., (Bazi Alahri et al., 2023) showed a secure nanosized dosage for the paclitaxel and 17-AAG drug delivery, which had limited response in phase 1 trials. Liu et al., (Afzal et al., 2022) created new polymeric microspheres and loaded them with two antitumor drugs for lung administration. The most studied SRC inhibitor referred as Dasatinib is currently being developed. NPs are crucial in medical imaging techniques such as magnetic resonance imaging (MRI), positron emission tomography (PET), and photoacoustic imaging (Fonseca-Santos et al., 2023). The versatile nanoparticles enable a plethora of prospects to solve intricate challenges and induce innovation in a variety of fields, thus leading to progress in medical care, diagnostics, and customized medicine.

7.1. Nanoparticles for various disease treatments

Polymeric NPs, liposomes, SLNs, nano-emulsions, micro-emulsions, and fluid crystals have all been used to treat Alzheimer's disease. For example, Tacrine was administered intravenously, and have the potential to increase drug delivery to neurons for dementia treatment (Mir Najib Ullah et al., 2023). Rivastigmine based on polymeric NPs and administered orally. An acidic matrix loaded with polysorbate and SLNs coated with taurodeoxycholate surfactant were utilized to safely administer diminazine and doxorubicin (Hersh et al., 2022). Piperine and huperzine drugs was applied to SLNs and given to the brain intraperitoneally in order to eliminate deposits and lumps and boost the activity of the enzyme AChE, the mouse skin did not exhibit any noticeable discomfort (S. Chen et al., 2021). The levodopa against Parkinson disorder has low absorption to the brain (Devi et al., 2020), While metallic, QDs, cerium oxide, carbon-based, and liposome-based NPs are used in Parkinson therapy, such particles allow medications to cross the blood-brain barrier (BBB) in a variety of ways (Hussein-Al-Ali et al., 2021). A study conducted by Bhattamisra et al., (Elsabahy et al., 2015) Rotigotine was encapsulated within chitosan NPs and delivered intranasally to a Parkinson's disease rat model. The study concluded that intranasal administration is an ideal method for delivering rotigotine to the rat brain (Zheng et al., 2021). PEGylation extends liposomes duration remain in blood vessels, whilst conjugating Magnetoliposomes (MLs) with antibodies enhances the possibility of active delivery. Namdari and colleagues tested various liposome modifications in a mouse model of MI. These adaptable liposomes are designed for effective pharmaceutical therapy, such as medication loading onto nanomaterials for enhanced intracellular transport. Modified nanocarriers include cationic liposomes with per fluorocarbon, polyelectrolyte, and polymeric content (W.-q. Wu et al., 2019).

7.2. Tissue engineering

Bone regeneration is a complex process that requires the integration of both nanomaterials and biological components in order to adequately mend damaged bones. The development of bone bioscaffolds by combining biomaterials and nanoparticles has significantly advanced bone implantation methods (Zheng et al., 2021). Polylactic acid (PLA), polyamide, PLA-glycolic acid copolymer (PLGA), polycaprolactone polyester, polyanhydride, polyglycolic acid (PGA), polysaccharides, polyurethane, polyacrylate, proteins, and other synthetic polymers have been synthesized for tissue engineering (Bag et al., 2023; Hashimoto et al., 2006). Initially, it became evident that collagen plays a key role in increasing the diffusion of bioactive molecules and cellular components essential for cardic renewal and regeneration (Zang et al., 2016). Zhang et al. (Bag et al., 2023) designed Au NPs with a diameter of about 20 nm, proved to be potent anti-angiogenic, blocking various heparin-binding hormones and reducing the growth of both ovarian and pancreatic cancers. Hashimoto et al. (Karmakar et al., 2023) originate TiO_2 particles could improve the attributes of composites, showing flexibility and elastic modulus of natural bone, as a result, they are widely utilized in tissue engineering of bones. Recently, chitosan, silicon dioxide and poly-caprolactone (PCL) nanomaterials are being used in dental tissue restoration (H. Liu et al., 2013).

Furthermore, the MSCs on the chitosan-based scaffold helped to generate fibrous cementum, woven/flaky bone, and periodontal ligament, revealing an efficacy in repairing important periodontal abnormalities (Nazeer et al., 2017). Recent advances have resulted in the production of gelatin nanomaterials mixed into polymeric scaffolds in neural engineering. Chesnutt et al. proposed a novel chitosan-based scaffold loaded with nanocrystalline calcium to enhance bone tissue growth. Several porous chitosan constructs are being shown to improve bone conduction, showing its versatility in tissue engineering (Cheng et al., 2018). A hydroxyapatite (nHAp/CTS) have received major interest in bone tissue regeneration (Zhu et al., 2018). Liu et al. (Miggiels et al., 2019) lead the effort to develop nanofiber composite scaffold from chitosan and hydroxyapatite was shown to improve osteogenic growth in the bone marrow, and this remarkable trait highlights its potential for boosting regeneration of bone tissue (X. Yang et al., 2023). Moreover, hydroxyapatite can be coupled with a variety of polymers to achieve good results in bone formation and repair. For instance, gold (Au), silver (Ag), and titanium oxide are all gaining popularity as bone scaffolding materials (Sinha et al., 2022).

7.3. Optical labeling in many colors for biological research

The increasing amount of sequence data generated by proteomic and genomics research has necessitated the onset of high-speed screening methods. One intriguing approach was based on the visual "barcoding" of polymer atoms in a solution. Its sole limitation is its ability to efficiently recognize unique tags. Furthermore, single QDs have proven to be excellent organic dye alternatives in a range of bio-tagging applications (Yaqoob et al., 2020). The multicolor visual encoding was achieved by precisely controlling the ratios of variably sized QDs, primarily zinc sulfide-capped cadmium selenide nanocrystals, within polymer microbeads. These QDs have special optical properties making them highly luminous and ideal fluorophores for wavelength and intensity multiplexing. Under favorable conditions, imaging methods and spectroscopy study validated the quantum tagged beads, exceptional homogeneity and consistency, resulting in about 99.99% accuracy in bead recognition. Notably, southern hybridization studies established the ability to detect both target and coding signals at the individual bead level. This color-coding approach has opened up new

opportunities in fields as diverse as gene expression analysis, high-throughput screening, medical diagnostics, and others. Its ongoing progress has the ability to offer up new avenues for investigation and innovation (Gargett et al., 2018; Wurz et al., 2014).

7.4. Protein recognition

Protein recognition is the process of determining the quantity and variety of proteins in a given sample for diagnostics, therapy, and biological research. Despite their slightly limited potential for concurrent detection, Au NPs frequently appear in immunohistochemistry research to find protein interactions. Surface-enhanced Raman scattering spectrometry is widely utilized to detect and identify single dye molecules and offers the potential to greatly enhance protein probe multiplexing. A sophisticated multifunctional probe developed by Prof. Mirkin's group, centers around a 13 nm Au NPs are coated with hydrophilic oligonucleotides having a Raman dye and a small molecule recognition element, such as Biotin. This probe has multiple advantages, including catalytic activity and the ability to be coated with Ag in a silver-hydroquinone solution. It may also be modified with antibodies on its surface for easy protein recognition and demonstrated no cross-reactivity. Sensor panels comprised of six non-covalent Au conjugates and fluorescent polymers quench polymer fluorescence, which is how this detection works; this quenching alters the interaction between nanoparticles and polymers. As a result, distinct and highly reproducible fluorescent response patterns emerge that are specific to specific proteins even at micro molar concentrations and can be quantitatively distinguished using linear discriminant analysis (DLA). This finding offers significant prospects for the growth of innovative nanomaterial-based protein detector arrays with applications in medical diagnosis, allowing for improved accuracy and specificity in a broad spectrum of therapeutic purposes (Young et al., 2011).

7.5. Economic exploration

A few companies are developing and advertising NPs for biological and medical applications. Many of them are spinoffs from various research universities, and while this list is not exhaustive, it does provide a representative sample that represents current market trends. A significant fraction of these companies are focused on medicinal uses and nano-ceramic materials in transplantation and orthopedics. For instance, colloidal silver is used in antimicrobial formulae and wound dressings, and titanium particles are used in filters for antibacterial response, especially when exposed to UV light. A few of them utilize quantum size effects in silicon nanocrystals to tag biomolecules, while others label diverse biological components with bio-conjugated gold nanoparticles. Moreover, the enhanced catalytic properties of nano-ceramic surfaces or noble metals such as platinum are employed to neutralize toxic toxins and other hazardous organic compounds. They collaborate to expand the landscape of biomedical nanotechnology applications (Ishikawa et al., 2021).

7.6. Significant Clinical Innovations

A better understanding of translational research is essential for assessing our current situation and developing effective treatment regimens for all types of cancer. Immunotherapy has substantially improved as a consequence of findings concerning cellular and humoral immune responses that focus on over-expressed tumor-associated antigens (TAAs), such as MUC1 that appears in many breast cancer patients and others with adenocarcinoma. Tecemotide particularly targets on MUC1, is now being examined in Phase III clinical studies, and its efficacy in treating non-

small cell lung cancer (NSCLC) in stages IIIA/IIIB. This is a crucial step toward realizing immunotherapy promise of providing more specific and effective cancer treatments (Luo et al., 2020). Lipovaxin-MM a dendritic based liposomal vaccine, is currently in a phase 1 trial for cancerous melanoma (Zhao et al., 2020), CRLX101 is an innovative nanopharmaceutical that employs cyclodextrin polymeric nanoparticle technology, which has immense promise in the pharmacy area and marks a major step toward offering clinical benefits (Schuerle et al., 2019). Individuals with benign tumors that exhibit the NY-ESO-1 antigen were given multiple doses of the vaccine IMF-001, which is formed up of a mixed polymeric particle such as a modified protein and a cholesteryl hydrophobized pullulan (CHP) complex (Nirmala et al., 2023). Several nanochemodrugs are being considered in medical trials, typically in combination with proteins and with high drug-binding potential, resulting in nanoparticle-conjugated albumin (Nab) complexes. When combined with gemcitabine, atezolizumab, and cyclophosphamide, nab-paclitaxel has the potential to treat both advanced and preliminary breast cancer. ABI-007, a different Nab-paclitaxel conjunction, has completed a Phase 3 study for non-small cell lung cancer and metastatic breast carcinoma in stage IV patients. Ongoing advances in cancer care include the development of pharmacological mimics capable of counteracting the cancer-inducing activities of altered regulator proteins. Multi-ion radiation radiotherapy employs pure beams of heavy ions, such as carbon ions, opening up new paths for tumor therapies. Such new discoveries lead the potential to improve cancer therapy and uncover more effective techniques for combating this challenging disease (Lin et al., 2020; Nirmala et al., 2023; Yan et al., 2020).

8. Future perspectives

The nanomedicine era is still evolving, with potential advances in tailored treatment and early cancer diagnosis. Tumor nanomedicine experts conducted extensive research into essential mechanisms including the design, description, and completion of an in vitro analysis, as well as the verification of anticancer characteristics through preclinical trials. There has been an increase in research activity in this domain during the past two decades, resulting in the submission of around 1,500 patents (Farjadian et al., 2019). Certain formulations have been approved, and a number of in vitro, in vivo, and clinical investigations are now being conducted (Chakravarty et al., 2017). Some of the nanomedicines being researched for tumor targeting, bio-imaging, and drug delivery include liposomes, extracellular vesicle nanoemulsions, Ag/Au NPs, magnetic NPs, carbon dots, and SLNs (Li & Burgess, 2020). The route of medicine from manufacturing to approval is complex and laborious. Moreover, advances in monitoring and characterization methods are required for the effective drug implementation (Gavas et al., 2021). The FDA and EMA analyze nanomedicines; however the lack of standard guidelines for evaluating nanomedicines complicates the process, and combination medicines to boost tumor efficacy. Studies are often scheduled to test immunotherapy, which might impede clinical trial development by enhancing cancer-targeting therapy (Farjadian et al., 2019). Furthermore, radio-labeled particles composed of iron oxide nanoparticles are widely utilized in diagnostic imaging, particularly for visualizing sentinel lymph nodes. They are appropriate for techniques like positron-emission tomography and single-photon emission computed tomography which improve the precision and accuracy of lymph node mapping in a variety of medical circumstances (Singh et al., 2018).

To maximize drug delivery, it is necessary to examine the intake, circulation, absorption into the tumor surroundings, adherence to the target, and cancer cell death.

The influence of nanoparticle thermodynamic characteristics on the biological reaction must be understood. Academics have concentrated on computational algorithms for effective drug administration in the last decade (Gavas et al., 2021; Li & Burgess, 2020). CytImmune, founded in 1988, is a pioneer in the creation of cancer-specific nanomedicines. The company began with diagnostics but has subsequently evolved into a clinical-stage nanomedicine company. Their primary mission is to discover, develop, and commercialize multifunctional tumor-targeting treatments. CytImmune's Aurimune nanomedicine platform has achieved global recognition in the nanomedicine field after successfully completing a Phase I clinical study for CYT-6091, representing a significant step forward in the evolution of nanotechnology-based cancer treatments (Sebastian, 2017b). It entails the use of colloidal Au NPs to deliver medications directly to malignant cells in order to improve their effectiveness. Molecules of tumor necrosis factor alpha are attached to the Thiol-derivatized polyethylene glycol (PEGTHIOL) molecules. PEG-THIOL cloaks the TNF-α containing nanoparticle, making it less apparent to the immune system. This coating allows the nanoparticle to travel through the bloodstream without being detected, eventually arriving at the tumor site for targeted therapy (Singh et al., 2018). T2 Biosystems use superparamagnetic crystals to bind to and aggregate cancer-associated proteins. The presence of cancer-related proteins is indicated by the production of a magnetic resonance signal by these clusters. In addition, some studies have employed quantum dots and fluorescent compounds to identify specific DNA strands that serve as early signs of cancer (C. Chen et al., 2022). Furthermore, nanoparticles are proving to be quite useful in the early identification of cancer. Nanoscale sensors, which frequently use nanoparticles or nanowires, have been developed to identify specific proteins linked with certain types of cancer cells in blood samples. This cutting-edge technology has the ability to detect cancer at an early stage, allowing for earlier intervention and improved patient outcomes. The flexibility of nanoparticles in cancer treatment and diagnosis highlights their significance in cancer biology.

Summary

Nanotechnology became known as a potential technique for detecting, identifying, and treating cancer cells, garnering significant interest in the scientific community. NPs have dramatically enhanced biomedical imaging, allowing cancer cells to be detected utilizing active, and passive targeted methods. Because of their small size and ability to accumulate contrast chemicals in tumor tissues, they have longer circulation and greater accumulation, making them ideal tools for early cancer detection. Several NP-based imaging techniques were created including magnetic resonance imaging (MRI), fluorescence imaging (FI), CT scans, X-ray radiography, and single photon emission computed tomography (SPECT). NPs are believed to be effective in tumor cell treatment. They allow traceable drug distribution as well as real-time monitoring of therapy response. NPs have acquired importance in radiotherapy because to their potent radiosensitizing abilities and image-guided treatment responses. Furthermore, NPs may be employed via phototherapy, gene therapy, and immunotherapy. While significant research on the biological use of NPs in oncology has been conducted, a number of obstacles must be solved before they can be widely used in clinical trials. Despite these obstacles, nanotechnology continues to hold considerable promise, with ongoing research focused on toxicity, cellular and physiological aspects of NP-based drug delivery, enhanced EPR effects, and pharmacokinetics. Clinical translation in NP-oriented cancer therapy appears to

have a promising future, with the possibility of groundbreaking advancements in cancer detection and treatment.

Supplementary Data

Figure S1. Nanocarrier types currently popular in cancer treatment

Table S1. The pros and cons of different nanoparticles utilized as routes of administration

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

Conflict of Interest

The authors declare no conflict of interest.

References

Afzal O, Altamimi AS, Nadeem MS, Alzarea SI, Almalki WH, Tariq A, Mubeen B, Murtaza BN, Iftikhar S, Riaz N (2022) Nanoparticles in Drug Delivery: From History to Therapeutic Applications. Nanomaterials 12: 4494.

Akakuru O, Louis H, Oyebanji O, Ita B, Amos P, Philip M (2018) Utility of nanomedicine for cancer treatment. J Nanomed Nanotechnol 9: 1-6.

Akanda M, Getti G, Nandi U, Mithu MS, Douroumis D (2021) Bioconjugated solid lipid nanoparticles (SLNs) for targeted prostate cancer therapy. International journal of pharmaceutics 599: 120416.

Alsaggar M, Liu D (2018) Organ-based drug delivery. Journal of Drug Targeting 26: 385-397.

Ansari MA, Chung I-M, Rajakumar G, Alzohairy MA, Alomary MN, Thiruvengadam M, Pottoo FH, Ahmad N (2020) Current nanoparticle approaches in nose to brain drug delivery and anticancer therapy-a review. Current pharmaceutical design 26: 1128-1137.

Anselmo AC, Mitragotri S (2019) Nanoparticles in the clinic: An update. Bioengineering & translational medicine 4: e10143.

Attia MF, Anton N, Wallyn J, Omran Z, Vandamme TF (2019) An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. Journal of Pharmacy and Pharmacology 71: 1185-1198.

Aulic S, Marson D, Laurini E, Fermeglia M, Pricl S (2020) Breast cancer nanomedicine market update and other industrial perspectives of nanomedicine Nanomedicines for Breast Cancer Theranostics. Elsevier, pp. 371-404.

Awad NS, Salkho NM, Abuwatfa WH, Paul V, AlSawaftah NM, Husseini GA (2023) Tumor vasculature vs tumor cell targeting: Understanding the latest trends in using functional nanoparticles for cancer treatment. OpenNano 11: 100136.

Bag J, Mukherjee S, Karati D (2023) Recent advancement of nanostructured materials: a compatible therapy of tissue engineering and drug delivery system. Polymer Bulletin: 1-24.

Barua S, Mitragotri S (2014) Challenges associated with penetration of nanoparticles across cell and tissue barriers: a review of current status and future prospects. Nano today 9: 223-243.

Batool SM, Hsia T, Beecroft A, Lewis B, Ekanayake E, Rosenfeld Y, Escobedo AK, Gamblin AS, Rawal S, Cote RJ (2023) Extrinsic and intrinsic preanalytical variables affecting liquid biopsy in cancer. Cell Reports Medicine.

Bazi Alahri M, Jibril Ibrahim A, Barani M, Arkaban H, Shadman SM, Salarpour S, Zarrintaj P, Jaberi J, Turki Jalil A (2023) Management of Brain Cancer and Neurodegenerative Disorders with Polymer-Based Nanoparticles as a Biocompatible Platform. Molecules 28: 841.

Beltrán-Gracia E, López-Camacho A, Higuera-Ciapara I, Velázquez-Fernández JB, Vallejo-Cardona AA (2019) Nanomedicine review: Clinical developments in liposomal applications. Cancer Nanotechnology 10: 1-40.

Bhadula RC, Sharma A, Srivastava V, Kala V, Singh SJ, Singh A (2023) Application of nanosensor in early diagnosis cancer disease AIP Conference Proceedings. AIP Publishing.

Bhagyaraj SM, Oluwafemi OS, Kalarikkal N, Thomas S (2018) Synthesis of inorganic nanomaterials. Woodhead publication: 1e18.

Bhurbhure O, Ghormade V, Katekar V, Sangule D, Dhage S, Boralkar V, Padole T (2022) Quantum Dots: A New Hope for the Pharmaceutical Field. Journal of Drug Delivery and Therapeutics 12: 236-244.

Brard C, Piperno-Neumann S, Delaye J, Brugières L, Hampson LV, Le Teuff G, Le Deley M-C, Gaspar N (2019) Sarcome-13/OS2016 trial protocol: a multicentre, randomised, open-label, phase II trial of mifamurtide combined with postoperative chemotherapy for patients with newly diagnosed high-risk osteosarcoma. BMJ open 9: e025877.

Bremer-Hoffmann S, Halamoda-Kenzaoui B, Borgos SE (2018) Identification of regulatory needs for nanomedicines. Journal of Interdisciplinary Nanomedicine 3: 4-15.

Bulbake U, Doppalapudi S, Kommineni N, Khan W (2017) Liposomal formulations in clinical use: an updated review. Pharmaceutics 9: 12.

Caputo D, Caracciolo G (2020) Nanoparticle-enabled blood tests for early detection of pancreatic ductal adenocarcinoma. Cancer Letters 470: 191-196.

Cardoso RV, Pereira PR, Freitas CS, Paschoalin VMF (2022) Trends in Drug Delivery Systems for Natural Bioactive Molecules to Treat Health Disorders: The Importance of Nano-Liposomes. Pharmaceutics 14: 2808.

Carretta A, Cardarelli F (2023) Monitoring drug stability by label-free fluorescence lifetime imaging: a case study on liposomal doxorubicin Journal of Physics: Conference Series. IOP Publishing, pp. 012009.

Chakravarty R, Shreya G, Ashutosh D, Weibo C (2017) Radiolabeled inorganic nanoparticles for positron emission tomography imaging of cancer: an overview. The quarterly journal of nuclear medicine and molecular imaging: official publication of the Italian Association of Nuclear Medicine (AIMN)[and] the International Association of Radiopharmacology (IAR),[and] Section of the Society of 61: 181.

Chen C, Ge J, Gao Y, Chen L, Cui J, Zeng J, Gao M (2022a) Ultrasmall superparamagnetic iron oxide nanoparticles: A next generation contrast agent for magnetic resonance imaging. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology 14: e1740.

Chen L, Huang J, Li X, Huang M, Zeng S, Zheng J, Peng S, Li S (2022b) Progress of Nanomaterials in Photodynamic Therapy Against Tumor. Frontiers in Bioengineering and Biotechnology 10: 920162.

Chen S, Huang S, Li Y, Zhou C (2021) Recent advances in epsilon-poly-L-lysine and L-lysine-based dendrimer synthesis, modification, and biomedical applications. Frontiers in Chemistry 9: 659304.

Chen Y-x, Wei C-x, Lyu Y-q, Chen H-z, Jiang G, Gao X-l (2020) Biomimetic drug-delivery systems for the management of brain diseases. Biomaterials science 8: 1073-1088.

Cheng H, Chabok R, Guan X, Chawla A, Li Y, Khademhosseini A, Jang HL (2018) Synergistic interplay between the two major bone minerals, hydroxyapatite and whitlockite nanoparticles, for osteogenic differentiation of mesenchymal stem cells. Acta biomaterialia 69: 342-351.

Cheng Z, Li M, Dey R, Chen Y (2021) Nanomaterials for cancer therapy: Current progress and perspectives. Journal of hematology & oncology 14: 1-27.

Chiang C-L, Cheng M-H, Lin C-H (2021) From nanoparticles to cancer nanomedicine: old problems with new solutions. Nanomaterials 11: 1727.

Chow JC (2022) Application of Nanomaterials in Biomedical Imaging and Cancer Therapy. MDPI, pp. 726.

Christodoulides N, McRae MP, Simmons GW, Modak SS, McDevitt JT (2019) Sensors that learn: The evolution from taste fingerprints to patterns of early disease detection. Micromachines 10: 251.

Chu S, Shi X, Tian Y, Gao F (2022) pH-responsive polymer nanomaterials for tumor therapy. Frontiers in Oncology 12: 855019.

Dai L, Liu J, Luo Z, Li M, Cai K (2016) Tumor therapy: targeted drug delivery systems. Journal of Materials Chemistry B 4: 6758-6772.

Das RP, Gandhi VV, Singh BG, Kunwar A (2019) Passive and active drug targeting: role of nanocarriers in rational design of anticancer formulations. Current Pharmaceutical Design 25: 3034-3056.

Desmond LJ, Phan AN, Gentile P (2021) Critical overview on the green synthesis of carbon quantum dots and their application for cancer therapy. Environmental Science: Nano 8: 848-862.

Devi L, Gupta R, Jain SK, Singh S, Kesharwani P (2020) Synthesis, characterization and in vitro assessment of colloidal gold nanoparticles of Gemcitabine with natural polysaccharides for treatment of breast cancer. Journal of Drug Delivery Science and Technology 56: 101565.

Đorđević S, Gonzalez MM, Conejos-Sánchez I, Carreira B, Pozzi S, Acúrcio RC, Satchi-Fainaro R, Florindo HF, Vicent MJ (2022) Current hurdles to the translation of nanomedicines from bench to the clinic. Drug delivery and translational research: 1-26.

Drago JZ, Modi S, Chandarlapaty S (2021) Unlocking the potential of antibody–drug conjugates for cancer therapy. Nature Reviews Clinical Oncology 18: 327-344.

Dreaden EC, Austin LA, Mackey MA, El-Sayed MA (2012) Size matters: gold nanoparticles in targeted cancer drug delivery. Therapeutic delivery 3: 457-478.

Du Y, He W, Xia Q, Zhou W, Yao C, Li X (2019) Thioether phosphatidylcholine liposomes: a novel ROS-responsive platform for drug delivery. ACS applied materials & interfaces 11: 37411-37420.

Ejigah V, Owoseni O, Bataille-Backer P, Ogundipe OD, Fisusi FA, Adesina SK (2022) Approaches to improve macromolecule and nanoparticle accumulation

in the tumor microenvironment by the enhanced permeability and retention effect. Polymers 14: 2601.

Elsabahy M, Heo GS, Lim S-M, Sun G, Wooley KL (2015) Polymeric nanostructures for imaging and therapy. Chemical reviews 115: 10967-11011.

Emami Nejad A, Najafgholian S, Rostami A, Sistani A, Shojaeifar S, Esparvarinha M, Nedaeinia R, Haghjooy Javanmard S, Taherian M, Ahmadlou M (2021) The role of hypoxia in the tumor microenvironment and development of cancer stem cell: a novel approach to developing treatment. Cancer Cell International 21: 1-26.

Farjadian F, Ghasemi A, Gohari O, Roointan A, Karimi M, Hamblin MR (2019) Nanopharmaceuticals and nanomedicines currently on the market: challenges and opportunities. Nanomedicine 14: 93-126.

Farzin A, Etesami SA, Quint J, Memic A, Tamayol A (2020) Magnetic nanoparticles in cancer therapy and diagnosis. Advanced healthcare materials 9: 1901058.

Fonseca-Santos B, Cazarin CA, da Silva PB, Dos Santos KP, da Rocha MCO, Báo SN, De-Souza MM, Chorilli M (2023) Intranasal in situ gelling liquid crystal for delivery of resveratrol ameliorates memory and neuroinflammation in Alzheimer's disease. Nanomedicine: Nanotechnology, Biology and Medicine 51: 102689.

Fu F, Li L, Luo Q, Li Q, Guo T, Yu M, Song Y, Song E (2018a) Selective and sensitive detection of lysozyme based on plasmon resonance light-scattering of hydrolyzed peptidoglycan stabilized-gold nanoparticles. Analyst 143: 1133-1140.

Fu N, Hu Y, Shi S, Ren S, Liu W, Su S, Zhao B, Weng L, Wang L (2018b) Au nanoparticles on two-dimensional MoS 2 nanosheets as a photoanode for efficient photoelectrochemical miRNA detection. Analyst 143: 1705-1712.

Fu S, Yang X (2023) Recent advances in natural small molecules as drug delivery systems. Journal of Materials Chemistry B.

Fulton MD, Najahi-Missaoui W (2023) Liposomes in Cancer Therapy: How Did We Start and Where Are We Now. International Journal of Molecular Sciences 24: 6615.

Gargett T, Abbas MN, Rolan P, Price JD, Gosling KM, Ferrante A, Ruszkiewicz A, Atmosukarto II, Altin J, Parish CR (2018) Phase I trial of Lipovaxin-MM, a novel dendritic cell-targeted liposomal vaccine for malignant melanoma. Cancer Immunology, Immunotherapy 67: 1461-1472.

Garrigue P, Tang J, Ding L, Bouhlel A, Tintaru A, Laurini E, Huang Y, Lyu Z, Zhang M, Fernandez S (2018) Self-assembling supramolecular dendrimer nanosystem for PET imaging of tumors. Proceedings of the National Academy of Sciences 115: 11454-11459.

Gavas S, Quazi S, Karpiński TM (2021) Nanoparticles for cancer therapy: current progress and challenges. Nanoscale research letters 16: 173.

Ghosh S, Javia A, Shetty S, Bardoliwala D, Maiti K, Banerjee S, Khopade A, Misra A, Sawant K, Bhowmick S (2021) Triple negative breast cancer and non-small cell lung cancer: Clinical challenges and nano-formulation approaches. Journal of Controlled Release 337: 27-58.

Gigliobianco MR, Casadidio C, Censi R, Di Martino P (2018) Nanocrystals of poorly soluble drugs: drug bioavailability and physicochemical stability. Pharmaceutics 10: 134.

Giri PM, Banerjee A, Layek B (2023) A Recent Review on Cancer Nanomedicine. Cancers 15: 2256.

Graumann J, Finkernagel F, Reinartz S, Stief T, Brödje D, Renz H, Jansen JM, Wagner U, Worzfeld T, Pogge von Strandmann E (2019) Multi-platform affinity proteomics identify proteins linked to metastasis and immune suppression in ovarian cancer plasma. Frontiers in oncology 9: 1150.

Grechnikov AA, Laptinskaya PK, Kuzmin II, Borodkov AS, Simanovsky YO, Nikiforov SM (2022) Laser-induced electron transfer desorption/ionization on MoO3 and WO3 surfaces for the determination of dithiocarbamates. Analytical and Bioanalytical Chemistry 414: 6929-6937.

Harshita, Barkat MA, Beg S, Pottoo FH, Ahmad FJ (2019) Nanopaclitaxel therapy: an evidence based review on the battle for next-generation formulation challenges. Nanomedicine 14: 1323-1341.

Hashimoto M, Takadama H, Mizuno M, Kokubo T (2006) Enhancement of mechanical strength of TiO2/high-density polyethylene composites for bone repair with silane-coupling treatment. Materials Research Bulletin 41: 515-524.

He H, Pham-Huy LA, Dramou P, Xiao D, Zuo P, Pham-Huy C (2013) Carbon nanotubes: applications in pharmacy and medicine. BioMed research international 2013.

Herbst RS, Gordon MS, Fine GD, Sosman JA, Soria J-C, Hamid O, Powderly JD, Burris HA, Mokatrin A, Kowanetz M (2013) A study of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic tumors. American Society of Clinical Oncology.

Hersh AM, Alomari S, Tyler BM (2022) Crossing the blood-brain barrier: advances in nanoparticle technology for drug delivery in neuro-oncology. International journal of molecular sciences 23: 4153.

Hussein-Al-Ali SH, Hussein MZ, Bullo S, Arulselvan P (2021) Chlorambucil-iron oxide nanoparticles as a drug delivery system for leukemia cancer cells. International journal of nanomedicine: 6205-6216.

Hussein HAA, Maraie NK (2021) Highlights on polymeric micelles as versatile nanocarriers for drug transporting. Al Mustansiriyah Journal of Pharmaceutical Sciences 21: 21-30.

Irvine DJ, Hanson MC, Rakhra K, Tokatlian T (2015) Synthetic nanoparticles for vaccines and immunotherapy. Chemical reviews 115: 11109-11146.

Ishikawa T, Kageyama S, Miyahara Y, Okayama T, Kokura S, Wang L, Sato E, Yagita H, Itoh Y, Shiku H (2021) Safety and antibody immune response of CHP-NY-ESO-1 vaccine combined with poly-ICLC in advanced or recurrent esophageal cancer patients. Cancer Immunology, Immunotherapy: 1-11.

Jabir NR, Tabrez S, Ashraf GM, Shakil S, Damanhouri GA, Kamal MA (2012) Nanotechnology-based approaches in anticancer research. International journal of nanomedicine 7: 4391.

Jhaveri AM, Torchilin VP (2014) Multifunctional polymeric micelles for delivery of drugs and siRNA. Frontiers in pharmacology 5: 77.

Jin C, Wang K, Oppong-Gyebi A, Hu J (2020) Application of nanotechnology in cancer diagnosis and therapy-a mini-review. International Journal of Medical Sciences 17: 2964.

Joshi SS, Badgwell BD (2021) Current treatment and recent progress in gastric cancer. CA: a cancer journal for clinicians 71: 264-279.

Kapse-Mistry S, Govender T, Srivastava R, Yergeri M (2014) Nanodrug delivery in reversing multidrug resistance in cancer cells. Frontiers in pharmacology 5: 159.

Karmakar R, Dey S, Alam A, Khandelwal M, Pati F, Rengan AK (2023) Attributes of Nanomaterials and Nanotopographies for Improved Bone Tissue Engineering and Regeneration. ACS Applied Bio Materials.

Katragadda U, Fan W, Wang Y, Teng Q, Tan C (2013) Combined delivery of paclitaxel and tanespimycin via micellar nanocarriers: pharmacokinetics, efficacy and metabolomic analysis. PloS one 8: e58619.

Kaur J, Mishra V, Singh SK, Gulati M, Kapoor B, Chellappan DK, Gupta G, Dureja H, Anand K, Dua K (2021) Harnessing amphiphilic polymeric micelles for diagnostic and therapeutic applications: Breakthroughs and bottlenecks. Journal of Controlled Release 334: 64-95.

Kemp JA, Kwon YJ (2021) Cancer nanotechnology: Current status and perspectives. Nano convergence 8: 34.

Khajavinia A, El-Aneed A (2023) Carbon-Based Nanoparticles and Their Surface-Modified Counterparts as MALDI Matrices. Analytical Chemistry 95: 100-114.

Kim H-K, Lee GH, Chang Y (2018) Gadolinium as an MRI contrast agent. Future Medicinal Chemistry 10: 639-661.

Knežević NŽ, Gadjanski I, Durand J-O (2019) Magnetic nanoarchitectures for cancer sensing, imaging and therapy. Journal of materials chemistry B 7: 9-23.

Koirala N, Butnariu M, Panthi M, Gurung R, Adhikari S, Subba RK, Acharya Z, Popović-Djordjević J (2023) Antibiotics in the management of tuberculosis and cancer Antibiotics-Therapeutic Spectrum and Limitations. Elsevier, pp. 251-294.

Krauss AC, Gao X, Li L, Manning ML, Patel P, Fu W, Janoria KG, Gieser G, Bateman DA, Przepiorka D (2019) FDA approval summary:(daunorubicin and cytarabine) liposome for injection for the treatment of adults with high-risk acute myeloid leukemia. Clinical Cancer Research 25: 2685-2690.

Kvistborg P, Yewdell JW (2018) Enhancing responses to cancer immunotherapy. Science 359: 516-517.

Li H, Jia C, Meng X, Li H (2019) Chemical synthesis and applications of colloidal metal phosphide nanocrystals. Frontiers in Chemistry 6: 652.

Li J, Burgess DJ (2020) Nanomedicine-based drug delivery towards tumor biological and immunological microenvironment. Acta Pharmaceutica Sinica B 10: 2110-2124.

Li P, Wang D, Hu J, Yang X (2022) The role of imaging in targeted delivery of nanomedicine for cancer therapy. Advanced Drug Delivery Reviews: 114447.

Li Z, Tan S, Li S, Shen Q, Wang K (2017) Cancer drug delivery in the nano era: An overview and perspectives. Oncology reports 38: 611-624.

Lin X, Li X, Lin X (2020) A review on applications of computational methods in drug screening and design. Molecules 25: 1375.

Liu H, Peng H, Wu Y, Zhang C, Cai Y, Xu G, Li Q, Chen X, Ji J, Zhang Y (2013) The promotion of bone regeneration by nanofibrous hydroxyapatite/chitosan scaffolds by effects on integrin-BMP/Smad signaling pathway in BMSCs. Biomaterials 34: 4404-4417.

Liu J, Li S, Wang J, Li N, Zhou J, Chen H (2023a) Application of Nano Drug Delivery System (NDDS) in Cancer Therapy: A Perspective. Recent Patents on Anti-Cancer Drug Discovery 18: 125-132.

Liu K, Chen W, Yang T, Wen B, Ding D, Keidar M, Tang J, Zhang W (2017) Paclitaxel and quercetin nanoparticles co-loaded in microspheres to prolong retention time for pulmonary drug delivery. International journal of nanomedicine: 8239-8255.

Liu R, Xu Y, Zhang N, Qu S, Zeng W, Li R, Dai Z (2023b) Nanotechnology for Enhancing Medical Imaging Nanomedicine. Springer, pp. 99-156.
Liu Z, Jiang W, Nam J, Moon JJ, Kim BY (2018) Immunomodulating nanomedicine for cancer therapy. Nano letters 18: 6655-6659.
Luo M-J, Palmieri M, Riffkin CD, Sakthianandeswaren A, Djajawi TM, Hirokawa Y, Shuttleworth V, Segal DH, White CA, Nhu D (2020) Defining the susceptibility of colorectal cancers to BH3-mimetic compounds. Cell death & disease 11: 735.
M Rabanel J, Aoun V, Elkin I, Mokhtar M, Hildgen P (2012) Drug-loaded nanocarriers: passive targeting and crossing of biological barriers. Current medicinal chemistry 19: 3070-3102.
Marasini N, Ghaffar KA, Skwarczynski M, Toth I (2017) Liposomes as a vaccine delivery system Micro and Nanotechnology in vaccine Development. Elsevier, pp. 221-239.
Martinelli C, Pucci C, Ciofani G (2019) Nanostructured carriers as innovative tools for cancer diagnosis and therapy. APL bioengineering 3: 011502.
Miggiels P, Wouters B, van Westen GJ, Dubbelman A-C, Hankemeier T (2019) Novel technologies for metabolomics: More for less. TrAC Trends in Analytical Chemistry 120: 115323.
Mikelez-Alonso I, Aires A, Cortajarena AL (2020) Cancer nano-immunotherapy from the injection to the target: The role of protein corona. International Journal of Molecular Sciences 21: 519.
Mir Najib Ullah SN, Afzal O, Altamimi ASA, Ather H, Sultana S, Almalki WH, Bharti P, Sahoo A, Dwivedi K, Khan G (2023) Nanomedicine in the Management of Alzheimer's Disease: State-of-the-Art. Biomedicines 11: 1752.
Mirkin CA, Meade TJ, Petrosko SH, Stegh AH 2015 Nanotechnology-based precision tools for the detection and treatment of cancer Springer, pp. Pages.
Mittal P, Saharan A, Verma R, Altalbawy F, Alfaidi MA, Batiha GE-S, Akter W, Gautam RK, Uddin MS, Rahman MS (2021) Dendrimers: a new race of pharmaceutical nanocarriers. BioMed Research International 2021.
Mubarak T, Mahmood O, Shatti W (2021) Synthesis of Iron-nickel Particles by Co-precipitation Technique and Used as a Contrast Medium in an MRI Machine IOP Conference Series: Materials Science and Engineering. IOP Publishing, pp. 012013.
Mundekkad D, Cho WC (2022) Nanoparticles in clinical translation for cancer therapy. International Journal of Molecular Sciences 23: 1685.
Nam J, Son S, Park KS, Zou W, Shea LD, Moon JJ (2019) Cancer nanomedicine for combination cancer immunotherapy. Nature Reviews Materials 4: 398-414.
Navya P, Kaphle A, Srinivas S, Bhargava SK, Rotello VM, Daima HK (2019) Current trends and challenges in cancer management and therapy using designer nanomaterials. Nano convergence 6: 1-30.
Nayak V, Singh KR, Paliwal R, Singh J, Pandey MD, Singh RP (2023) Introduction to nanotechnological utility in the pharmaceutical industry Nanotechnology for Drug Delivery and Pharmaceuticals. Elsevier, pp. 337-355.
Nazeer MA, Yilgör E, Yilgör I (2017) Intercalated chitosan/hydroxyapatite nanocomposites: Promising materials for bone tissue engineering applications. Carbohydrate polymers 175: 38-46.
Nicoud MB, Ospital IA, Táquez Delgado MA, Riedel J, Fuentes P, Bernabeu E, Rubinstein MR, Lauretta P, Martínez Vivot R, Aguilar MdlÁ (2023) Nanomicellar formulations loaded with histamine and paclitaxel as a new

strategy to improve chemotherapy for breast cancer. International Journal of Molecular Sciences 24: 3546.

Nirmala MJ, Kizhuveetil U, Johnson A, Balaji G, Nagarajan R, Muthuvijayan V (2023) Cancer nanomedicine: a review of nano-therapeutics and challenges ahead. RSC advances 13: 8606-8629.

Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W (2022) Liposomes: Structure, composition, types, and clinical applications. Heliyon.

Parodi A, Kolesova EP, Voronina MV, Frolova AS, Kostyushev D, Trushina DB, Akasov R, Pallaeva T, Zamyatnin Jr AA (2022) Anticancer nanotherapeutics in clinical trials: The work behind clinical translation of nanomedicine. International Journal of Molecular Sciences 23: 13368.

Pei X, Zhu Z, Gan Z, Chen J, Zhang X, Cheng X, Wan Q, Wang J (2020) PEGylated nano-graphene oxide as a nanocarrier for delivering mixed anticancer drugs to improve anticancer activity. Scientific reports 10: 2717.

Pereira P, Serra AC, Coelho JF (2021) Vinyl Polymer-based technologies towards the efficient delivery of chemotherapeutic drugs. Progress in Polymer Science 121: 101432.

Powles T, Vogelzang NJ, Fine GD, Eder JP, Braiteh FS, Loriot Y, Cruz Zambrano C, Bellmunt J, Burris HA, Teng S-lM (2014) Inhibition of PD-L1 by MPDL3280A and clinical activity in pts with metastatic urothelial bladder cancer (UBC). American Society of Clinical Oncology.

Punu GF, Harahap Y, Anjani QK, Hartrianti P, Donnelly RF, Ramadon D (2023) Solid Lipid Nanoparticles (SLN): Formulation and Fabrication. Pharmaceutical Sciences and Research 10: 1.

Qazi MS, Fayaz HB NANOTECHNOLOGY AND IT'S UTILISATION IN THE HEALTHCARE INDUSTRY.

Rani R, Malik P, Dhania S, Mukherjee TK (2023) Recent Advances in Mesoporous Silica Nanoparticle-Mediated Drug Delivery for Breast Cancer Treatment. Pharmaceutics 15: 227.

Rasool M, Malik A, Waquar S, Arooj M, Zahid S, Asif M, Shaheen S, Hussain A, Ullah H, Gan SH (2022) New challenges in the use of nanomedicine in cancer therapy. Bioengineered 13: 759-773.

Righetti P, Boschetti E (2023) Pillaging plucking plundering ransacking proteomes via CPLL technology. Open J Proteom Genom 8: 001-010.

Rodríguez F, Caruana P, De la Fuente N, Español P, Gamez M, Balart J, Llurba E, Rovira R, Ruiz R, Martín-Lorente C (2022) Nano-based approved pharmaceuticals for cancer treatment: Present and future challenges. Biomolecules 12: 784.

Saifuddin N, Raziah A, Junizah A (2013) Carbon nanotubes: a review on structure and their interaction with proteins. Journal of Chemistry 2013.

Salehi B, Mishra AP, Nigam M, Kobarfard F, Javed Z, Rajabi S, Khan K, Ashfaq HA, Ahmad T, Pezzani R (2020) Multivesicular liposome (Depofoam) in human diseases. Iranian journal of pharmaceutical research: IJPR 19: 9.

Sato Y, Nakamura T, Yamada Y, Harashima H (2021) The nanomedicine rush: new strategies for unmet medical needs based on innovative nano DDS. Journal of controlled release 330: 305-316.

Schuerle S, Soleimany AP, Yeh T, Anand G, Häberli M, Fleming H, Mirkhani N, Qiu F, Hauert S, Wang X (2019) Synthetic and living micropropellers for convection-enhanced nanoparticle transport. Science advances 5: eaav4803.

Scott AM, Allison JP, Wolchok JD (2012) Monoclonal antibodies in cancer therapy. Cancer immunity 12.

Sebastian R (2017a) Nanomedicine-the future of cancer treatment: a review. J Cancer Prev Curr Res 8: 00-265.

Sebastian R (2017b) Nanomedicine-the future of cancer treatment: a review. J Cancer Prev Curr Res 8: 00265.

Sharma P, Kumar Mehra N, Jain K, Jain N (2016) Biomedical applications of carbon nanotubes: a critical review. Current drug delivery 13: 796-817.

Shrivas K, Nirmalkar N, Thakur SS, Deb MK, Shinde SS, Shankar R (2018) Sucrose capped gold nanoparticles as a plasmonic chemical sensor based on non-covalent interactions: Application for selective detection of vitamins B1 and B6 in brown and white rice food samples. Food chemistry 250: 14-21.

Siddique S, Chow JC (2020) Gold nanoparticles for drug delivery and cancer therapy. Applied Sciences 10: 3824.

Singh P, Pandit S, Mokkapati V, Garg A, Ravikumar V, Mijakovic I (2018) Gold nanoparticles in diagnostics and therapeutics for human cancer. International journal of molecular sciences 19: 1979.

Singh R (2019) Nanotechnology based therapeutic application in cancer diagnosis and therapy. 3 Biotech 9: 415.

Sinha A, Simnani FZ, Singh D, Nandi A, Choudhury A, Patel P, Jha E, Kaushik NK, Mishra YK, Panda PK (2022) The translational paradigm of nanobiomaterials: Biological chemistry to modern applications. Materials Today Bio: 100463.

Smith DM, Simon JK, Baker Jr JR (2013) Applications of nanotechnology for immunology. Nature Reviews Immunology 13: 592-605.

Song J, Sokoll LJ, Zhang Z, Chan DW (2023) VCAM-1 complements CA-125 in detecting recurrent ovarian cancer. Clinical Proteomics 20: 1-8.

Sonju JJ, Dahal A, Singh SS, Jois SD (2021) Peptide-functionalized liposomes as therapeutic and diagnostic tools for cancer treatment. Journal of Controlled Release 329: 624-644.

Stathopoulos P (2017) Galen's contribution to head and neck surgery. Journal of Oral and Maxillofacial Surgery 75: 1095-1096.

Steinberg HE, Bowman NM, Diestra A, Ferradas C, Russo P, Clark DE, Zhu D, Magni R, Malaga E, Diaz M (2021) Detection of toxoplasmic encephalitis in HIV positive patients in urine with hydrogel nanoparticles. PLoS Neglected Tropical Diseases 15: e0009199.

Tiwari H, Rai N, Singh S, Gupta P, Verma A, Singh AK, Kajal, Salvi P, Singh SK, Gautam V (2023) Recent advances in nanomaterials-based targeted drug delivery for preclinical cancer diagnosis and therapeutics. Bioengineering 10: 760.

Tran S, DeGiovanni P-J, Piel B, Rai P (2017) Cancer nanomedicine: a review of recent success in drug delivery. Clinical and translational medicine 6: 1-21.

Tucci ST, Kheirolomoom A, Ingham ES, Mahakian LM, Tam SM, Foiret J, Hubbard NE, Borowsky AD, Baikoghli M, Cheng RH (2019) Tumor-specific delivery of gemcitabine with activatable liposomes. Journal of Controlled Release 309: 277-288.

Tzogani K, Penttilä K, Lapveteläinen T, Hemmings R, Koenig J, Freire J, Márcia S, Cole S, Coppola P, Flores B (2020) EMA review of daunorubicin and cytarabine encapsulated in liposomes (Vyxeos, CPX-351) for the treatment of adults with newly diagnosed, therapy-related acute myeloid leukemia or acute myeloid leukemia with myelodysplasia-related changes. The Oncologist 25: e1414-e1420.

Van Trimpont M, Peeters E, De Visser Y, Schalk AM, Mondelaers V, De Moerloose B, Lavie A, Lammens T, Goossens S, Van Vlierberghe P (2022) Novel

insights on the use of L-asparaginase as an efficient and safe anti-cancer therapy. Cancers 14: 902.
Vickers NJ (2017) Animal communication: when i'm calling you, will you answer too? Current biology 27: R713-R715.
Wagner AM, Knipe JM, Orive G, Peppas NA (2019) Quantum dots in biomedical applications. Acta biomaterialia 94: 44-63.
Wahren-Herlenius M, Dörner T (2013) Immunopathogenic mechanisms of systemic autoimmune disease. The Lancet 382: 819-831.
Walling MA, Novak JA, Shepard JRE (2009) Quantum dots for live cell and in vivo imaging. International journal of molecular sciences 10: 441-491.
Wan X, Song Y, Song N, Li J, Yang L, Li Y, Tan H (2016) The preliminary study of immune superparamagnetic iron oxide nanoparticles for the detection of lung cancer in magnetic resonance imaging. Carbohydrate research 419: 33-40.
Wang H, Qu R, Chen Q, Zhang T, Chen X, Wu B, Chen T (2022a) PEGylated Prussian blue nanoparticles for modulating polyethyleneimine cytotoxicity and attenuating tumor hypoxia for dual-enhanced photodynamic therapy. Journal of Materials Chemistry B 10: 5410-5421.
Wang S, Cheng K, Chen K, Xu C, Ma P, Dang G, Yang Y, Lei Q, Huang H, Yu Y (2022b) Nanoparticle-based medicines in clinical cancer therapy. Nano Today 45: 101512.
Wang X, Liu Y, Xu W, Jia L, Chi D, Yu J, Wang J, He Z, Liu X, Wang Y (2021) Irinotecan and berberine co-delivery liposomes showed improved efficacy and reduced intestinal toxicity compared with Onivyde for pancreatic cancer. Drug Delivery and Translational Research: 1-12.
Wang Y-C, Rhéaume É, Lesage F, Kakkar A (2018) Synthetic methodologies to gold nanoshells: an overview. Molecules 23: 2851.
Wu J (2021) The enhanced permeability and retention (EPR) effect: The significance of the concept and methods to enhance its application. Journal of personalized medicine 11: 771.
Wu W-q, Peng S, Song Z-y, Lin S (2019) Collagen biomaterial for the treatment of myocardial infarction: an update on cardiac tissue engineering and myocardial regeneration. Drug Delivery and Translational Research 9: 920-934.
Wurz GT, Kao C-J, Wolf M, DeGregorio MW (2014) Tecemotide: an antigen-specific cancer immunotherapy. Human vaccines & immunotherapeutics 10: 3383-3393.
Yan L, Shen J, Wang J, Yang X, Dong S, Lu S (2020) Nanoparticle-based drug delivery system: a patient-friendly chemotherapy for oncology. Dose-Response 18: 1559325820936161.
Yang J, Bae H (2023) Drug conjugates for targeting regulatory T cells in the tumor microenvironment: guided missiles for cancer treatment. Experimental & Molecular Medicine: 1-9.
Yang J, Feng J, Yang S, Xu Y, Shen Z (2023a) Exceedingly Small Magnetic Iron Oxide Nanoparticles for T1-Weighted Magnetic Resonance Imaging and Imaging-Guided Therapy of Tumors. Small: 2302856.
Yang J, Wang X, Wang B, Park K, Wooley K, Zhang S (2022) Challenging the fundamental conjectures in nanoparticle drug delivery for chemotherapy treatment of solid cancers. Advanced Drug Delivery Reviews: 114525.
Yang X, Childs-Disney JL, Paegel M, Disney MD (2023b) DNA-Encoded Libraries and Their Application to RNA. Israel Journal of Chemistry: e202300073.
Yaqoob AA, Ahmad H, Parveen T, Ahmad A, Oves M, Ismail IM, Qari HA, Umar K, Mohamad Ibrahim MN (2020) Recent advances in metal decorated

nanomaterials and their various biological applications: A review. Frontiers in chemistry 8: 341.

Ying N, Liu S, Zhang M, Cheng J, Luo L, Jiang J, Shi G, Wu S, Ji J, Su H (2023) Nano delivery system for paclitaxel: Recent advances in cancer theranostics. Colloids and Surfaces B: Biointerfaces: 113419.

Young C, Schluep T, Hwang J, Eliasof S (2011) CRLX101 (formerly IT-101) a novel nanopharmaceutical of camptothecin in clinical development. Current Bioactive Compounds 7: 8-14.

Yuan H, Jiang W, Von Roemeling CA, Qie Y, Liu X, Chen Y, Wang Y, Wharen RE, Yun K, Bu G (2017) Multivalent bi-specific nanobioconjugate engager for targeted cancer immunotherapy. Nature nanotechnology 12: 763-769.

Zang S, Jin L, Kang S, Hu X, Wang M, Wang J, Chen B, Peng B, Wang Q (2016) Periodontal Wound Healing by Transplantation of Jaw Bone Marrow-Derived Mesenchymal Stem Cells in Chitosan/Anorganic Bovine Bone Carrier Into One-Wall Infrabony Defects in Beagles. Journal of periodontology 87: 971-981.

Zanganeh S, Hutter G, Spitler R, Lenkov O, Mahmoudi M, Shaw A, Pajarinen JS, Nejadnik H, Goodman S, Moseley M (2016) Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues. Nature nanotechnology 11: 986-994.

Zeng Z, Gao H, Chen C, Xiao L, Zhang K (2022) Bioresponsive nanomaterials: recent advances in cancer multimodal imaging and imaging-guided therapy. Frontiers in Chemistry 10: 881812.

Zhang C, Zhou X, Zhang H, Han X, Zhou X (2022) Recent progress of novel nanotechnology challenging the multidrug resistance of cancer. Frontiers in Pharmacology: 122.

Zhang W, Zhang Z, Zhang Y (2011) The application of carbon nanotubes in target drug delivery systems for cancer therapies. Nanoscale research letters 6: 1-22.

Zhang Y, Fu J, Shi Y, Peng S, Cai Y, Zhan X, Song N, Liu Y, Wang Z, Yu Y (2018) A new cancer immunotherapy via simultaneous DC-mobilization and DC-targeted IDO gene silencing using an immune-stimulatory nanosystem. International Journal of Cancer 143: 2039-2052.

Zhang Y, Yang H, An X, Wang Z, Yang X, Yu M, Zhang R, Sun Z, Wang Q (2020) Controlled synthesis of Ag2Te@ Ag2S Core–Shell quantum dots with enhanced and tunable fluorescence in the second near-infrared window. Small 16: 2001003.

Zhao C-Y, Cheng R, Yang Z, Tian Z-M (2018) Nanotechnology for cancer therapy based on chemotherapy. Molecules 23: 826.

Zhao DK, Liang J, Huang XY, Shen S, Wang J (2023) Organoids technology for advancing the clinical translation of cancer nanomedicine. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology: e1892.

Zhao X-Y, Wang X-Y, Wei Q-Y, Xu Y-M, Lau AT (2020) Potency and selectivity of SMAC/DIABLO mimetics in solid tumor therapy. Cells 9: 1012.

Zheng X, Zhang P, Fu Z, Meng S, Dai L, Yang H (2021) Applications of nanomaterials in tissue engineering. RSC advances 11: 19041-19058.

Zhu C, Qiu J, Pongkitwitoon S, Thomopoulos S, Xia Y (2018) Inverse opal scaffolds with gradations in mineral content for spatial control of osteogenesis. Advanced Materials 30: 1706706.

Zhu Y, Li J, Li W, Zhang Y, Yang X, Chen N, Sun Y, Zhao Y, Fan C, Huang Q (2012) The biocompatibility of nanodiamonds and their application in drug delivery systems. Theranostics 2: 302.

About The Authors

Miss Khadija NASIR doing MPhil in Microbiology and molecular genetics from the institute of Microbiology and molecular genetics, University of the Punjab, Lahore, Pakistan. She has interest in Genetics and plants. She is doing research on plant micro. Growth of microorganisms at different stages of Vigna radiata.
E-mail: khadijanasir438@gmail.com , ORCID: 0009 0009 1980 1346

Dr. Saba SABA holds a PhD in Microbiology and Molecular Genetics. She currently serves as an Assistant Professor at The Women University in Multan, Pakistan. Dr. Saba's research interests revolve around Human and Microbial Genetics, specifically focusing on Host-Microbe Interactions, Cancer Genetics, and Microbial Genetics Moculation to Environment. She has a strong academic background and a dedication to exploring this intricate relationship. Her contributions to international publications include articles and book chapters, highlighting her commitment to advancing scientific knowledge in this field.
Email: mmgpu03@gmail.com, ORCID: 0000-0002-9182-4667

Dr. Abid HUSSAIN holds a doctoral degree in Microbiology and Molecular Genetics. He is currently working at the University of Okara, Pakistan. His research interests include Immune Regulation, Immune Genetics, Host-Microbiome Interaction, and Drug Delivery. Dr. Hussain has a strong scientific record with numerous international publications such as articles and book chapters.
Email: abihqureshi@gmail.com, ORCID: 0000-0001-8661-9522

Kainat RAMZAN holds a Master's degree in Biochemistry and boasts a strong academic record. Presently, she serves as a visiting lecturer at the University of Okara in Pakistan. Her research focus revolves around Human Genetics and Bioinformatics.
Email: kainatramzan54@gmail.com,

Dr. Sobia MUSHTAQ received her PhD in 2015 in biochemistry/molecular biology from Quaid-i-Azam University, Islamabad, Pakistan. She is working at University institute of Biochemistry and Biotechnology, Arid Agriculture University, Rawalpindi. Her research interests includes working on molecular mechanisms of diseases especially cardiac disorders and diabetes, nano-particles delivery as drugs to control cardiac disorders and use of microRNAs as therapeutic agents. She has publications in peer-reviewed journals. She also has written many book chapters for BEd course Biology at Allama Iqbal open university, Islamabad.
E-mail: sobiamushtaq26@gmail.com, ORCID: 0009 0001 2086 8304

To Cite This Chapter:
Nasir, K., Saba, S., Hussain, A., Ramzan, K., & Mushtaq, S. (2023). **Nanoparticle based drug delivery in cancer therapy.** In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 169-206). ISRES Publishing**.**

VIRAL BIOLOGICAL THREATS FROM CBRN AGENTS

Ali SERT
Mümin POLAT

Introduction

Microbiological agents are universally distributed in nature and form a dominant part of life on earth. They are found in soil and water as well as in plants and animals, but also in the most hostile habitats such as hot springs and deep ocean vents. The vast majority of microbial agents are harmless and many are beneficial. Turning this benefit into harm is mostly done by human beings. While some of them have been used for fermentation in food processes for centuries , some of them are applied in biotechnological processes. However, some of these agents are pathogenic or toxic . They can cause disease in humans, plants or animals. Some may be considered biological warfare agents. Infectious diseases, which have always affected people since the past, still show their effects as agents that negatively affect human health today. Despite improvements in treatment, care, and disease prevention measures, these new infections, which arise due to the emergence of new agents or the periodic transformation of previous agents, are becoming increasingly complex problems. Because the usual pathogens are more virulent or more resistant to existing drugs; It creates difficulties in dealing with these agents. Multi-resistant or completely resistant microorganisms make treatment almost impossible. Recently, increasing socio - cultural mobility and changes in climate and nature, either spontaneously or by human hand, cause the emergence of new infectious agents or their spread to very distant regions when they are limited to a certain geographical region. The fact that a significant part of the new infectious agents is of zoonotic origin has resulted in a better understanding of the close connection between human and animal health and the concept of "one health". On the other hand, the developments in diagnosis and surveillance techniques and the international cooperation provided by the awareness created facilitate the timely diagnosis of new or re-emerging infections and taking appropriate measures at the international level. Experiences in which the infectious diseases we have encountered recently have been effective all over the world have shown that it is necessary to be prepared for new viral infections in the future as well.

MERS- CoV, Ebola, avian, whose names we have heard frequently for more than 15 years around the world Some new or re-emerging viral diseases such as influenza , and finally Zika virus pose threats to human health and adversely affect the patient care system. (Hancı et al. 2001).

Pandemic : An epidemic that crosses international and natural borders or at least spreads over a large region. A pandemic can begin when three conditions are met :

- ✓ the emergence of a new or old disease with a low level of protection in a population
- ✓ infect humans and cause serious disease;
- ✓ It can spread easily and sustainably among people.

Deadly epidemics in human history; It has had devastating effects on the economy and society. Understanding the mechanism of action of biological agents as possible biological weapons is closely linked to breakthroughs in science and industrial production over the past 80 years. Therefore, future breakthroughs in the natural sciences, especially in gene and biotechnology , are likely to lead to further leaps in

the development of new biological agents tailored for specific tasks. A small outbreak of disease can be an early warning of a serious biological attack. The agents we encounter in this way can be characterized in the context of epidemic time, place and person after the case definition is determined. The definition of an epidemic as endemic, epidemic , or pandemic may vary, depending in part on the "expected" situation. As they describe diseases that are limited but local and common in a particular geographic area or population, epidemics refer to diseases that involve many more people than normal in a given community or that spread to areas where they are naturally found. In the 21st century, when epidemics are mentioned, epidemics such as cholera and plague come to mind, but in the century we live in, these epidemics are replaced; It has taken agents that will cause great destruction on a global scale such as Ebola, SARS, MERS, Coronaviruses . If we consider these biological agents in terms of CBRN, we know that they are used more than Chemical or Nuclear warfare agents, especially in the 1st and 2nd World Wars. Based on this information, it brings to mind the question of whether the biological agents that emerged in today's time can be used like other agents used in the wars of the past. In the light of these dangers, the concept of bioterrorism has entered the literature.

If we talk about why biological agents are used more than other agents in the past and the possibility of using today's agents, the underlying factors are ; These agents are preferred more or are likely to be preferred because they are low cost, affect large masses, and cause an environment of fear and panic.

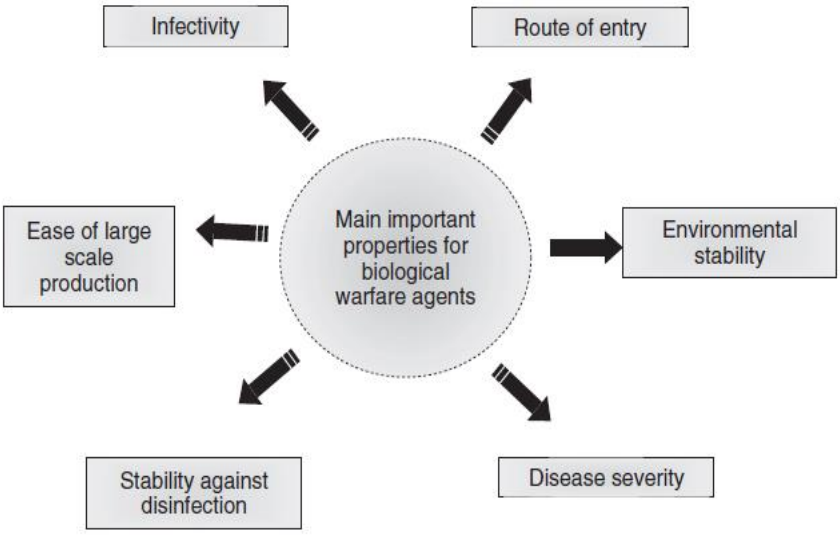


Figure 1. Some features that should be included in the agenda to be used for biological warfare

Categories of Biological Agents Considered for Potential Biological Warfare

- ✓ Category A agents are highly contagious, easily transmitted, and cause high mortality .
- ✓ Category B agents, second highest priority agents, completely inactivate living things, but have a lower mortality rate than category A agents.

- ✓ Category C agents include emerging pathogens that may be engineered for mass spread in the future because of their ease of production and spread, and their high potential for morbidity, mortality, and major health effects.

COVID-19 Coronaviruses

It is a family of RNA viruses that typically cause mild respiratory illness in humans. Severe acute respiratory disease (SARS), which emerged in 2003, showed that coronaviruses can also cause outbreaks of serious infections in humans. Later in 2012, Middle East respiratory syndrome coronavirus (MERS- CoV) emerged in Saudi Arabia. After two previous coronavirus outbreaks, a new type of coronavirus was identified in 2019 in Wuhan , China . On 31 December 2019, China's National Health Commission notified the World Health Organization (WHO) about the detection of pneumonia cases of unknown etiology in the city of Wuhan , Hubei province , and the total number of cases increased to 44 by January 3, 2020. It was stated that the factor causing the disease could not be defined yet at the time of the notification. Following the closure of the Huanan seafood market on January 1, 2020, on January 11 and 12, WHO received detailed information from the Chinese National Health Commission that the outbreak was related to the seafood market in Wuhan city. On January 7, 2020, it was determined by experts in China that the cause of the epidemic was a new type of coronavirus and it was isolated in the laboratory environment.

At the same time , diagnostic kits have been developed for the new type of coronavirus, which is called 2019-nCoV, so that it can be used in other countries. On January 11, the first death due to the new coronavirus occurred, and on January 13, the first case of laboratory-confirmed new coronavirus in Thailand, in a country other than China, was reported by the Ministry of Public Health of Thailand. On January 12, China shared the genome sequence of the novel coronavirus with WHO. WHO consultants called the type of virus that causes the disease “Covid-19” to create the official name of the epidemic. It spread to other countries not long after the first case of the epidemic was reported. On January 30, 2020, the WHO Director-General declared a “Global Emergency” due to the spread of the epidemic that started in China to other countries. It is stated that the purpose of the emergency, which was declared for the 6th time in the 21st century, is to prevent the spread of the epidemic to countries with weak health systems. In order to control the epidemic, travel movements in China and other countries have been restricted and strict control measures have been implemented. On March 11, 2020, the WHO Director-General declared that they considered the Covid-19 outbreak caused by SARS-CoV-2 as a pandemic, as the number of Covid-19 cases outside of China increased by 13 times and the number of affected countries by 3 times The coronavirus outbreaks in the past were limited to the epidemic, but Covid-19 became a global nightmare and caused a pandemic . WHO declared the epidemic caused by the H1N1 virus, a deadly subtype of the influenza virus, as a pandemic in 2009 . Coronaviruses are known to cause zoonotic diseases by passing from animals to humans . Since the human body does not develop immunity to these pathogens, zoonotic diseases can be fatal. Information about the source of SARS-CoV-2 is not yet clear. Since the first case was related to the Huanan seafood market, different studies on animals continue. After analyzing the Covid-19 genome sequence, the virus gene sequence was compared with those in the database. It was concluded that the two coronaviruses originating from bats were 88% similar to Covid-19. In addition, 79% similarity with the coronavirus causing SARS and 50% with MERS- Cov was detected. Research continues on the endangered scaly anteater (Pangolin) in terms of being a reservoir host for SARS-CoV-2, apart from bats (Yücel and Görmez 2019).

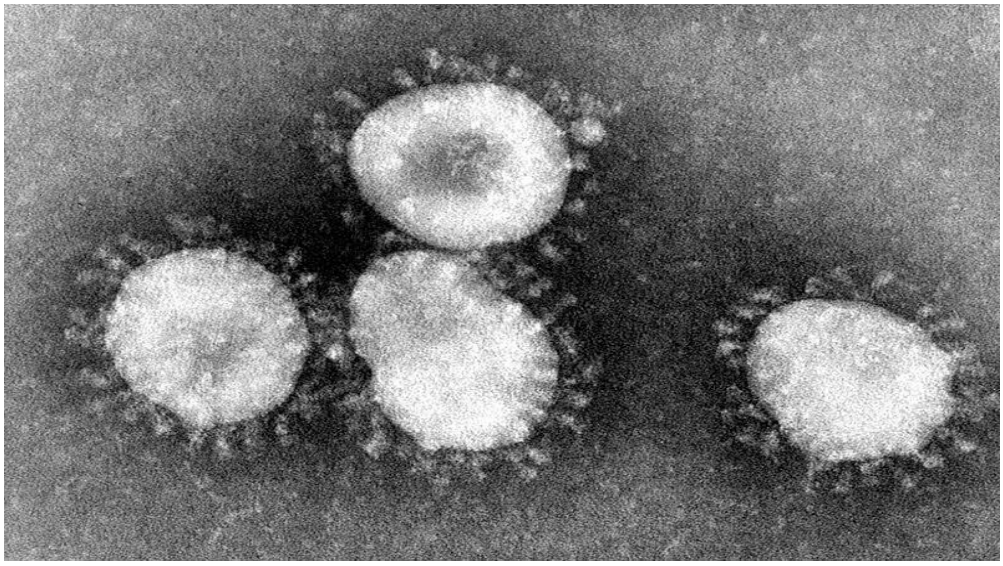


Figure 2. Coronavirus microscope image

is stated that there is a 99% similarity in genome sequences between the virus isolate in pangolins and SARS-CoV-2 obtained from an infected person. The coronavirus and causes disease in humans is a very important step in preventing the reoccurrence of the disease in the future, as well as preventing the reoccurrence of the experienced situations by preventing the occurrence of an epidemic. The genomes of coronaviruses have great genetic diversity due to frequent recombination.

Coronaviruses, which have gained the feature of infecting new cell types and new species, are the cause of inter-species infections and create an epidemic among humans like the pandemic we live in. Due to increasing levels of human animal interaction, it is anticipated that coronaviruses may periodically emerge in humans. There is information that individuals of all ages have reported Covid-19 cases. The severity of the infection has been observed to vary from asymptomatic to critical diseases, and the clinical severity of Covid-19 has been defined in 5 groups. It has been observed that although the Covid-19 test is positive in asymptomatic cases, it does not show any signs and symptoms, and in mild cases, symptoms of acute upper respiratory tract infection such as high fever, fatigue, myalgia, cough, sore throat are observed. While it is stated that patients with moderate disease have pneumonia , fever, cough symptoms but no shortness of breath, it is stated that there are signs and symptoms such as dyspnea , cyanosis , and saturation level less than 92%, in addition to the rapid progression of the disease in severe cases. It is stated that critically ill patients have ARDS or respiratory failure, shock and multiple organ failure. It is known that the mortality rate in adults with a critical disease can reach up to 50%, of coronaviruses having auxiliary proteins, they can escape from the innate immune system of the host, and the immune system's response may this time harm the body instead of protecting it. For this reason, it is important to have an underlying disease in the infected person . It has been observed that the progression of the disease to the critical level can occur in less than 5 days in individuals infected with SARS-CoV-2 in advanced ages.

It has been obtained as a result of studies that people with hypertension, diabetes, cardiovascular and respiratory diseases, and chronic kidney failure accounted for 2.8% of those who lost their lives due to Covid-19 (Yücel and Görmez 2019). In the results of an analysis , it has been reported that especially having hypertension at an

advanced age, severe course of the disease in case of being infected with Covid-19, and the risk of mortality increases approximately 2.5%. The increase in the financial power of terrorist organizations, which is one of the negative consequences of globalization, and the fact that terrorism, supported by some states, has become a widespread method of struggle, has paved the way for various organizations to expand their targets. In today's world; It is a fact that everyone should accept and get used to that there may be terrorists with viruses, bacteria, dangerous chemical weapons or nuclear bombs that can kill thousands of people. We know that; Biological weapons are bacteria, viruses, toxins, etc., which are used to create destructive effects on other living things. are infectious agents (10).

A bioterrorism epidemic has the same characteristics as a natural epidemic. It can spread like an ordinary disease and it is not possible to predict when it will start, and the treatment protocols of some diseases are not clear. For example, the emergence of Ebola in Guinea or the threat posed by the MERS epidemic in the Middle East are uncertain. Therefore, early measures should be taken with fast and accurate detection methods (diagnostic kits), and it is important for endemic and pandemic situations to prevent the spread of diseases. The easier and cheaper the production, transportation and use of biological weapons, the more expensive and difficult are the methods of prevention and treatment. Due to the incubation times, it takes time to be detected after an attack, making the biological weapon more dangerous. For this reason, unusual situations should be carefully examined. For an effective defense against these agents, a complete communication network, organized health institutions, scientists, detailed information and organization such as health statistics are needed.

Conclusion

The use of viruses as biological weapons leaves deep traces by affecting large masses, causing social destruction and showing its effect with chronic diseases. Since their production does not require high technology and their costs are low, their malicious use is always possible. Especially when aerosolized bacteria, viruses, spores or toxins are sprayed to the environment by airplanes or by different methods, it has the feature of causing serious morbidity and mortality . Although there are very detailed studies on diseases and epidemics of microorganisms that are biological warfare agents in the literature, there are limited data on the clinical picture and treatment that may occur when these microorganisms are used as biological warfare agents, especially in aerosol form. All countries of the world and healthcare professionals should closely follow all these re-emerging factors and carry out global studies to combat these factors.

References

Aslan FG, Altındiş M, 2016. Current viral factors; zika , chikungunya , ebola, enterovirus d68, mers cov , influenza . Kocaeli University Journal of Health Sciences, 2, 11-16. Barras , V. , & Greub , G. (2014). History of biology warfare and bioterrorism _ clinical microbiology and infection , 20(6), 497-502.

Cheng , Z. (2020). clinical features of patients infected with 2019 novels coronavirus in Wuhan , China . the lancet , 395(10223), 497-506.

Conenello , GM ., Zamarin , D., Perrone , LA, Tumpey , T., and Palese , P. (2007) *PLoS pathog* _ , **3** (10), 1414–1421.

Erkekoğlu P, Koçer- Gümüşel B, 2018. Biological warfare agents: their history, pathophysiology , diagnosis, treatment and precautions. fabad journal of pharmaceuticals sciences , 43, 81-111.

Guillemin , J. (2005) *Biological Weapons : From the Invention of State-Sponsored Programs to Contemporary bioterrorism* , Columbia University Press , New York. ISBN: 978-0231129428 .

Hancı İH ., Özdemir, Ç, Bozbıyık , A., & Tuğ, A. (2001). Biological weapons: effects, methods of prevention . *sted , 10* , 330-332.

He, F. , Deng , Y., & Li , W. (2020). Coronavirus Disease 2019 (COVID-19): What we know ? Journal of medical virology _

Henderson DA, Inglesby TV, Bartlett et al: Smallpox as a biological weapon . medical and public health management . jama 1999; 281, 2127-2137.

Khardori , N. (2006) *Bioterrorism Preparedness* , WILEY-VCH, Weinheim . ISBN: 3-527-31235-8.

Kircicek , A. , Arslantaş , D., İncedere , O., Öztaş, D., & Ateş, A. Biological Threats, Novel Coronavirus Disease and Its Place in CBRN. of *and Education 5th International Congress on Woman And Child Health and Education* (P. 27).

Mayor , A. (2003) Greek Fire, Poison arrows and scorpion Bombs : Biological and Chemical Warfare in the Ancient World, Overlook Duckworth . ISBN: 1585677348X.

Midilli K, 2013. New infectious agents and new threats Ankem Journal , 27, 91-94.

Özkuyumcu C, 2004. Viral zoonoses . molecular , clinical and diagnostic virology. sun bookstore, 2004, 293-324

Roy , C. , Reed , D., & Hutt , J. (2010). aerobiology and inhalation exposure to biological select agents and toxins . veterinary pathology , 47(5), 779-789. Huang , C. , Wang , Y., Li , X., Ren, L., Zhao , J., Hu, Y., &

Thompson , A. , Taylor, BN, (2008), The International System of Units (SI), United States version of the English text of the eighth edition (2006) of the International Bureau of Weights and measures publication .

TR Ministry of Health: Notification System of Infectious Diseases, Standard Diagnosis, Surveillance and Laboratory Guide, 4th Edition, Ankara, 2005: 79-88.

Viral agents as biological weapons . Turkish Journal of hygiene biology, Turkiye , 63, 67-78.

Watson, DJ ., Baker, TA, Bell , SP, Gann , A., and Losick , R. (2008) *Molecular Biology of the Gene* , Benjamin Cummings .

Wecht , CH (2004) *Forensic Aspects of Chemical and biological Terrorism* , Lawyers & Judges Publishing, Tucson , AR, USA. ISBN: 978-1930056671 .

Yücel B, Görmez AA, 2019. An overview of the Sars- corona virus. Turkish Journal of Technology and Applied Sciences, 2, 32-39.

To Cite This Chapter:
Sert, A., & Polat, M. (2023). **Viral biologıcal threats from cbrn agents.** In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 207-212). ISRES Publishing**.**

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT PROPERTIES OF THREE COMMON MEDICINAL HERBS

G. SHOBANA
D. CHANDRASEKAR
N. Agnel Arul JOHN
G. HARİHARAN

1. Introduction

Chemical entities with one or more unpaired electrons in atomic or molecular orbitals are referred to be free radicals **(Halliwell and Gutteridge, 1999)**. Free radicals have the ability to damage healthy cells in the body, stripping them of their structural integrity. They play a part in the aging process as well as degenerative illnesses of the aging process, including cancer, heart disease, cataracts, weakened immune systems, and brain dysfunction. In total, at least fifty different illnesses have been linked to the pathophysiology of free radicals.

Antioxidants are necessary to maintain optimal cellular and systemic health and well-being because they can neutralize free radicals before they attack cells. Two main mechanisms of action have been proposed for antioxidants: the first is a chain-breaking mechanism in which the primary antioxidants donate electrons to the free radicals in the system, such as lipid radicals; the second mechanism involves the removal of ROS (reactive oxygen species) and RNS (reactive nitrogen species) initiator by quenching chain initiator catalyst **(Sies 1997)**. Antioxidants can be either enzymatic or nonenzymatic; examples of enzymatic antioxidants are catalase, superoxide dismutase, and glutathione, which catalyze the neutralization of many types of free radicals **(Jacob R.A,1995),** while the nonenzymatic antioxidants include Vitamin C, selenium, vitamin E, carotenoids, and polyphenols. Antioxidants are shown to be essential in preventing heart disease, cancer, DNA deterioration, lung illness, and neurological disorders **(Percival M., 1998).** This evidence is growing.

The medicinal use of plants as antioxidants to lessen oxidative tissue damage has garnered attention recently. Rich in phenolic compounds such as flavonoids, plants, herbs, and spices have been shown to possess antioxidant qualities that help them fight cancer and have anti-inflammatory, antiallergenic, antiviral, and antiaging effects **(Aqil *et al*., 2006).** In India, people from diverse ethnic backgrounds and geographical locations have their own unique cultures, customs, and medical knowledge. Throughout human history, plants have been used to treat illnesses and have also served as a catalyst for the development of novel, side-effect-free pharmaceuticals. Previous research on antioxidant activity in plants primarily concentrated on vitamin content; however, phytochemicals that have been linked to increased levels of antioxidant activity have been the subject of extensive investigation to improve disease conditions. As a result, an appropriate technique was required to measure an agent's antioxidant capacity using in vitro antioxidant assays that call for a small amount of the material.

A study was conducted on three common plants, namely *Euphorbia hirta* Linn. (Leaves), *Centella asiatica* L. (Leaves), and *Calendula officinalis* Linn. (Flower). An aromatic, upright annual herb of the Asteraceae family is called Calendula officinalis Linn. (Thulukka samanthi in Tamil). According to **Parente *et al.* (2012)**, it is frequently used to treat minor wounds, skin infections, burns, bee stings, sunburns, and other conditions. Appearing in the Apiaceae family, *Centella asiatica* L. (Tamil name: vallarai).**As per Vijayashalini and Abirami (2018)**, it treats skin infections

and illnesses, inflammation, and persistent ulcers. The Euphorbiaceae family includes *Euphorbia hirta* Linn. (Tamil name: Amman pachcharisi). Conjunctivitis, wounds, and sores are treated with it as an antiseptic.The herb is known for its analgesic properties, which can be used to relieve colic, rheumatism, intense headaches, and toothaches**(Kumar and others, 2010)**. The current investigation therefore sought to assess the antioxidant and phytochemical screening capabilities.

2. Material and Methods

2.1. Collectıon of Plant Material

The plant materials utilized in this study were gathered in and around Trichy. They included *Calendula officinalis* Linn. (Flower), *Centella asiatica* L. (Leaves), and *Euphorbia hirta* Linn. (Leaves) (Voucher number: BISH0000619230; FRI50032; 11077). Rev. Fr. Dr. John Britto, the taxonomist and director of the Rapinart Herbarium at St. Joseph College in Trichy, identified and verified the materials.

2.2. Preparatıon of Aqueous Extracts

After being shade dried, the leaves of *Euphorbia hirta* Linn., *Centella asiatica* L., and *Calendula officinalis* Linn. were coarsely powdered using an electrical blender. A mixture of 200g of each plant material and 1.2 litre of water was prepared. Subsequently, it was reduced to one-third by boiling and filtered. Dryness was reached by evaporating the filtrate. Additional research was done on the extracted paste form.

2.3. Prelımınary Phytochemıcal Screenıng

Using standard procedures, alkaloids, glycosides, saponins, tannins, terpenoids, phenolic compounds, protein, and carbohydrates were tested for various phytoconsituents in the dry powder and aqueous extract of *Calendula officinalis Linn.* (Flower), *Centella asiatica* L. (Leaves), and *Euphorbia hirta* Linn. (Leaves) **(Brindha *et al*., 1981).**

2.4. *In vıtro* Antıoxıdant Actıvıty

The DPPH radical scavenging assay **(Brand Williums *et al*., 2000)**, the ABTS radical scavenging **assay (Robert Re *et al*., 1999)**, the reducing power assay **(Manmohan Snghal *et al*., 2011)**, and the nitric oxide radical inhibition assay **(Sreejayan and Rao, 1996)** were used to assess the free radical scavenging activity of the aqueous extract of *Calendula officinalis* Linn. (Flower), *Centella asiatica* L (Leaves), and *Euphorbia hirta* Linn. (Leaves).

2.4.1. DPPH radical scavenging assay (Brand williums *et al*., 2000)

DPPH was used to assess the chosen plants ability to scavenge free radicals. A 200µM DPPH solution was made with 95% methanol. Five test tubes containing 100 and 1000 µg/ml of the stock plant extract solution were used. Using a spectrophotometer, the absorbance was measured at 517 nm after 10 minutes of incubation of 0.5 ml of freshly prepared DPPH solution with the test drug. The reference was standard ascorbic acid.

Calculation

% scavenging of the DPPH free radical was measured using following equation

2.4.2. ABTS radical scavenging assay (Robert Re *et al.*, 1999)

Different concentrations of standard ascorbic acid and plant extract (ranging from 50 to 250 µg) were added to a set of test tubes. Phosphate buffer was used to create a 2.5 ml volume with various concentrations. For the Control, two milliliters of phosphate buffer were added.Then, each test tube received 0.3 milliliter of ABTS solution.At 734 nm, the solution was immediately read.

Calculation:

The percentage scavenging activity of plant extract on ABTS

$$\mathbf{I\ (\%) = (A_0 - A_1)/A_0 \times 100}$$

2.4.3. Reducing power assay (ManmohanSnghal*et al.*, 2011)

1 ml of varying concentrations (100-500 µg/ml) of aqueous plant extracts was mixed with 2.5 ml phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Aliquots of 2.5 ml of trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10min. The upper layer of the solution (2.5 ml) was mixed with 0.5ml of freshly prepared ferric chloride solution was added and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power.

2.4.4. Nitric oxide radical inhibition assay (Sreejayan and Rao, 1996)

Aqueous plant extracts at varying concentrations were incubated with sodium nitro prusside solution, with ascorbic acid serving as the standard.Ascorbic acid was added to 5 ml of 0.025M phosphate buffer and various concentrations of plant extracts are present in the volume.The tubes spent three hours being incubated at 25°C.While sodium nitro prusside and buffer were used in equal amounts, the test compounds were not used in the control experiment.Griess reagent (0.5ml) was combined with 0.5ml of the reaction mixture after three hours.A 546 nm absorbance measurement was made.

Calculation:

$$\mathbf{NO_2\ Scavenging\ activity\ (\%) = A_{Control} - A_{Test} / A\ Control \times 100}$$

3.Result and Dıscussion

Biologically active naturally occurring chemical substances called phytochemicals are present in plants. These compounds also give plants their flavor, color, and aroma. Their primary function is to shield the plants from disease and harm from biotic and abiotic stresses. These plant compounds, also known as phytochemicals, are created to shield plant cells from environmental dangers like UV radiation, pathogenic invasion, drought, stress, and pollution. Presently, they play a crucial role in preserving human health and preventing disease, which eventually improves quality of life.

There is a wide-ranging dietary phytochemical that are found and obtained from edible fungi, vegetables, fruits, nuts, whole grains, seeds, herbs, spices and legumes. They are accumulated in various plant portions such as in the seeds, roots, stems, leaves, flowers, and fruits.

Table 1a. Preliminary Phytochemical analysis of *Calendula officinalis* Linn.

S.No	Test	Drug Powder	Aqueous Extract
1	Saponin	-	-
2	Tannin	+	+
3	Sterol	+	+
4	Terpenoids	+	+
5	Flavonoid	+	+
6	Coumarin	+	-
7	Quinines	-	+
9	Alkaloids	+	+
10	Glycosides	+	+
11	Sugar	-	-
12	Phenols	+	+

Table 1a shows the results of the preliminary phytochemical analysis of *Calendula Officinalis* Linn. It was discovered that the medicinal powder and watery extract of *Calendula Officinalis* Linn. contained sterols, alkaloids, phenols, glycosides, flavonoids, and terpenoids. The antioxidant properties of *Calendula Officinalis* Linn. may have been caused by the presence of flavonoids, terpenoids, and phenols.

Table 1b. Preliminary Phytochemical analysis of *Centella asiatica* Linn.

S.No	Test	Drug Powder	Aqueous Extract
1	Saponin	+	+
2	Tannin	-	-
3	Sterol	+	+
4	Terpenoids	-	-
5	Flavonoid	+	+
6	Coumarin	+	-
7	Quinines	-	+
9	Alkaloids	+	+
10	Glycosides	+	+
11	Sugar	-	-
12	Phenols	+	+

Result obtained for qualitative screening of phytochemicals in the leaves of *C. asiatica* L. and its bioactivity are presented in **Table 1b.** The drug powder and aqueous extracts of *C. asiatica* L.showed the presence of major phytoconstituents such as phenol, alkaloids, flavonoids, sterol and saponin which may responsible for pharmacological actions and medicinal properties.

Table 1c. Preliminary Phytochemical analysis of *Euphorbia hirta* Linn.

S.No	Test	Drug Powder	Aqueous Extract
1	Saponin	-	-
2	Tannin	+	+
3	Sterol	+	+
4	Terpenoids	+	+
5	Flavonoid	+	+
6	Coumarin	+	-
7	Quinines	-	+
9	Alkaloids	+	+
10	Glycosides	+	+
11	Sugar	-	-
12	Phenols	+	+

The results of the phytochemical screening of drug powder and aqueous extract of leaves of ***Euphorbia hirta* Linn.** showed the presence of alkaloids,phenol, Flavonoids,terpenoids,sterol and tannin. The presence of these compounds suggests that ***Euphorbia hirta* Linn.** having various medicinal properties which can be used in the treatment of various diseases.

Based on the preliminary phytochemical results, alkaloids,phenol, glycosides and flavonoids was found in both drug powder and aqueous extract of *C. officinalis* , *Centella asiatica* L. and *Euphorbia hirta* Linn. The saponin was totally absent in *C. officinalis* and *Euphorbia hirta* Linn.**(Table 1a,b and c).**

In Vitro Antıoxidant Activity

Owing to the complexity of phytoconstituents, the antioxidant activity of herbal drugs or plant extracts cannot be assessed by a single assay; thus, the study must use widely recognized assays to assess the antioxidant activity of plant extracts. Numerous techniques for assessing antioxidant activity and elucidating the mechanism of antioxidants have been developed. The assays most frequently used to assess the antioxidant activity of plant extracts are DPPH assay, ABTS, Nitric oxide, superoxide anion hydroxyl radical, and AAPH scavenging. Therefore, the goal of the current study is to demonstrate the chosen plants' capacity for antioxidants.

Table 2: Effect of aqueous extracts of calendula officinalis linn., Centella asiatica l. and euphorbia Hirta linn. on DPPH scavenging activity

Concentration of plant extract and standard (µg)	DPPH Radical scavenging Assay(%)			
	AECO	AECA	AEEH	Std (Ascorbic Acid)
100	29.72	19.09	30.11	27.52
200	43.24	23.63	42.56	42.87
300	56.75	37.27	52.35	57.85
400	67.56	50.9	69.37	68.92
500	72.97	75.45	78.23	80.12

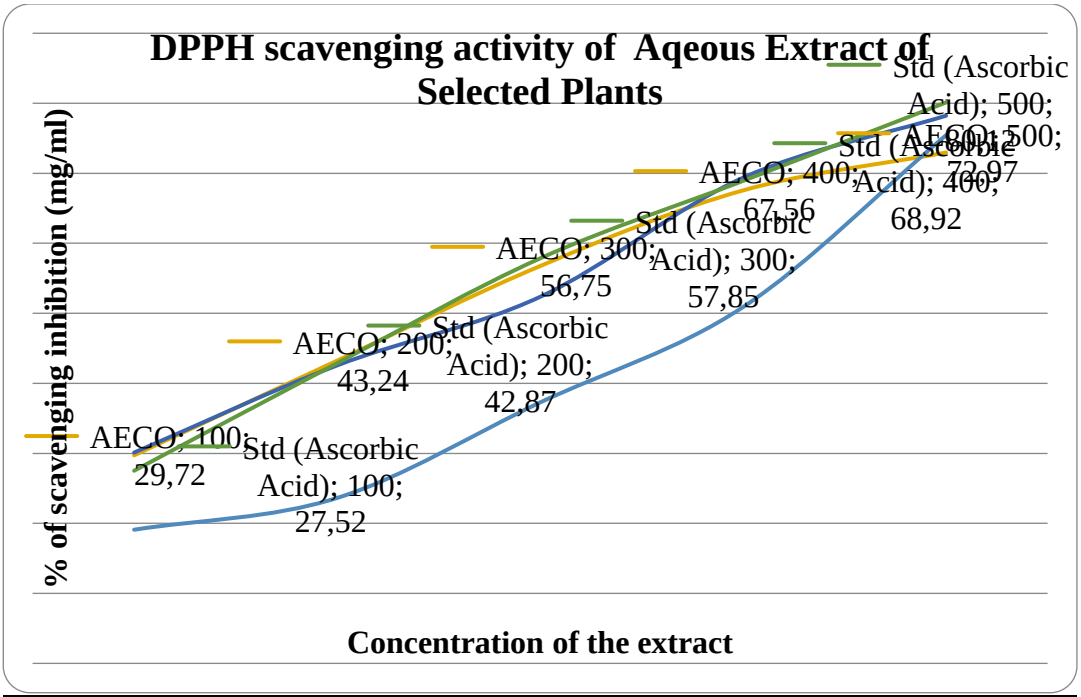


Figure 1. Effect of aqueous extracts of calendula officinalis linn., centella asiatica l. and euphorbia hirta linn. on dpph scavenging activity

DPPH is a relatively stable free radical with a nitrogen center that can readily take on an electron or a hydrogen radical to transform into a stable diamagnetic molecule.The concentration of the plant extract was raised to enhance this activity. The foundation of the DPPH antioxidant assay is the ability of the stable free radical 1,1,di phenyl -2 picryl hydrazyl to become decolored when antioxidants are present.An odd electron found in the DPPH radical is what causes the absorbance at 517 nm as well as the noticeable deep purple color.Changes in absorbance provide a quantitative measure of the decolorization of DPPH, which occurs when it accepts an electron donated by an

antioxidant compound. The ability of substances or plant extracts to function as free radical scavengers has been extensively tested using this kind of reactivity. In the current study , the DPPH radical scavenging activity of aqueous extracts of *Calendula officinalis* Linn., *Centella asiatica* L. and *Euphorbia hirta* Linn. was studied and the levels are given in **Table 2 and figure 1.** The DPPH free radical scavenging ability, the aqueous extracts of those plants showed strong radical scavenging activity with percentage of 72.97% -AECO , AECA- 75.45 %, AEEH-78.23% at higher concentration (500 **µg/ml).** The order of scavenging activity was maximum in *Euphorbia hirta* Linn. followed by and *C. officinalis*, *Centella asiatica* L. The values are also comparable with standard ascorbic acid at the same concentration **(Table 1a & Fig.1a).** This result of the present study suggested that the *C. officinalis* , *asiatica* L. and *Euphorbia hirta* Linn. contain flavonoids that can donate electron/hydrogen easily.

Table 3. *Effect of Aqueous Extracts Of* Calendula Officinalis *Linn.,* Centella Asiatica *L. And* Euphorbia Hirta *Linn. on Abts Radical Scavenging Activity*

Concentration of plant extract and standard (µg)	**ABTS Radical scavenging Assay(%)**			
	AECO	**AECA**	**AEEH**	**Std (Ascorbic Acid)**
100	29.56	15.62	22.31	29.32
200	43.91	31.25	32.07	42.37
300	58.26	37.5	46.7	56.25
400	72.6	46.87	49.14	70.3
500	82.6	65.62	66.2	82.1

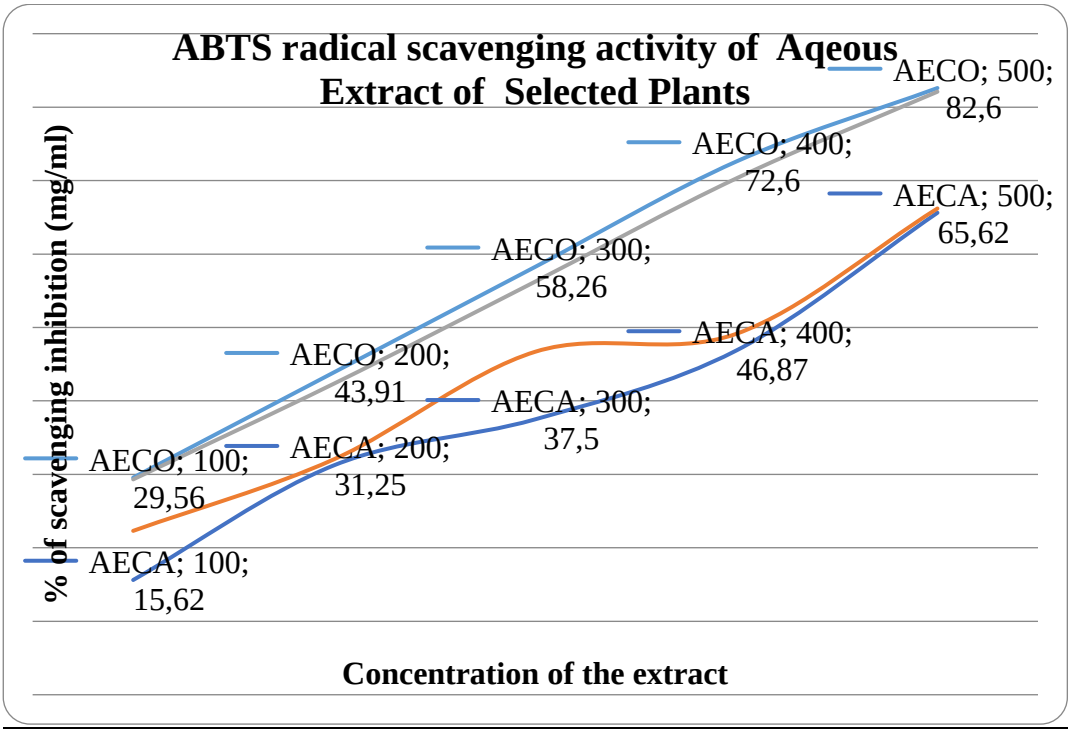


Figure 2. EFFECT OF AQUEOUS EXTRACTS OF Calendula *officinalis* Linn., *Centella asiatica* L. and *Euphorbia hirta* Linn. ON ABTS RADICAL SCAVENGING ACTIVITY

Compared to other antioxidant assays, the ABTS assay is more suitable for evaluating the antiradical capacity of both hydrophilic and lipophilic antioxidants because it can be used in both organic and aqueous solvent systems. The ABTS radical cation can be reduced by antioxidants, which is the basis for this method **(Pellegrini *et al.*, 1999)**. The basis of the ABTS assay is the way antioxidants inhibit the radical cation, ABTS+, which has a distinctive wavelength of 734 nm.The method's basic idea is that ABTS and potassium persulphate react to form the blue-green chromogen known as ABTS radical cation (ABTS+). The color radical is changed into a colorless state when an antioxidant reductant is present **(Sreejayan and Rao, 1996).** Aqueous extracts of *Euphorbia hirta* Linn, Centella asiatica L., and C. officinalis Linn.were used in this study.

From the table 3 and Fig.2 , The observed ABTS.+ cation radical showed a significant increase, which could be attributed to scavenging activity. of *C. officinalis* Linn. , *Centella asiatica* L. and *Euphorbia hirta* Linn. and the inhibition is dependent on the concentration of plant extracts. The maximum activity was noted at the higher concentration of (500µg) aqueous extracts of the selected plants. Among the four plants tested, the aqueous extracts of *C. officinalis* has better $ABTS^{\cdot+}$ cation radical scavenging activity.The aqueous extracts of *C. officinalis* , *Centella asiatica* L. and *Euphorbia hirta* Linn. at concentration of 500µg exhibited a maximum inhibition of $ABTS^{\cdot+}$ cation formation at a percentage of 82.60%, 65.62%, 66.20% repectively.

Certain chemical compounds have the ability to inhibit the potassium persulfate activity, which in turn reduces the amount of ABTS•+ produced. According to the aforementioned findings, flavonoids with greater ability to lower free radicals (ABTS•+) are present in the aqueous and ethanol extracts of *calendula officinali* Linn., *Centella asiatica* L., and *Euphorbia hirta* Linn. These extracts have the highest antioxidant activity.

Table 4. *EFFECT OF AQUEOUS EXTRACTSOF* Calendula officinalis *Linn.*, Centella asiatica *L. And* Euphorbia hirta *Linn. ON REDUCING POWER ASSAY*

Concentration of plant extract and standard (µg)	Reducing Power Assay(%)			
	AECO	AECA	AEEH	Std (Ascorbic Acid)
100	50	33.3	33.3	48.25
250	66.6	50	50	62.7
500	72.7	60	55.55	71.65
750	80	77.7	71.42	78.95
1000	94.2	81.8	73.33	80.85

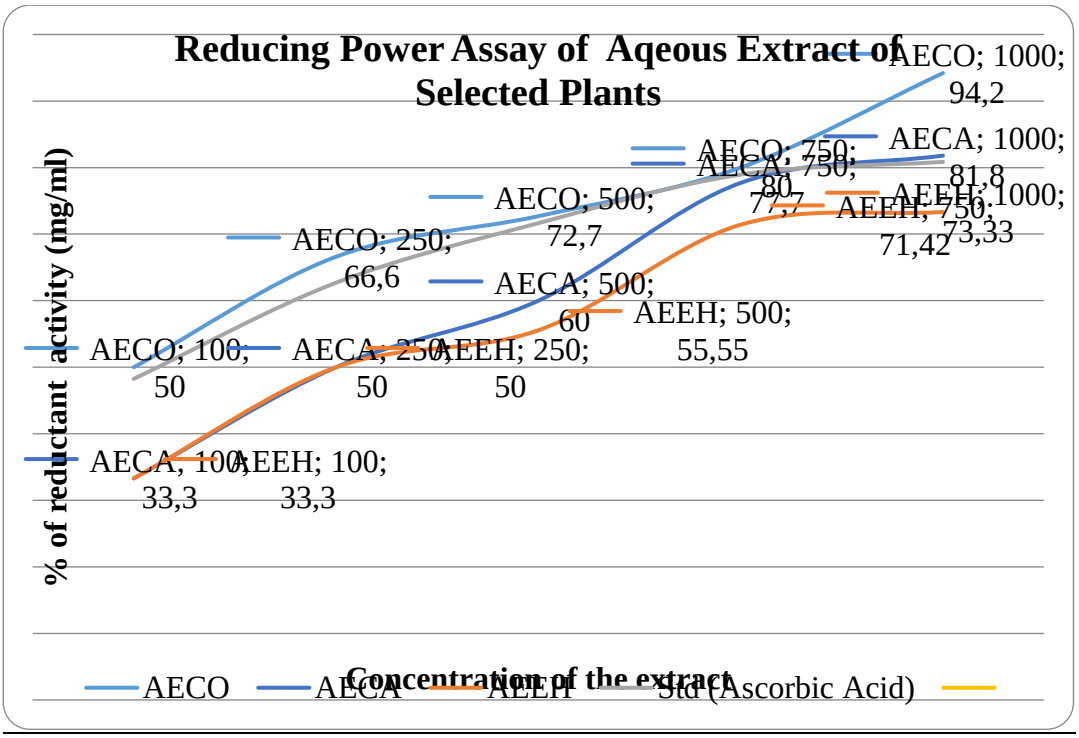


Figure 3. *EFFECT OF AQUEOUS EXTRACTS OF* Calendula officinalis *Linn.*, Centella asiatica *L. and* Euphorbia hirta *Linn. ON REDUCING POWER ASSAY*

The reduction of the Fe3+ -ferricyanide complex to the ferrous (Fe2+) ions creates a chromogenic complex, which is measured at 700 nm of the resulting blue-green colored solution. This absorbance is proportional to the amount of Fe2+ in the mixture. The reducing power assay measures the electron donating capacity of reducing agents, i.e. antioxidants. The ferric reducing antioxidant power of the aqueous extracts of Euphorbia hirta Linn., Centella asiatica L., and C. officinalis was assessed; the results are shown in Table 4 and Figure 3. The current investigation

shows that certain plant extracts have the capacity to lower ferric ions. For C. officinalis, Centella asiatica L., and Euphorbia hirta Linn., the ferric reducing antioxidant value measured at 1000 µg was regarded as100%. It could be found that aqueous extracts of all the selected plants exhibited highest activity and the values are 94.2%(*C. officinalis*) followed by 81.8% (*Centella asiatica* L.) and 73.33% (*Euphorbia hirta* Linn.).

From the above results, it was suggested that the aqueous extracts of *C. officinalis* , *Centella asiatica* L. and *Euphorbia hirta* Linn. have significant antioxidant activity which could be attributed to the presence of flavanoids and phenolic content.

Table 5. EFFECT OF AQUEOUS EXTRACTS OF *Calendula officinalis* Linn., *Centella asiatica* L. and *Euphorbia hirta* Linn. ON NITRIC OXIDE INHIBITION ASSAY

Concentration of plant extract and standard (µg)	**NO Inhibition assay (%)**			
	AECO	**AECA**	**AEEH**	**Std (Ascorbic Acid)**
100	27.85	25.7	27.25	28.02
200	30.38	28.23	29.78	30.4
300	38.87	36.72	38.27	39.45
400	50.36	48.21	49.76	50.7
500	79.4	77.25	78.8	79.25

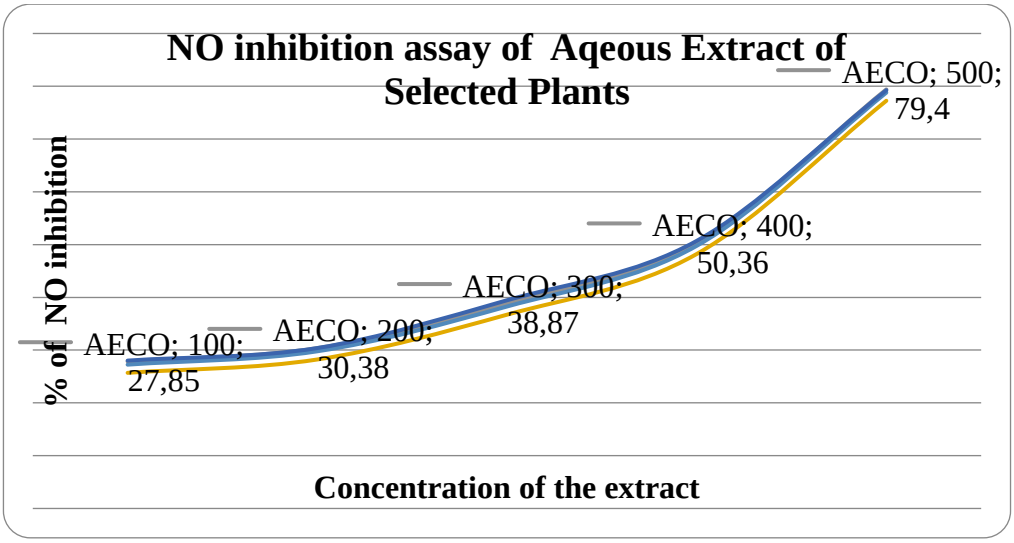


Figure 4. EFFECT OF AQUEOUS EXTRACTSOF Calendula officinalis Linn., Centella asiatica L. And Euphorbia hirta Linn. ON NITRIC OXIDE INHIBITION ASSAY

Strong pleiotropic inhibitors of physiological processes, including neuronal signaling, smooth muscle relaxation, platelet aggregation inhibition, and cell-mediated toxicity regulation, are exhibited by nitric oxide (NO). As an effector molecule in various biological systems, such as neuronal messenger, vasodilatation, and antimicrobial and antitumor activities, it is a diffusible free radical.Nitrate and peroxy nitrite anions, which function as free radicals, are produced when oxygen combines with excess nitric oxide. The nitric oxide in this study was produced by the reaction of sodium nitro prusside with oxygen to form nitrite, which then reacts with griss reagent at physiological PH. The nitrite was then incubated with varying concentrations of plant extracts to form a chromophore, which was measured at 546 nm.

Table 5 & Figure 4. shows the measure of nitric oxide scavenging activity of aqueous extracts of *C. officinalis* , *Centella asiatica* L. and *Euphorbia hirta* Linn. The aqueous extracts of selected plant sources produced moderate to good nitric oxide scavenging activity and the activity was found to be less significant with respect to the inhibition observed at concentration of 100,200 and 300 µg/ ml concentration, while at higher concentration it was found to be highly significant(400,500 µg/ ml). The aqueous extracts of all the selected plants at higher concentration(500 µg) exhibited highest activity and the values are 79.40% (*C. officinalis*) followed by 77.25% (*Centella asiatica* L),78.8% (*Euphorbia hirta* Linn.). Among the three selected plants, *C. officinalis* had significant nitric oxide radical scavenging activity.

From the above results , it was noticed that the aqueous extracts of *C. officinalis* , *Centella asiatica* L. and *Euphorbia hirta* Linn. showed high flavonoid content which has contributed directly to the antioxidant activity by neutralising the free radicals.

Summary

From the results of the present study was concluded that the aqueous extract of flowers of *Calendula officinalis* Linn., leaves of *Centella asiatica* L. and leaves of *Euphorbia hirta* Linn. exhibited optimum levels of antioxidant activities in all the models tested.The antioxidants efficacy of different solvent extract from these plants will be evaluated in the future. And also further in depth studies coupled with *in vivo* model resulted in the development of safe, efficacious and cost effective natural antioxidant.

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

Acknowledgement

The authors are thankful to the Management of Srimad Andavan Arts and Science College (Autonoumous) for providing the research facilities and Dr. G. Jothi, Dean of Life-Sciences and Head, Teaching and Nonteaching Faculty of Department of Biochemistry, Srimad Andavan Arts and Science College (Autonoumous), Triuchirappalli, Tamil Nadu, India. For giving guidance to complete this research work.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this chapter.

References

Aqil, F., Ahmad, I., and Mehmood, Z., (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkish Journal of Biology,* 30 (3), 177–183.

Bhuiyan, M.A.R., Hoque, M,Z., Hossain, S.J.,(2009). Free Radical Scavenging Activities of *Zizyphusmauritiana*. *World J. Agr. Sci*, 5:318-322.

Brand-Williams, W., Cuvelier, M., and Berset, C., (2000). Use of a free radical method to evaluate antioxidant activity. *LWT – J Food Sci. Tech*, 28(1):25–30.

Brindha, P., Sasikala, and Bhimarao, (1981). Pharmacognostic studies on *Coleus Aromatic Benth,*IndianBerage, B.M.E.B.R,12; 17-31.

Duh, P.D., (1998) Antioxidant activity of burdock (*Arctium lappa* Linne.): Its scavenging effect on free-radical and active oxygen. *J. Am. Oil Chem. Soc*, 75, 455-461.

Halliwell, B., and Gutteridge, J.M.C., (1999). Free Radicals in Biology and Medicine (3rd ed.). Oxford University Press,1-25.

Jacob, R.A., (1995). The integrated antioxidant system. *Nutrition Research*, 15 (5): 755–766.

Kumar, S., Malhotra, R., Kumar, D., (2010). *Euphorbia hirta*: its chemistry, traditional and medicinal uses, and pharmacological activities. *Pharmacogn Rev*, 4: 58-61.

Manmohan Singhal, Arindam Paul, HemendraPratap Singh, Sushil Kumar Dubeyaandkalpesh Gaur, (2011).Evaluation of reducing power assay of chalcone semicarbazones. *J. Chem. Pharm. Res*, 3(3): 639-645.

Meir, S., Kanner, J., Akiri, B., Hada, S.P., (1995). Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaf. *J. Agri. Food chem*. 43:1813-1819.

Mojab, F., Kamalinejad, M., Ghaderi, N., Vanidipour, H.R., (2003). Phytochemicals screening of some species of Iranian plants. *Iran J. Pharm. Res*, 3: 77-82.

Moncada, A., Palmer, R.M.J., Higgs, E.A., (1991). Nitric oxide: Physiology, pathophysiology and pharmacology. *Pharmacol Rev*, 43: 109-142.

Oyaizu, M., (1986). Studies on product of browning reaction prepared from glucose amine. *Jpn J Nutr*, 44: 307-315.

Pan, Y., Wang, K.., Huang, S., Wang, H., Mu, X., He, C., et al., (2008). Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longum* Lour.) peel. *Food Chemistry*, 106, 1264-1270.

Parente, L.M., Andrade, M.A., Brito, L.A., Moura, V.M., Miguel, M.P., Lino-Junior Rde, S., Tresvenzol, L.F., Paula, J.R.,, Paulo, N.M., (2011). Angiogenic activity of *Calendula officinalis* flowers L. in rats. *Acta Cir Bras*, 26, 19–24.

Percival, M., (1998). Antioxidants, Clinical Nutrition Insight, *Advanced Nutrition.*,31:201-205.

Raquibul Hasan, S.M., Mokarram Hossain, M.D., Raushanara, A., Mariam, J., Ehsanul Hoque Mazumder, M.D., Shafiqur Rahman, (2009). DPPH free radical scavenging activity of some Bangladesh medicinal plants. *Full length Research paper*. 3 (11): 875-879.

Rekka, E., Kourounakis, P.N., (1991). Effect of hydroxyethyl rutenosides and related compounds on lipid peroxidation and free radical scavenging activity-some structural aspects. *J. Pharm Pharmacol*, 43: 486-491.

Robert, R.E., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine,* 26, 1231-1237.

Sies, H., (1997). Oxidative Stress: Oxidants and Antioxidants. *Experimental Physiology,* 82, 291-295.

Sreejayan, N., Rao, M.N.A., (1996). Free radical scavenging activity of curcuminoids. *J Drug Res*, 46:169-171.

Vijayashalini, P., and Abirami, P., (2018). Diversity of medicinal plants in Eratti Hill, Thamarakarai Beat of Bargur Reserve forest, Western ghost in Erode District, Tamilnadu, India. *Asian J Pharm Clin Res,* 11, (10): 78-85.

Wolfenden, B.S., Willson, R.L., (1982). Radical-cations as reference chromogens in kinetic studies of one-electron transfer reactions: pulse radiolysis studies of [2,2-azinobis-(3-ethylbenzthiazoline-6-sulphonate),]. *J. Chem. Soc. Perkin Trans,* 2, 805-812.

Yildirim, A., Mavi, A., Oktay, M., Kara, A.A., Algur, O.F., Bilaloglu, V., (2000). Comparison of antioxidant and antimicrobial activities of tilia (*Tilia arentea* Desf. Ex. D.C.) sage (*Salvia triloba* L.) and black tea (*Camellia sinensis* L.) extracts. *J. Agr. Food Chem,* 2000; 48(10): 5030-5034.

About The Authors

Dr. SHOBANA G formerly working as a Assistant professor in the Department of Biochemistry, Srimad Andavan Arts and Science College (Autonomous), Trichy, Tamilnadu. She received her Ph.D -Biochemistry from the Bharathidasan university in the field of Wound Healing using herbal medicine. She has 8 years of teaching experience. Her area of research interests are pharmacology, phytochemistry, bioinformatics etc. To her credit she has published 25 research articles and also written eight book chapters and has five conference proceedings.She completed one Minor Research Project Funded by UGC in the year of 2014-2016. She has guided 20 Ug and Pg students.

E-mail: shobana.gunasekaran9@gmail.com, ORCID: **0000-0002-4793-6178**

Dr. AGNEL ARUL JOHN N currently works as an Associate Professor and is also the Research Director in Srimad Andavan Arts & Science college(Autonomous), Trichy,Tamilnadu. He has 21 years of teaching experience and has won the Best Faculty award by Novel Research Academy in 2019. To his credit he has published 50 research articles. He has guided 30 M. Phil students and guided 5 Ph.D students.He also written many edited book chapters.He is an reviewer of various national and international journals.

E-mail: aruljohnagnel@gmail.com, ORCID: **0000-0003-0626-531X**

Mr. HARI HARAN G, is an Assistant Professor in Department of Biochemistry at "Srimad Andavan Arts & Science College (Autonomous)", Affiliated to Bharathidasan University, Thiruvanaikovil, Trichy. He is pursuing his Ph.D., in the field of Diabetes and Pharmacology from Bharathidasan University, Tiruchirappalli. He has 8 years of teaching experience and 9 years of research experience. His area of research interests are Diabetes, Cancer biology, Bioinformatics, Phytochemistry and Arthritis. He has published 12 research articles in national and international journals. He has also published a book chapter in Taylor and Francis group. He has supervised 09 M.Sc., students. He has 2 conference proceedings at International level conference.

E-mail: haribiochemistry@gmail.com,ORCID: 0000-0003-0805-9245

To Cite This Chapter :
Shobana, G., Chandrasekar, D., Agnel Arul John, N., & Hariharan, G., (2023). **Phytochemıcal Screenıng and Antıoxıdant Properties of Three Common Medicinal Herbs**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 213-227). ISRES Publishing

REPRODUCTIVE HEALTH NEEDS OF WOMEN WITH DISABILITIES: A NON-PHARMACEUTICAL INTERVENTIONS APPROACH

Abiola Adiat OMOKHABI

Introduction

Women with Disabilities (WWD) have the same reproductive health-care needs as other women, particularly modern contraceptives, family planning and childbirth. There have been misconceptions about the reproductive health needs of WWD hence there have been offered limited reproductive health-care services. Although disability has historically been underrepresented in development efforts, there is growing acceptance of the concept of disability-inclusive development, which is based on the belief that people with disabilities experience underdevelopment differently and thus requires different solutions (Grech, 2021).

Disability is explicitly mentioned in the World Health Organization's rationale for taking a rights-based approach to providing contraceptive information and services (World Health Organisation, 2014). Disability is mentioned in the Sustainable Development Goals including 3.7 on universal access to reproductive health-care services and 10.2 on inclusion of all regardless of among other things, disability (United Nations, 2015). There is also disability in Equity and Non-discrimination Rights Principle of Family Planning (FP) 2020 (now FP2030) (Stover & Sonneveldt, 2017; Family Planning, 2020). However, the reproductive health needs of WWD are underserved in research and practice (Carew, Braathen, Swartz, Hunt, & Rohleder, 2017; Hameed, Maddams, Lowe, Davies, Khosla, & Shakespeare, 2020). Whereas there is an ideological commitment to inclusiveness in the provision of reproductive health services, it can be undermined by a lack of actionable data about the needs of WWD (Abdul Karimu 2018).

Nigeria is an important country, particularly in light of the global contraceptive goal known as 120 by 20, which aims to provide modern contraception to 120 million more women by 2020 (Brown, Druce, Bunting, Radloff, Koroma, Gupta, Siems, Kerrigan, Kress & Darmstadt 2020). Nigeria has struggled to meet its country goal of increasing contraceptive uptake by more than 1.5% per year to meet the Sustainable Development Goal target of 3.7 (Fagbamigbe, Afolabi, & Idemudia. 2018; Mercer, Lu & Proctor, 2019). However,low contraceptive uptake in the country has been linked to cultural and religious factors and a lack of adequate knowledge and attitudes (Akamike, Madubueze Okedo-Alex, Anyigor, Azuogu, Umeokonkwo, & Mbachu, 2020; Bolarinwa & Olagunju 2020).

According to Zandam, Mitra, and Mitra (2022), several initiatives have been implemented to improve contraceptive uptake, including mass media awareness campaigns. According to the research of Speizer, Guilkey, Escamilla, Lance, Calhoun, Ojogun, and Fasiku (2019), increasing awareness through mass media can influence people to take action. The impact evaluation of the Nigerian Urban Reproductive Health Initiative revealed that between 2015 and 2020, awareness campaigns via radio, television, and community events increased the use of modern contraceptives from 21.1% to 30.1% in the states where the programme was implemented (Atagame,

Benson, Calhoun, Corroon, Guilkey, Iyiwose, Kebede, Lance, O'Hara, Ojogun, Speizer, Stewart, & Winston, 2017).

Despite the potential of education and awareness interventions, programme shortcomings have been identified, particularly in targeting harder-to-reach groups of women (Mercer Lu & Proctor, 2019). A review of contraception programme and policy documents revealed that WWD were not included in the design and planning of family planning programmes in Nigeria (Federal Ministry of Health, 2014; Family Planning, 2020). A recent review calls for more research into the barriers to good reproductive health that WWD may face and non-pharmaceutical interventions to overcome such barriers (Fraser, Corby, & Meaney-Davis 2021). This is the purpose of this paper.

Women with Disabilities

Women with disabilities do not make up a homogeneous group. They suffer from a variety of impairments, including physical, psychosocial, intellectual, and sensory conditions. These conditions may or may not be accompanied by functional restrictions. Women and girls with disabilities come in a variety of shapes and sizes, including those who have multiple and intersecting identities, such as being from a particular social class or ethnic, religious, or racial background. There are migrants, refugees, or those who seek asylum. There are internally displaced. There are those who are young and old. There are also those who are widowed. Women with Disabilities face lower economic and social status, increased risk of abuse and violence, including sexual violence, discrimination and harmful gender-based discriminatory practices, and barriers to accessing justice, information, and services, as well as civic and political participation because of systemic marginalization, attitudes, and environmental barriers. Consequently, WWD find it challenging to participate on an equal basis with others (United Nations(UN) Women, 2018).

Reproductive Health of Women with Disabilities

Reproductive health is defined as a state of complete physical, mental, and social well-being, not simply the absence of disease or infirmity in all matters relating to the reproduction system, its function, and processes (UNDP/UNFPA WHO, 2010 in Omokhabi, 2014). One of the fundamental components of development is promoting and achieving women's health, particularly reproductive health (Omokhabi,2016). Reproductive health is an important component of overall health and well-being at all stages of life, and it is regarded as a prerequisite for social, economic, and human development by the United Nations. Menarche and menstruation, fertility, pregnancy and childbirth, gynecological cancers, sexually transmitted infections, sexuality, and sexual health and function are all aspects of women's reproductive health.

According to the review of literature on the barriers to the reproductive health needs of women with disabilities, WWD are exposed to more likely to delay initiation of prenatal care and have an increased risk for adverse health conditions that impact maternal and infant outcomes, such as gestational diabetes, obesity, chronic hypertension, hypertensive disorders of pregnancy (such as preeclampsia), cesarean section, and infant low birthweight. Women with disabilities report that health care providers have limited knowledge about specific support needs and other resources for women with disabilities during and after pregnancy, or that providers have

negative attitudes and misconceptions about women with disabilities' preferences or abilities to have children.

Women should be informed, empowered, and enabled to choose whether and when to become pregnant, to receive timely testing for reproductive cancers, to protect themselves against sexually transmitted diseases and infections, and to have a satisfying sexual life to achieve optimal reproductive health (United Nations Population Information Network, 2019). Reproductive health, sexuality, and sexual health are all important components of wellness for all women, and women with disabilities are no exception. Furthermore, research on healthcare shows that people with disabilities (PWD) have poorer health outcomes than their non-disabled counterparts. Women with disabilities are more likely than women without disabilities to have unmet healthcare needs among PWD according to Matin, Williamson, Karyani, Rezaei, Soofi and Soltani (2021).

Barriers to Reproductive Health Needs of Women with Disabilities

Young women with hearing impairments who use sign language in Nigeria reported hearing disability-specific challenges in interactions with Health Care Workers (HCW), such as the rarity of interpretation services being available at the facility, not being able to make themselves understood, not understanding everything an HCW said, missing a turn to be seen due to not hearing their name called, having concerns about confidentiality, and not receiving all the services. These women also reported not seeking care for reasons related to hearing loss, such as communication difficulties, a lack of someone to accompany them (and interpret), and dissatisfaction with the way they had previously been treated by an HCW (Arulogun, Titiloye, Afolabi, Oyewole, & Nwaorgu. 2013 in MacQuarrie, & Fleuret, 2022).

Complex health conditions and mobility impairments can also have direct and indirect effects on pregnancy (Iezzoni, Yu Wint, Smeltzer, & Ecker, 2014), miscarriage risks (Horner-Johnson, Kulkarni-Rajasekhara, Darney, Dissanayake, and Caughey, 2017), postpartum depression (Mitra, Iezzoni, Zhang, Long-Bellil, Smeltzer, & Barton, 2015, sexual health and function, (Eisenberg, Andreski &Mona, 2015). Negative disability stereotypes and misperceptions about the needs and preferences of this female population can have a direct impact on access, utilisation, and quality of care (Streur, Schafer, Garcia, Quint, Sandberg, Kalpakjian, & Wittmann,2020). Although most people are aware of the special health care needs of this population (Taouk, Fialkow, & Schulkin, 2018), few receive information or training on how to care for women with disabilities (Smeltzer, Mitra, Long-Bellil, Iezzoni, & Smith, 2018).

Health care providers struggle with their own ambivalence or discomfort, as well as limited knowledge of disability to guide their care (Tarasoff 2017; Streur, Schafer Garcia, & Wittmann. 2018; Mitra, Smith, Smeltzer -Long-Bellil, Sammet Moring & Iezzoni 2017). There are incorrect assumptions that people with disabilities are not sexual beings, resulting in a lack of sexual health education, barriers to access and uptake of family planning, sexual abuse and exploitation, and risk factors for HIV and other sexually transmitted diseases (Rohleder, Braathen, & Carew,2019). Studies highlight how girls and young women with disabilities are infantilised, disempowered and lack voice, choice and control to make decisions about their own bodies and sexualities (Plan International, 2017; Jones, Presler-Marshall, & Stavropoulou, 2018).

There is a lack of comprehensive knowledge about appropriate family planning practices for women and girls with disabilities (Kassa, Luck, Bekele, & Riedel-Heller ,2016; FHI360, 2017). High costs of accessing family planning services for people with disabilities, several studies have highlighted the financial barriers to family planning services faced by people with disabilities, particularly adolescent girls (Tanabe, Nagujjah, Rimal, Bukania, & Krause, 2015). People with disabilities are largely invisible in monitoring and evaluation activities, according to a recent scoping study commissioned by DFID (Buchy, Resch, Wapling, Jones, & Singh, 2017). Women with disabilities, particularly those with physical and sensory disabilities, face barriers to accessing maternity care facilities (Ganle, Otupiri, Obeng, Edusie Ankomah, & Adanu,2016; Tarasoff, 2017; Mazurkiewicz Stefaniak, & Dmoch-Gajzlerska 2018; Nguyen, King, Edwards, & Dunne ,2020).

Women with physical disabilities who used wheelchairs reported that they could not enter offices, restrooms, and washrooms without facilities that acknowledge their disabilities and recognize their inclusions (Mitra, Long-Bellil, Lezzoni, Smeltzer, & Smith, 2016; Tarasoff, 2017; Nguyen et al., 2020). Women with sensory impairment have difficulty finding their way around, especially if no one is available to assist them (Mazurkiewicz et al., 2018). Mitra et al. (2016) and Tarasoff (2017) discovered that women with physical disabilities have limited access to adapted equipment. There is strong evidence that healthcare providers are unaware of the link between physical disability and pregnancy (Smeltzer, Mitra, Iezzoni, Long-Bellil & Smith ,2016 Tarasoff, 2017: Tran, Nippita, Nguyen, Nguyen, Huynh, Le Hua & Roberts ,2018; Nguyen et al., 2020).

Non-Pharmaceutical Interventions Approach in Women Reproductive Health

Non-Pharmaceutical Interventions Approach i**s** *any method used to promote good reproductive health and behaviour of women generally without requiring pharmaceutical* drug treatment. Non-pharmaceutical intervention (NPI) refers to any type of health intervention that is not primarily based on medication. Non-pharmacological interventions may be used to prevent or treat disease or other health conditions, as well as to improve public health. They can be educational or involve various lifestyle or environmental changes (Abraha, Rimland, Trotta, Dell'Aquila, Cruz-Jentoft., Petrovic,Gudmundsson, Soiza., O'Mahony, Guaita & Cherubini,2017).Complex or multicomponent interventions employ multiple strategies *(Boutron, & Ravaud ,2012)* and they frequently entail the participation of multiple care providers *(Boutron, Altman, Moher,Schulz Ravaud ,2017).*

Interventions for Promotion Reproductive Health Needs of Women with Disabilities

Several interventions can increase the access of women with disabilities to high-quality medical care. Family Planning Education in Ghana is made accessible through sign language, and copies of the Behavioural Change Communication (BCC) materials are printed in braille, according to the State Report (2018). A successful campaign for deaf youth in three Latin American countries used posters with barcodes gave access to videos in sign language addressing sexuality and sexual health. Other pilots include the UNFPA-funded Deaf Elimu, a web and mobile-based application that targets 800,000 deaf youth users in Kenya who search for sexual-related health

information in sign language, and that was also launched in 2016 (Plan International, 2017).

The World Health Organization and John Hopkins University (2018) recommend physically accessible facilities (such as wheelchair ramps and large bathrooms with grab bars), community outreach programmes for people with limited mobility, and print materials with straightforward graphs to ensure that family planning services are accessible to women and girls with disabilities. For disabled girls and young women who live in remote or other isolated areas, in Uganda, the Straight Talk Foundation established mobile clinics with trained multidisciplinary teams (Plan International, 2017).

Consequent upon these comparative analyses of these interventions from three selected countries in Africa (Ghana, Kenya, and Uganda) and three Latin American countries, women with disabilities, health care providers, organisations, communities, and governments have different roles to play in non-pharmaceutical interventions of the reproductive health needs of women with disabilities which include the following:

Health Care Providers (Obstetrician–gynecologists)(HCP-OG) **can d**iscuss preventive health screenings with WWD and ensure they have access to recommended screenings. They can educate each women with disabilities, about how to live a healthy, long life. The health concerns of WWD should be taken into account even if the WWD do not request it and they should be provided the information needed to prevent or treat a health condition. HCP-OG can maintain clear and direct communication with the WWD. Providers should repeat their questions or instructions to WWD if they are unable to understand what they have said. HCP-OG can increase **clinical awareness and care of WWD conditions, functional effects of these conditions, and effective interventions.** They can o**btain information regarding a WWD's sexual history, sexual violence, reproductive preferences, and pregnancy intention for them. Make sure they get preventive health screenings.**
HCP-OG should respect WWD's preferences, needs, and values by providing patient-centered care. They should respect WWD as individuals and value their knowledge of disability experiences.

HCP-OG can attend education and training programmes for persons with disabilities which have the capacity to improve disability and inclusion awareness among care givers to elicit positive attitudes and comfort about WWD. Women with disabilities experience stigmatisation and HCP-OG should tackle these stigma attitudes towards them. HCP-OG should advise and refer WWD to screening or mammography or other preventive medical examinations. They should plan for and address any unique requirements for people with disabilities (WWD), such as the need for specialized medical equipment, examination tables with accessible heights, and other equipment or supports to facilitate gynecological and other health examinations. HCP-OG can provide reproductive health information and promote acceptance of a woman's right to consensual gender expression, including the rights of women with intellectual and other disabilities. They should assist WWD to identify and plan pregnancy intent preferences as well as provide infant care materials, such as cribs or changing tables, available to women with disabilities.

Organisations and communities can reduce structural barriers for persons with disabilities. They can follow state and federal statutes that govern accessibility for

persons with disabilities such as the Disabilities Act and the Affordable Care Act to achieve this responsibility. Recently, the government of Nigeria enacted the Discrimination against Persons with Disabilities (Prohibition) Act, 2018. The Act addresses the promotion and protection of the rights of persons with disabilities as it relates to; accessibility, women with disabilities, right to inclusive quality education, right to living independently and being included in the community and right to equality and non-discrimination. Organisations and communities can design accessible treatment areas with features like parking, signage, and height-adjustable exam tables.

Government at all levels should make sure that resources for breast cancer screening are available to women with disabilities (WWD). The government should create health education materials to encourage women with disabilities to get screened for breast cancer and raise awareness of the disease. The government should ensure that advocacy for increased access to sexual and reproductive health services, including prenatal and postnatal care for women with disabilities. It is critical to provide accurate and verified information to pregnant women with disabilities, their partners, and families in collaboration with specialist NGOs, so that they can make responsible decisions about their health and the health of their children regarding delivery and birth period, as well as their right to give their free and informed consent to any birth-related surgical procedure. They can create national policies and laws that ensure people with disabilities have access to sexual and reproductive health care and reproductive rights.

Furthermore, the government should provide sexual and reproductive health-care facilities and information to WWD, educate sexual and reproductive health workers about disability inclusion, combat discrimination, and improve service delivery to WWD, and create a monitoring and evaluation mechanism to track the implementation of policies and programmes on sexual and reproductive health access for people with disabilities. The government should enhance research and data collection in order to monitor, evaluate, and strengthen sexual and reproductive health and services for people with disabilities.

Women with disabilities can explore and use resources and opportunities available at their disposal to expand their reproductive health knowledge, including preparing for a healthy pregnancy. These women should discuss their reproductive health concerns and available services with health care providers, including contraception, pregnancy, and menopause. They should share reproductive health preferences with intimate partners, including contraceptive methods, pregnancy intention, and sexual activity. Women with disabilities should discuss **cancer screening with a medical professional because all women, including those who have disabilities, are at risk for cervical cancer.**

Summary

This paper has demonstrated that women with disabilities face a number of obstacles regarding their reproductive health, including challenges in accessing reproductive health needs and information as well as the ignorance and unfavourable attitudes of

healthcare professionals based on prior research findings. This chapter proposed a first-step non-pharmaceutical approach to providing women with disabilities with better reproductive health care. In essence, women with disabilities require reproductive health care that is specific to women's needs and traits. There is also a need for training for healthcare professionals to understand the special requirements of WWD patients. This training should focus on patient-centered care, working effectively with women, and the link between disabilities and pregnancy is necessary for healthcare professionals. Education and resources should also be made available to enhance respect for women with disabilities and promote their safety to meet the population's needs for reproductive health.

References

Abdul Karimu, A. T. F. (2018). Disabled Persons in Ghanaian Health Strategies: Reflections on the 2016 Adolescent Reproductive Health Policy. *Reproductive Health Matters* 26 (54): 20–24.

Abraha I, Rimland J..M, Trotta FM, Dell'Aquila G., Cruz-Jentoft A., Petrovic M., Gudmundsson A, Soiza R., O'Mahony D., Guaita A, & Cherubini A (2017). Systematic review of systematic reviews of non-pharmacological interventions to treat behavioural disturbances in older patients with dementia. The SENATOR-OnTop series. BMJ Open.;7(3):e012759. doi: 10.1136/bmjopen-2016-012759.7(7):e012759corr1. PMID: 28302633; PMCID: PMC5372076.

Akamike. I. C., Madubueze, U. C., Okedo-Alex, I. N., Anyigor, C. J., Azuogu, B. N., Umeokonkwo, C. D., & Mbachu C .O. (2020). Perception, pattern of use, partner support and determinants of uptake of family planning methods among women in rural communities in Southeast Nigeria. *Contraception and Reproduction Medicine*. 5(1):14.

Arulogun O. S. Titiloye M. A. Afolabi N. B. Oyewole O. E. ,& Nwaorgu O. G. B . (2013). 'Experiences of girls with hearing impairment in accessing reproductive health care services in Ibadan, Nigeria'. *African Journal of Reproductive Health* , 17 , 85 – 93 . doi: 10.4314/ajrh.v17i1

Atagame, K. L., Benson, A., Calhoun, L., Corroon, M., Guilkey, D., Iyiwose, P., Kebede, E., Lance, P., O'Hara, R., Ojogun, O. T., Speizer, I. S., Stewart, J. F. & Winston, J. (2017) Evaluation of the Nigerian urban reproductive health initiative (NURHI) program. *Studies in Family Planning.* 48(3):253–68.

Bolarinwa, O. A., & Olagunju, O. S. (2020). Knowledge and factors influencing long-acting reversible contraceptives use among women of reproductive age in Nigeria. Gates Open Res. 3, doi: 10.12688/gatesopenres.12

Boutron I, & Ravaud P (2012). Introduction. In Boutron I, Ravaud P, Moher D (eds.). Randomized clinical trials of non-pharmacological treatments. Boca Raton: CRC Press. pp. xi–xii. ISBN 9781420088021.

Boutron I, Altman DG, Moher D, Schulz KF,& Ravaud P (2017). "CONSORT Statement for Randomized Trials of Non-pharmacologic Treatments: A 2017 Update and a CONSORT Extension for Non-pharmacologic Trial Abstracts". *Annals of Internal Medicine.* ***167*** *(1): 40–47.* doi:10.7326/M17-0046. PMID 28630973.

Brown, W., Druce, N., Bunting, J., Radloff, S., Koroma, D., Gupta, S., Siems, B., Kerrigan, M., Kress, D. & Darmstadt, G. L. (2020). Developing the "120 by 20" goal for the Global FP2020 Initiative. *Studies in Family Planning* 45(1):73-84 doi:10.1111/j.1728-4465. 2014.00377.x

Buchy, M, Resch, E, Wapling, L, Jones, S. & Singh, P. (2017). Scoping Study: Donor Support for Disability Inclusive Country Led Evaluation Systems and Processes. Synthesis Report. Oxford: Oxford Policy Management

Carew, M. T., Braathen, S. H., Swartz, L., Hunt, X., & Rohleder, P. (2017). 'The sexual lives of people with disabilities within low-and middle-income countries: A scoping study of studies published in English'. *Global Health Action.* 10(1), 1337342 https://doi.org/ 10.1080/16549716.2017.1337342

Eisenberg, N.W., Andreski, S-R, & Mona, L.R. (2015). Sexuality and physical disability: A disability-affirmative approach to assessment and intervention within health care. Current Sex Health Rep 7:19–29.

Fagbamigbe A.F., Afolabi R.F., & Idemudia, E.S. (2018). Demand and unmet needs of contraception among sexually active in-union women in Nigeria: distribution, associated characteristics, barriers, and programme implications. Sage Open. 8(1) http://journals.sagepub.com/doi/10.1177/2158244017754023.

Family Planning (2020). http://www.family planning2020.org/nigeria.

Federal Ministry of Health Nigeria Family Planning Blueprint (Scale-Up Plan) (2014). Federal Government of Nigeria

FHI 360 (2017). Assessment on Family Planning Needs of People Living with Disabilities: Case of Addis Ababa, Ethiopia. USAID, FHI 360, Ministry of Health.

FP2020. (2015). *Family Planning 2020: Rights and Empowerment Principles for Family Planning. Washington, DC: FP2020 Rights & Empowerment Working Group*. http://www.familyplanning2020.org/sites/default/files/rightsbasedfp/FP2020_Statement_of_Principles_ FINAL.pdf

Fraser, E, Corby, N & Meaney-Davis, J (2021). Family Planning for women and girls with disabilities. Disability Inclusion Helpdesk Research Report No. 60. London, UK: Disability Inclusion Helpdesk

Ganle J.K, Otupiri E., Obeng B., Edusie A.K. Ankomah, A. & Adanu, R. (2016). **Challenges women with disability face in accessing and using maternal healthcare services in Ghana: A qualitative study.** PLoS One, 11 10.1371/journal.pone.0158361.

Grech, S. (2021). Critical Thinking on Disability and Development in the Global South in Brown, R., Maroto, M. and Pettinichio, D. (eds.). The Oxford Handbook of the Sociology of Disability, Oxford University Pres https://doi.org/10.1093/oxfordhb/9780190093167.013.9

Hameed, S., Maddams A., Lowe H., Davies L., Khosla, R., & Shakespeare T. (2020). From Words to Actions: Systematic Review of Interventions to Promote Sexual and Reproductive Health of Persons with Disabilities in Low-and Middle-Income Countries. BMJ Global Health 5 (10): e002903. http://dx.doi.org/10.1136/bmjgh-2020-002903

Horner-Johnson, W., Kulkarni-Rajasekhara, S., Darney, B. G., Dissanayake, M., & Caughey, A. B. (2017). Live birth, miscarriage, and abortion among U.S. women with and without disabilities. *Disability Health Journal*. 10:382–386

Iezzoni L.I., Yu, J., Wint, A.J., Smeltzer, S.C., & Ecker, J.L.(2014). General health, health conditions, and current pregnancy among U.S. women with and without chronic physical disabilities. *Disability Health Journal*. 7(2):181-8.

Jones, N., Presler-Marshall, E. & Stavropoulou, M. (2018). Adolescents with Disabilities: Enhancing Resilience and Delivering Inclusive Development,

London: GAGE Programme. https://www.odi.org/sites/odi.org.uk/files/resource-documents/12323.pdf

Kassa, T.A., Luck, T., Bekele, A . & Riedel-Heller, S. G. (2016). Sexual and reproductive health of young people with disability in Ethiopia: a study on knowledge, attitude and practice: a cross-sectional study. *Global Health* **12**, 5 https://doi.org/10.1186/s12992-016-0142-3

MacQuarrie, Kerry L. D. & Fleuret, J. (2022). Patterns of Reproductive Health among Women with Disabilities. DHS Analytical Studies No. 80. Rockville, Maryland, USA: ICF.

Matin, B.K., Williamson, H. J., Karyani, A. K. Rezaei, S, Soofi, M.,& Soltani, S. (2021). Barriers in access to healthcare for women with disabilities: a systematic review in qualitative studies. *BMC Women's Health* **21**, 44 https://doi.org/10.1186/s12905-021-01189-5

Mazurkiewicz B., Stefaniak, M., & Dmoch-Gajzlerska, E. (2018). Perinatal care needs and expectations of women with low vision or total blindness in Warsaw Poland. *Disability. Health Journal.,* 11, 618-623, 10.1016/j.dhjo.2018.05.005

Mercer, L. D., Lu, F., & Proctor, J. L. (2019). Sub-national levels and trends in contraceptive prevalence, unmet need, and demand for family planning in Nigeria with survey uncertainty. *BMC Public Health*. 19(1):1–9.doi: 10.1186/s12889-019-8043

Mitra M., Smith, L. D., Smeltzer, S.C., Long-Bellil, L. M, Sammet Moring, N., & Iezzoni L. I. (2017). Barriers to providing maternity care to women with physical disabilities: Perspectives from health care practitioners. *Disability Health Journal*;10:445–450

Mitra, M., Iezzoni L. I., Zhang, J., Long-Bellil, L. M., Smeltzer, S. C., Barton, B. A. (2015). Prevalence and risk factors for postpartum depression symptoms among women with disabilities. *Maternal Child Health Journal* 19:362–372

Mitra, M., Long-Bellil, L.M., Iezzoni, L.I., Smeltzer, S.C., & Smith, L.D. (2016). Pregnancy among women with physical disabilities: Unmet needs and recommendations on navigating pregnancy *Disability. Health Journal.* 9. 457-463, 10.1016/j.dhjo.2015.12.007

Nguyen, T.V., King, J., Edwards, N., & Dunne, M. P. (2020). "Nothing suitable for us": experiences of women with physical disabilities in accessing maternal healthcare services in Northern Vietnam Disability. Rehabilitation. pp. 1-9, 10.1080/09638288.2020.1773548

Omokhabi, A. A. (2014). Determinants of Reproductive Health Behaviour among Female Workers in Tertiary Institutions in Southwestern Nigeria. Unpublished Ph.D. Thesis, Department of Adult Education, Faculty of Education, University of Ibadan, Ibadan, Nigeria

Omokhabi, A. A. (2016). Factors Influencing Reproductive Health Behaviour of Female Non-Academic Staff in the Nigerian Universities. *Ibadan Journal of Educational Studies.* Vol. 13. No. 1: 89-102.

Plan International (2017). Let me decide and thrive: Global discrimination and exclusion of girls and young women with disabilities, Working: Plan International

Ramjan, L., Cotton A., Algoso, M., & Peters, K. (2016). Barriers to breast and cervical cancer screening for women with physical disability: A review. *Women Health*.;56(2):141-56.

Rohleder, P., Braathen, S., & Carew, T (2019). Disability and Sexual Health: A Critical Exploration of Key Issues, Abingdon: Routledge.

Smeltzer, S. C., Mitra, M., Long-Bellil, L., Iezzoni, L. I., Smith, L. D. (2018). **Obstetric clinicians' experiences and educational preparation for caring for pregnant women with physical disabilities: A qualitative study** *Disability. Health Journal*. 11. 8-13.

Smeltzer, S. C., Mitra, M.. Iezzoni, L.I., Long-Bellil, L,.& Smith, L. D. (2016). Perinatal Experiences of Women With Physical Disabilities and Their Recommendations for Clinicians. *Journal of. Obstetrician. Gynecological neonatal Nursing*. 45 pp. 781-789.

Speizer, I. S., Guilkey, D. K., Escamilla, V., Lance, P. M., Calhoun, L.M., Ojogun, O. T., & Fasiku, D. (2019). On the sustainability of a family planning program in Nigeria when fundingends. Anglewicz P, editor. PLoS One. 14(9):e0222790. doi: 10.1371/journal. pone.0222790. https://dx.plos.org/10.1371/journal.pone.0222790.

Stover, J., & Sonneveldt, E. (2017). Progress toward the Goals of Family Planning 2020. *Studies in Family Planning*. 48 (1): 83–88.

Streur, C. S., Schafer, C. L., Garcia, V. P., & Wittmann, D. A. (2018). "I Don't Know What I'm Doing I Hope I'm Not Just an Idiot": The need to train pediatric urologists to discuss sexual and reproductive health care with young women with spina bifida. *Journal of Sex Medicine*. 15:1403–1413

Streur, C. S., Schafer, C. L., Garcia, V. P., Quint, E. H., Sandberg, D. E., Kalpakjian, C. Z., & Wittmann, D. A. (2020). He told me it would be extremely selfish of me to even consider (having kids): The importance of reproductive health to women with spina bifida and the lack of support from their providers. *Disability Health Journal*. 13(2):100815.

Tanabe, M., Nagujjah, Y., Rimal, N., Bukania, F. & Krause, S. (2015) 'Intersecting sexual and reproductive health and disability in humanitarian settings: risks, needs, and capacities of refugees with disabilities in Kenya, Nepal, and Uganda' Sexuality and Disability 33(4): 411–427.

Taouk, L. H., Fialkow, M. F., & Schulkin, J. A. (2018). Provision of reproductive healthcare to women with disabilities: A survey of obstetrician-gynecologists' training, practices, and perceived barriers. *Health Equity*. 2:207–215.

Tarasoff, L.A. (2017). "We don't know. We've never had anybody like you before": Barriers to perinatal care for women with physical disabilities. *Disability Health Journal*. 10:426–433

Tran, H. T., Nippita, T., Nguyen, P. T. K., Nguyen, P. T. T., Huynh, T. T. D., Le Hua, O. T, & Roberts, C. L. (2018). Knowledge, experience and attitudes towards skin-to-skin contact following Caesarean sections among health professionals in Vietnam Acta Pediatrician 107 1917-1923.

UN Women (2018). *The Empowerment of women and girls with disabilities towards full and effective participation and gender equality* https://www.unwomen.org/sites/default/files/ Headquarters/Attachments/Sections/Library/Publications/2018/Empowerment-of-women-and-girls-with-disabilities-en.pdf

United Nations Population Information Network (2019). Guidelines on Reproductive Health. : www.un.org/popin/unfpa/taskforce/guide/iatfreph.gdl.html

WHO (2014). *Ensuring Human Rights in the Provision of Contraceptive Information and Services: Guidance and Recommendations*. Geneva, Switzerland: World Health Organization

World Health Organization Department of Reproductive Health and Research (WHO/RHR) and Johns Hopkins Bloomberg School of Public Health/Center

for Communication Programs (CCP), Knowledge for Health Project. Family Planning:
A Global Handbook for Providers (2018 update). Baltimore and Geneva: CCP and
WHO,

Zandam, H., Mitra M., & Mitra, S. (2022). Awareness and access to mass media sources
of information about modern family planning methods among women with disabilities in Nigeria: An analysis of 2018 demographic and health survey. *Frontier in Global Women's Health*. G 3:746569.doi: 10.3389/fgwh.2022.746569.

To Cite This Chapter:
Omokhabi, A. A. (2023). **Reproductive health needs of women wıth disabilities: a non-pharmaceutıcal ınterventıons approach.** In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 228-239). ISRES Publishing**.**

NON-CODING RNAS: RECENT ADVANCEMENTS IN THE DEVELOPMENT AND BIOCHEMISTRY AS NOVEL ANTI-TUMOR DRUG MOLECULES

Muhammad Farrukh NISAR
Amir JAVED
Asif ALI
Sania SHAHEEN
Muhammad RAFIQ
Muhammad Yasir WAQAS
Zahid MANZOOR
Shaukat Hussain MUNAWAR
Kang XU

Introduction

The number of non-coding RNA (ncRNAs) in human tissues is three times that of protein-coding RNAs[1]. Another type of ncRNAs, dubbed long non-coding RNAs (lncRNAs), were recently discovered by a comprehensive review of data from RNA sequencing from human tissue, and its number is roughly multi-fold than the protein coding mRNAs[1-2]. The lncRNAs may function in the nucleus or either within the cytoplasm, while in the nucleoplasm it help reorganizing and modifying the chromatin, modulates transcription, RNAs processing, while as soon it comes outside of the nucleus it interacts with other related molecules (RNAs) and fully mature proteins [3]. More typically, lncRNAs can also influence many processes including mRNA degradation, translation, and protein kinetics, though the study of lncRNAs is still in its early years, but it has already shown their significance in both development and roles in disease treatment regimen[4].

Huge literature evidenced lncRNAs role in controlling RNAs expression at transcriptional levels by modulating gene silencing and or gene activation [5]. The lncRNAs in association with transcriptional start sites, help recruit lncRNAtranscription factors, transcriptional co-activators, and certain protein factors. Similarly, lncRNAs may behave as molecular decoys by entangling transcription factors and preventing them from reaching DNA binding sites for direct contacts[6-7]. Moreover, lncRNAs play crucial roles in a wide range of biological processes and diseases, including cancer. Despite the fact that recent advances in our understanding of lncRNA-mediated gene expression have focused mostly on the critical cell signaling pathways controlled by lncRNAs in cancer [8]. Several lncRNAs are related with carcinogenesis, metastasis, and prognosis and includeslncRNA HOTAIR, MALAT1, GAS5, PC3, and H19[9-13]. Natural phytochemicals and small drug molecules with a novel mechanism of action have anticancer properties may not harms the normal cells are a dire need to be explored in order to control the rising number of cancer patients across the globe[14].

Particularly in case of oncology and various infectious and chronic diseases, phytochemicals (PCs) are essential in the development of novel therapeutics [15-16]. In addition, PCs have also great role in controlling cardiovascular diseases (with drugs like statins) and multiple sclerosis (with drugs like fingolimod) [17-19]. In the last several decades (1940-2002) about half of the approved anticancer drugs were of natural source from phytochemicals. Some effective anticancer drug molecules such as alkaloids originally isolated from *Catharanthus roseus* (Family: Apocynaceae) and certain terpenes such as paclitaxel of *Taxus baccata* [20-21] are proven novel pharmaceuticals compared to their synthetic analogs. Since the onset of current

century, huge list of phytochemicals have been came from plants. Inspite of the recent advancements in synthetic drug formulations, about 64% of antihypertensive drugs are sourced from natural sources [20]. Traditional medicines (TMs) are extremely valuable since they rely on the usage of natural ingredients based on long traditional knowledge of usage. Traditional medication systems spaning thousand of year of knowledge based experience are still under wide use in traditional Chinese (TCM), Unani, Ayurvedic, Kampo, and in traditional Korean medicine (TKM a still thought superior over many known medication systems [22-23] are superior to use over many others.

A majority of transcripts in the human genome comprised of lncRNAs [24], and have low-level and tissue-specific expression lncRNAs while tend to be less conserved across the species [25-27]. Although this term initially led to label lncRNAs as "transcriptional noise" [27], but latest findings shown that the majority of lncRNAs are crucial in a wide array of cell physiological and signaling procedures such as transcriptional regulations, may target RNA polymerase II, gene splicing, and even contributing to epigenetic events[28]. Furthermore, the endogenous RNA competition theory describes a competitive interaction between lncRNAs and microRNAs (miRNAs), suggesting that former plays a key role in numerous biological activities [29].

lncRNAs

The lncRNAs are non-coding, consisting of 200 nucleotides or more (often between 1000 and 10,000), and they provide both RNA- and protein-like activities [6, 31], and gained much attention recently as important gene regulators [30]. lncRNAs play a role in organelle and nuclear condensate creation and regulation [14]. The unique processes by which lncRNAs are regulated confine them to particular tissue types and sometimes even subcellular locations. These lncRNAs can be sense (transcripts of "right" mRNA strand) or antisense (transcripts of "wrong" mRNA strand), intronic (transcripts of introns in protein coding genes) or intergenic (transcripts found between the genes) [32]. In contrast to messenger RNAs (mRNA), lncRNAs resided inside nucleus in association with the genomic DNA [33][30], where they are engaged in transcriptional control of gene expressions, genome reorganizations and multiple cytoplasmic activities.

Although they share some structural similarities with short RNA guides utilised in RNA interference (RNAi) processes, but lncRNAs are fundamentally distinct molecules in terms of structural, catalytic, or regulatory functions in gene expression for a range of biological phenomenon. The lncRNAs can alter the promoters, untranslated sections, exons, introns, and terminators of genes, in addition to the accessibility of chromatin, transcription, splicing, and translation [34]. Furthermore, lncRNAs have been reported to aid in angiogenesis as lncRNA MVIH promotes angiogenesis in liver cancer. Studies claim that lncRNAs may alter cellular physiology and constitutes a significant class of cellular regulatory molecules [35]. In the transcription of mRNAs, post transcriptional events such as RNA splicing, molecular stability, cytoplasmic export, protein translation, are all under the influence of different lncRNAs [5].

Huge information on lncRNAs came after excessive research in sequencing, expression patterns analyses, and functions of these molecules. Therefore, it is crucial to organize and annotate this data to maintain indepth mechanism of action of

lncRNAs. Several lncRNA databases do really facilitate research involving lncRNAs [36-37], and especially the links between lncRNAs with that of different diseases. Numerous illnesses have been found linked with lncRNA dysfunctions [27] and includes cancers [38], cardiovascular diseases [39] and neurodegeneration syndromes [40]. The increased expression of lncRNA PCA3 is regarded as potential biomarker in aggressive prostate cancers [41]. Hepatocellular carcinoma has been linked to an uptick in lncRNA expression. A better prognosis is seen in liver transplant patients with higher homeobox transcript antisense intergenic RNA (HOTAIR) scores [42]. The lncRNAUCA1 was shown to have good specificity and sensitivity in the identification of bladder cancer from urine samples of patients would be considered as a biomarker for this disease [43]. By stimulating cellular proliferation rate either in the presence of absence of multiple anti-estrogens, forced expression of lncRNA BCAR4 in breast cancer cells is thought as potential targeted therapy aghainst anti-estrogen resistant breast cancer cells.

Mechanism of action of lncRNAs

Some pharmacological agents can enhance the function of effector molecules or cellular pathways for therapeutic benefit, however most existing medications and drug molecules work by blocking their target's ability to do something. Countless genes, including those that regulate tumour suppression, cell development, transcription, and the repair of DNA and RNA. The lncRNAs emerged as significant modulators in gene expression, but also aid in rearranging chromatin architecture. After the role of lncRNAs is confirmed in multiple disease conditions, and this knowledge help the creation of inhibitors of the natural antisense transcripts (NAT).

Multiple cellular and molecular mechanisms contribute to lncRNAs' method of action. Many ncRNAs, including acute insulin response (AIR) and KCNQ1 opposite strand antisense transcript 1(KCNQ1OT1), recruits protein complexes (PRC1 & PRC2) along with euchromatic histone lysine N-methyltransferase 2 (EHMT2 or G9A) to reconstruct heterochromatin and silence placentally imprinted genes via the scaffold mechanism. KCNQ1OT1 inhibits widely expressed genes by methylation of DNA and histone modifications. Transcriptional inhibition is presumably the mechanism by which lncRNAAIR represses the adjacent insulin-like growth factor 2 receptor (IGF2R) gene [44]. PRC2 links to the lncRNA taurine upregulated 1 (TUG1) when DNA is damaged to cause a transcriptional reduction in the expression of genes involved in cell cycle regulation [45]. The HOX anti-sense intergenic RNA (HOTAIR) transcript is the initial lncRNAs discovered revealed a comparable role it engages PRC2 to mediate transcriptional modulation of numerous gene loci distant from its locus of origin (trans-regulation) [46-47]. Both *cis* and *trans* mechanisms can be used by lncRNAs. For instance, short interfering RNAs (siRNAs) that affect on one or more neighboring transcripts on the same chromosome are said to be *cis*-acting RNAs. While trans-acting RNAs, can be activated far from their site of synthesis and operate directly on a wide variety of chromatin locations, even those on other chromosomes. Therapeutic discovery strategies that prioritise *cis*-regulatory mechanisms (in case of locus specificity) over *trans*-regulatory ones are expected to significantly increase the probability of discovering target-oriented therapeutic compounds. In addition to protein-coding genes, a locus may also have multiple lncRNAs that act in concert to counteract or adapt to external stresses. For example, DNA damage triggers the transcriptional activation of cyclin dependent kinase inhibitor 1A (CDKN1A or P21) through the lncRNAs p21-associated ncRNA DNA damage activated (PANDA) and lincRNA-p21 [47]. Both PANDA and CDKN1A regulate the nuclear transcription factor Y subunit (NFYA) that took over the control of cell cycle progression and

apoptotic cell death [47]. Additionally, lincRNA-p21 induces gene silencing by activating heterogeneous nuclear ribonucleoprotein K (HNRNPK) [48]. Moreover, lncRNAs can also affect transcriptional activity via multiple cellular levels. DLX6 antisense RNA 1 (DLX6-AS1 or Evf-2) promotes the activity of the enhancer region shared by DLX5 and DLX6 in conjunction with distal-less homeobox 2 (DLX2), a homeodomain-containing transcription factor [49]. DLX6-AS1 has been offered as an example of a novel class of conserved lncRNAs that regulate development via interactions with homeodomain proteins. Assembly transcript 1 (NEAT1) is a paraspeckle-localized lncRNA that facilitates A-to-I editing and subsequent mRNA cleavage by binding to paraspeckle component 1 (PSPC1) and non-POU domain containing octamer-binding protein (NONO; also known as p54). Allosteric modulation of the RNA-binding protein TLS (Translated in liposarcoma protein) by the lncRNA cyclin D1 (CCND1) has been demonstrated [50-51]. The “competing endogenous RNA” concept propose that RNAs can exchange information with one another depending on the density of occupation of their shared miRNA response regions [29]. According to this theory, the expression or silencing of a target is determined by the availability of binding sites on the mRNA for miRNAs. lncRNAs like linc-MD1 may operate as a "sponge" to limit the ability of miRNAs to govern muscle development by going after alternate targets by binding to a miRNA on its own miRNA response elements [52]. Many mammalian genome non-coding transcripts may act as transcriptional enhancers [53] Cell-lineage-specific factors e.g., forkhead box A1 (FOXA1), which functions on structurally and functionally diverse groups of enhancers, may facilitate and restrict the expression of essential regulatory transcription factors particularly androgen receptors. Therefore, down regulation of FOXA1 is not an authentic prognostic sign in prostate tumours because it dramatically reorganizes the hormonal responses by altered bonding of androgen receptor to specific enhancer molecules [54]. Enhancer-templated ncRNAs are produced, and these ncRNAs can regulate transcription [55].

Gene regulation by lncRNA

The cellular expression of lncRNAs is higher because of their crucial roles in gene regulation [14] , which are the major regulators of gene expression activating in the immune system [56]. Cancer, immune responses, and neurological illnesses are only some of the biological and physiopathological contexts in which these mechanisms ultimately affect gene expression [14]. Lineage-specific regulation of differentiation and function by lncRNAs in innate and adaptive cell types [56]. Their expression patterns, which are tissue- and condition-specific, lend credence to their potential as biomarkers, making it appropriate to target lncRNAs in the clinic. Potential medicinal applications, mechanisms of lncRNA production, their modulating role in transcription, post transcriptional events, and other forms of gene control, and more are all being investigated [14].

The expression levels of various genes encoding certain protein molecules are associated with the immunoregulatory responses by lncRNAs in multiple cellular pathways including through direct linkage with chromatin, RNAs, and a variety of protein molecules. RNA modifications, enhancer RNAs, and circular RNAs all play important roles in biology beyond only long noncoding RNAs [56]. Some lncRNAs have the potential to affect even more facets of gene expression by being translated into functional peptides154. To exert their effects, lncRNAs must interact with other RNAs and proteins, as is the case with other ncRNAs [14].

Epigenetically altering NEAT1 expression has been shown to affect the transcription of genes involved in downstream processes in Alzheimer's disease and herpes simplex virus infection, which suggests that signal transducer and activator of transcription 3 may regulate NEAT1 expression. These findings establish NEAT1 as a critical early-stage stress and disease effector [56]. New disease-associated lncRNAs, such as C6orf3 for psoriasis and LINC01475/RP11-129J12.1 for ulcerative colitis, were also found, as was differential regulation of lncRNAs and protein-coding genes. The findings of this study will aid in the annotation and analysis of the significance of lncRNA genes for a wide range of cell types and human traits [57].

lncRNAs control gene activity in a lot of different ways. lncRNAs can change the structure and function of chromatin by interacting with DNA, RNA, and proteins. They can also change the transcription of nearby and faraway genes and the stability, translation, and splicing of RNA [14]. Splicing suppression via RNA-RNA hybrid formation with target pre-mRNAs, fine-tuning of target-gene splicing via chromatin remodelling, and lncRNA regulation of post-translational modifications of splicing components are some additional mechanisms of lncRNA-mediated splicing control demonstrated by these studies [58].

New research on the functions of circRNAs and lncRNAs in controlling components of myogenesis is summarised in studies. Because of these discoveries, we now have a much better grasp on the gene regulatory systems governing muscle proliferation and differentiation, which bodes well for the creation of novel preventative, diagnostic, and therapeutic strategies for muscle problems. [59].
Some lncRNAs have the ability to base-pair directly with other RNAs, bringing in proteins that aid in the breakdown of mRNA. The double-stranded RNA-binding protein STAU1 (which binds to the 3′ untranslated region of translating mRNAs) is a good example of a mediator of this type of mRNA decay, known as Staufen-mediated mRNA decay [60]. The novel lncRNA LOC554202 has been shown to have an exact match with the intronic sequence of miR-31. The transcriptional activity of miRNA-31 (miR-31) is controlled by LOC554202. The host gene LOC554202 and miRNA-31 are both overexpressed in luminal TNBC cell lines compared to basal TNBC cell lines. An epigenetic mechanism for the silence of these two genes by promoter hypermethylation was suggested by the substantial increase in miR-31 and its host gene following treatment of TNBC cell lines with a de-methylation agent alone or in conjunction with a de-acetylating agent [61]. Next, we'll talk about the molecular processes that regulate lncRNA transcription, with a focus on how transcription begins and ends. These processes show how lncRNA expression differs significantly from that of proteins. Miss regulation of lncRNAs leads to genomic stress, such as transcription-replication conflict and R-loop-mediated DNA damage [62]. Prostate cancer cells proliferate and respond to ligands more strongly when the lncRNAs PRNCR1 (also known as PCAT8) and PCGEM1 are overexpressed, as both of these lncRNAs regulate androgen receptor signalling [63].

Lung cancer-associated transcript 1 (LUCAT1) was initially discovered in cigarette-related lung cancer and is located on the antisense strand of chromosome 5's q14.3 region. Thyroid, breast, ovarian, and renal cell carcinoma are only few of the cancers where evidence is growing suggesting LUCAT1 has a role. It has been proven to cause many malignant tumours, and its expression is particularly high in liver cancer. It is associated to the clinicopathological aspects of cancer patients and affects tumour development, invasion, and migration through a myriad of mechanisms. Therefore,

LUCAT1 has potential as a therapeutic target and a predictive biological marker in cancer [64].

Small ncRNAs

The RNAs responsible for RNA interference (RNAi) are typically between 19-30 nucleotides (nt) long, found among the many tiny cellular ncRNAs (such as tRNAs, snRNAs, snoRNAs, and many others given below) (Figure 1). The capacity of foreign RNA to cause silencing of homologous sequences in *Caenorhabditis elegans* (*C. elegans*) was the first evidence for this method of RNA-dependent gene silencing [65]. A relatively new molecular tool for *in vivo* gene silencing, the RNAi which is linked with numerous conserved silencing pathways playing crucial role in regulation of gene expressions at transcriptional levels, help maintain genomic stability, protection against transposons and retroviruses. Precursors and mechanisms of action help classify short RNAs into three broad categories viz. miRNAs, PIWI-interacting RNAs (piRNAs), and siRNAs [66] (Figure 1).

Compared to miRNAs and siRNAs, on an average, piRNAs comprised of somewhat larger size (24-31 nt) [67] derived from single-stranded RNA (ssRNAs) by surpassing the involvement of Dicers (Figure 1). The PIWI-clade of Argonaute proteins is found in mammals, which is how the piRNAs got their name. Their presence in the germline cells shows they have a role in defending against parasite chromosomal sequences. Moreover, , tiny RNA molecules such as RNAi constitute a novel group of mammalian small transcripts, however, their regulatory mechanism involved in certain cellular physiological pathways has not yet been proven. The terminators are linked to the short RNAs known as promoters [24, 68] associated with the centrosome, telomeres, and specifically initiation of the transcription [69].

The miRNA and small interfering RNAs **(siRNAs)**

The therapeutic application of siRNAs and miRNAs are being used as an alternate to treat various genetic illnesses followed with the findings that RNAi actively and precisely suppress the expressions of target genes in *C. elegans* by employing large sized double stranded RNAs (dsRNAs) [70-71], The ability of RNAi to suppress gene expression has led to its intensive investigations indie mammalian cells, while miRNAs and siRNAs both appeared as an excellent alternative therapeutic drugs with a great implementation to treat cancers, viral diseases, and multiple disorders associated with genetics or environmental factors [72]. In addition to its capacity to generate a strong and powerful knockdown of the targeted genes, RNAi also has the enticing property of having a high sequence specificity [73-74]. Extra- and intracellular hurdles that restrict the knockdown efficiency of RNAi treatments have been demonstrated. Many alternative methods have been proposed as potential solutions to these problems, and some of them have even advanced to clinical testing.

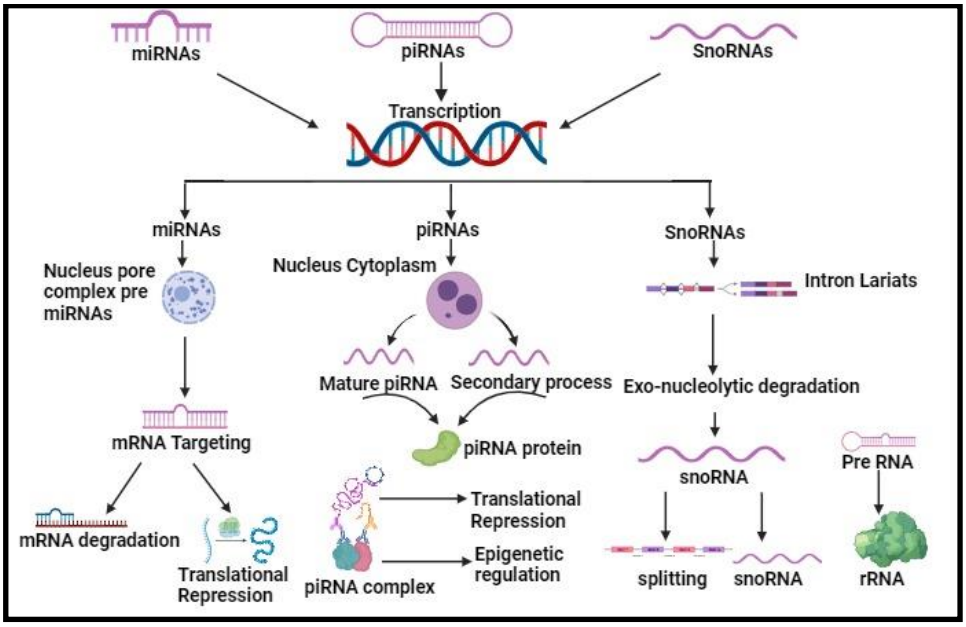


Figure 1. Small ncRNAs (rRNA, tRNAs, snRNAs, snoRNAs, piRNA etc.)

Mechanism of action of RNAi for siRNA and miRNA

For deeper understanding, how and where these molecules need to be delivered, a brief overview of the key mechanistic characteristics of RNAi will be provided (Figure 2).The in-depth analyses on RNAi processes are still needs to be done as a part of their innate immunological defense mechanisms against damaging effects or invading nucleic acids from microbial infections, protozoa, fungi, plants, and mammals all employ RNAi to silence certain genes [75]. Despite their structural and molecular similarities, siRNA and miRNA use distinct mechanisms to identify and silence their target genes in an RNAi response [76]. For example, miRNAs and siRNAs comprised of 21-25 base pair (bp) oligonucleotides with the ability to silence genes. When linked to the RNA-induced silencing complex (siRISC for siRNAs and miRISC for miRNAs), siRNAs and many miRNAs result in target destruction. While siRNA are prevalent in both plants and animals, miRNA are an endogenous form of gene regulation. A second distinction between miRNA and siRNA is that miRNA can regulate the production of many mRNAs by attaching to them with a variety of mismatches, whereas siRNA is exclusive to a single mRNA target through perfect complementarity [76]. Both miRNAs and siRNAs can initiate RNAi when they are transcribed from different vectors, introduced in the cells via injecting or transfection, or generated "naturally" by processing stem-loops precursors encoded within the host genome [75]. In the RNAi mechanism of miRNAs, the mature miRNA sequence is encased in one arm of the stem-loop structure, which is initially transcribed by RNA polymerase-II to generate a very long primary miRNA (pri-miRNA) transcript. This pri-miRNA is cleaved in the nucleus by the Drosha nuclease in conjunction with Pasha to form the homologous precursor of miRNA (premiRNA), which consists of a stem loop of 65 nucleotides [77-78]. The pre-miRNA is actively transported from the nucleus to the cytoplasm by exportin-5. Here, the pre-miRNA is processed by the RNase III Dicer into mature duplexes of roughly 21 nucleotides, which are then loaded into the miRISC in a way identical to siRNA to bind the relevant mRNAs and impede translation or promote their destruction. It has been shown that the efficiency of miRISC-mediated gene silencing is dependent on the degree of complementarity between miRNA and mRNA. Site-specific cleavage, as explained above, appears to only occur when the miRNA and target RNA are highly complementary. However,

mRNA degradation and translational inhibition have also been linked to mechanisms like mismatched miRNA/target sequences. It has been shown that the default method by which miRNAs suppress gene expression is the combination of these activities, called noncleavage repression [79-80].

To counteract carcinogenic activity, miRNAs can be mimicked by introducing synthetic duplexes into cells (or miRNA replacement therapy). Adeno-associated viruses (AAVs) are another method for boosting miRNA levels [81]. The conjugate of polyethylenimine (PEI) with miR-145 when applied systemically or even localized actively reduces tumour proliferation by 60% while increasing apoptosis *in vivo* using xenograft tumour model in mice. Moreover, adenovirus mediated targeting of let-7 pose a mimicing impact *in vivo*nonsmall cell lung carcinoma (NSCLC) model [82]. These findings were further confirmed in a NSCLC mouse model by systemic administration of miR-34a and let-7b that help reducing tumour size, and slow proliferation of tumor cells along with the high expression levels of both miR-34a and let-7b [83].

ncRNAs in human diseases

Researchers have discovered that small RNAs perform regulatory functions in virtually every stage of development. Therefore, it should come as no surprise that abnormal small RNA expression has been associated with several human illnesses such as cancer, neurological disorders, and cardiovascular conditions [84]. To be more specific, we can say that some miRNAs regulate genes with oncogenic features while others work on tumour suppressor genes, and that both types of miRNAs contribute to cancer development [85]. However, miR-21, which is overexpressed in a wide variety of malignancies, acts adversely on a number of tumor suppressor targets [86]. In addition, abnormal expression of long intergenic ncRNAs (lincRNAs) has been linked to several severe human disorders [84]. Cancers of the lung, breast, and cervix have all been associated to MALAT-1 ncRNA overexpression [86]. In almost two third of leukemia cases, an upregulated p15 antisense ncRNA was found. This ncRNA inhibits the production of the tumor suppressor p15 sense mRNA by forming heterochromatin [87]. The INK4b/ARF/INK4a tumor suppressor locus is overlapped by the long antisense transcript ANRIL [88], whose expression is increased in prostate cancer [89]. The lincRNA HOTAIR is also highly abundant in both primary breast tumors and metastases; it plays a role in the epigenetic trans-silencing of homeobox (HOX) genes. Intriguingly, HOTAIR induces epigenetic silencing of metastasis suppressor genes by directing the PRC2 repressive complex to these genes [90]. Neurodevelopmental and neurodegenerative diseases have also been linked to aberrant lincRNA expression [91]. The -secretase-1 (BACE1) mRNA is a critical element in the etiology of Alzheimer's disease, and its expression is rapidly upregulated in response to numerous cellular stressors (including amyloid 1-42 exposure). High levels of BACE1-AS and BACE1 mRNA are consistent with Alzheimer's disease [92].

Diagnostic and Prognostic Biomarkers

Several ncRNAs are specific and possible biomarkers for early detection and tracking of carcinogenesis due to their expression changes in tumor cells. The lncRNAs are highly specific, and easily detected within a range of pathological samples (blood, tissues, and urine) , and hence gaining attention to have an important role in cancer patients. Many forms of cancer, including bladder, prostate, gastric, pancreatic, and breast cancer, may benefit from using lncRNAs as a biomarker. In the clinic, lncRNAs

like PCA3 are utilized frequently to make a diagnosis of prostate cancer. Cancer risk may also be predicted using single nucleotide polymorphisms (SNPs) in lncRNAs, hence these molecules are rapidly gaining attention as potential diagnostic/prognostic indicators, therapeutic targets, and non-invasive biomarkers [93].

Diagnostic lncRNABiomarkers

If found early enough, most forms of cancer can be treated successfully. Methods and markers for detecting and diagnosing cancer include magnetic resonance imaging (MRI), computed tomography (CT), X rays, ultrasound, histology, molecular pathology, and the identification of circulating tumour cells. Prostrate cancer (PCa) is frequently diagnosed cancers in men, and is among the sixth leading cause of cancer mortalities. The majority of lncRNA applications have been in prostate cancer [94]. PCA3 is highly sensitive assay for teh detection of PCa than PSA test and digital rectal examination [95], and hence the former became a routine diagnostic tool. Human cells express two types of mitochondrial (mt) lncRNAs with stem-loop structures i.e., sense mitochondrial ncRNA (SncmtRNA) and antisense mitochondrial ncRNA (AsncmtRNA) [96-98]. For the detection of bladder cancer, fluorescent in situ hybridization is used to compare SncmtRNA and AsncmtRNA expression levels in the isolated cells from urine samples [99]. Urine cells from patients with bladder cancer consistently contained both SncmtRNA and AsncmtRNA, despite the later being repressed. SncmtRNA and AsncmtRNA expression is characteristic of tumour cells but not of normal cells. HOTAIR and other lncRNAs (HOX-AS2, MALAT1, lincRoR) are enriched in the urinary exosomes of patients with urothelial bladder cancer, suggesting a potential diagnostic application [100]. Furthermore, a set of lncRNAs that are dysregulated in the bone marrow of pediatric AML patients has been described and may be useful as a diagnostic marker [101]. Certain other biomarkers for the detection of multiple myeloma is MEG3, which have reduced expression levels in mesenchymal stromal cells of bone marrow origin [102]. Patients with squamous cell carcinoma (SCC) may also be easily identifieed with reduced expression levels of MEG3 in tumor biopsies [103]. Another type of lncRNA, the X-inactive specific transcript (XIST) which is highly expressed in most of the cancers could also be a potential diagnostic tools [104-106]. Combining serum XIST and HIF1AAS1 improves their diagnostic utility for non-small cell lung cancer (NSCLC) [107]. On the contrary, breast cancer, ovarian cancer and cervical cancer have shown a down regulation of XIST [108]. All of the aforementioned are only a few instances where lncRNAs have shown diagnostic promise. The lncRNA PCA3 is considered safe for human consumption. When it comes to other lncRNAs, further research is needed before they may be suggested for usage in humans.

Prognostic lncRNA Biomarkers

A prognosis (from the Greek words for "before" and "knowledge") is a diagnosis or forecast of how a disease will progress and the patient's prognosis for recovery or survival. Several factors are involved in prognosis, and is identified by the type of cancers, where it is located, how big and bad it is, its response to the therapies, age of the patients, and the overall performance of the patients. Tumor grade is associated with the highly modulated expression levels of lncRNAs in cancer patients. SNHG3, PANDAR, hPVT1,78, and HOTAIR are some examples of lncRNAs with established prognostic value for hepatocellular carcinoma (HCC) [109]. Gallbladder cancer patients' HOTAIR expression may also have prognostic value [110],acute myeloid leukemia[111], small-cell lung cancer[112], colon cancer,[113],[114],breast cancer[115], and oesophageal SCC[114]. Some other lncRNAs that relate with cancer

prognosis include ANRIL for GBC[116], AFAP1-AS1 for pancreatic cancer[117], LINC00472, H19 and KCNQ101T for breast cancer[115, 118], lncRNA-ATB for colorectal cancer[119], SChLAP1 for prostate cancer[119], HIF1A-AS2 for TNBC[120], and NEAT1 for glioma[121]. lncRNAs have also been proposed as a biomarker for the molecular subtyping of triple-negative breast cancer [122]. Suppressed expression of lncRNAs has also been linked to worse cancer outcomes. Reduced levels of AI364715 expression [123], GACAT1[124], and GACAT2[125] in gastric cancer tissues, PCAT29 in prostate cancer[126], AC026166.2–001 and RP11-169D4.1–001 in larynx SCC [127], lncRNA-LET [128] and MEG3[116] as a predictive indicator in GBC tissue samples. Epigenetic alterations in lncRNAs may potentially be used for illness prognosis prediction. Multiple myeloma subtype and stage have both been linked to MEG3 promoter hypermethylation, for instance [129], PVT1[130] and PCAT1 [131] might be useful for both detecting cancer and predicting its progression. In conclusion, changes in lncRNA expression, either up or down, could have predictive importance.

Challenges and Limitations

Although ncRNAs show tremendous promise in cancer treatment, several obstacles still need to be overcome. Some of the obstacles that need to be solved for successful clinical translation include off-target effects, administration techniques, and challenges relating to stability and specificity. Obstacles in distribution and targeting prevent the full utilization of ncRNAs therapeutic potential in cancer. Effective, precisional and target oriented transportation of lncRNAs system is highly demanded to formulate ncRNA-based cellular therapeutic system for advanced tumors with least side effects or systemic toxicity . It is challenging to give long-lasting therapeutic advantages due to the intricate intracellular machinery that regulates ncRNAs. Regulatory variables are essential for the translation of ncRNA-based therapeutics from the laboratory to the clinics. Because of certain novel properties particularly least off targeting specifically the unwanted interactive disturbance within the host intracellular signaling procedures. Moreover, ncRNAs demand exceptional care to follow regulatory instructions along with the safety measures. In this regard, rigorous preclinical testing and oversight by authorities are essential. In order to overcome these obstacles, researchers from different fields must work together, and cutting-edge tools for ncRNA characterization and rigorous clinical investigations are required. Despite the challenges, there is great potential for improved cancer diagnostics, prognoses, and therapeutic approaches if ncRNAs can be better understood and their potential harnessed. The development of efficient ncRNA-based diagnostics and therapies for cancer still faces considerable obstacles, including a lack of selectivity, a lack of knowledge of processes, and delivery issues[132].

Lack of specificity: The greatest challenge in identifying ncRNAs role in tumor biology is the formation of preferred therapies having target orientation and precision, without any lacks in terms of, mismatching or failed actions. The major hurdle being caused is the lower specificity for ncRNAs in tumor cells which is being eradicated by improving the technology. The single cell sequencing technique along with the spatial transcriptomics are being focused recently to modulate the expression levels and targeted functionality of ncRNAs. Furthermore, in the highly target oriented therapeutic systems that can help modulate the functions or expression levels of ncRNAs in tumor cells are also being developed [133].

Chemical modifications related to stability: Huge number of cellular endogenous ribonucleases make RNAs quite unstable molecule with poor pharmacological characteristics. RNA is hydrophilic and contains a negative charge. Furthermore, the catalytic activity of ribose sugar is due to its 2′-OH moiety. For improving the stability of RNA-based treatments without diminishing their biological efficacy, chemical modification is required**.** [134].

Mechanistic understanding: A big challenges in the study of cancer cell ncRNAs is limited understanding of the processes that underpin their activities. Lack of mechanistic insights has hampered the study of ncRNAs biology in the tumor cells, thus variety of approaches are being employed to overcome this issue. Cutting-edge technologies like CRISPR/Cas9 genome editing system, and RNA sequencing helping the precise identification of target genes and cellular networking cascades being governed by these ncRNAs. *In vivo* and *in vitro* models have also been developed to study the roles of ncRNAs to achieve precision and accuracy to target hitting and least off the target impacts [135-137].

Delivery: Since ncRNAs need to be delivered to specific tissues and inside the cells for tumor treatment, studying them presents a substantial difficulty. The problem of ncRNA delivery has prompted the development of a number of solutions.Protecting ncRNAs by enclosing them in a nanoparticle, such as a liposome or one comprised of biodegradable polymers, is one strategy. Preclinical studies have shown that this method is successful for delivering many ncRNAs to tumor cells *in vivo* and *in vitro*, including miRNAs and siRNAs. Another area of study is the use of polyethyleneimine (PEI)-modified liposomes to transport the tumor-suppressing miRNA miR-34a to PCa cells *in vitro* and in a rat model, where it suppresses tumour growth and metastasis [138-140].

• Viral vectors, e.g. lentiviruses or AAVs, provide another option for transporting ncRNAs to their intended recipients. The delivery of ncRNAs to cells using viral vectors is efficient, but it is not without drawbacks, such as the possibility of immunological responses and the risk of insertional mutagenesis [141].

• Recently, there has been a lot of interest in using exosomes to transport ncRNAs. It is possible to extract exosomes, which are tiny vesicles pinched off by cells, and modify them such that they transport ncRNAs. Since exosomes are not immunogenic and are biologically compatible, they can efficiently transport miRNAs and siRNAs to their intended recipients. For instance, in vitro and in a mouse model, exosomes were used to deliver the miRNA miR-193a to lung cancer cells, where it inhibited tumour growth and increased survival. Nature Communications published the results of this study in 2019 [142-143].

The use of exosomes for the transport of ncRNAs is a potential new strategy. Exosomes are cellular vesicles that may be extracted and manipulated to transport ncRNAs. The efficiency with which exosomes deliver miRNAs and siRNAs to their targets is impressive, and they pose little risk of immune response. Reduced tumour growth and increased survival were observed when exosomes were used to carry a miRNA called miR-193a in an *in vivo* and *in vitro* models of lung cancer [144].

Therapeutic Potentialof RNAi

The biological phenomenon known as RNA interference (RNAi) occurs when some RNA molecules prevent the production or translation of particular genes [65]. siRNAs, lncRNAs, and miRNAs are all ncRNAs that may induce the sequence-specific silencing of cognate genes through RNAi. Regulatory ncRNAs have been shown to promote or repress target gene expression; aberrant ncRNA expression plays a critical role in carcinogenesis [145]. Gene expression or function issues might result from miRNAs being out of whack. RNA interference (RNAi) has therapeutic potential for supplying transcription-suppressive factors, subsets of kinases, and other signalling molecules to restore expression of these damaged genes. RNA interference's potential as a cancer therapy is High specificity compared to other cancer therapies like chemotherapy; induction of silencing in the advanced phases of growth; transmission of silenced gene to the next generation; low cost compared to other methods of gene therapy [146]. As was previously indicated, cancer is a primary focus for RNAi-based treatment. Oncogenes, mutated tumour suppressor genes, and a number of other genes involved in tumour progression are good targets for gene silencing by RNAi-based therapy because of the precise functional mechanism, high potential, and high specificity of RNAi-based gene silencing. An advantage of RNAi as a cancer treatment is that it can target several genes in multiple cellular pathways all at once [147]. Inhibiting numerous genes at once is an efficient method for treating cancer and decreasing the likelihood of medication resistance due to chemical drug overuse. The creation of patient-specific, tailor-made pharmaceuticals is yet another benefit of this approach. The development of tumors can be slowed or stopped with greater success when using personalized medications. An important phase in the pathway leading to the degradation and translational suppression of target mRNA is the RNA-induced silencing complex (RISC), a multiprotein complex. Dicer, an endoribonuclease, catalyzes the conversion of lengthy dsRNA and complex hairpin precursors into shorter duplexes or siRNA [148]. Next, RISC is programmed to use the siRNAs. Double-stranded siRNA is processed during incorporation into passenger and guide strands. The latter is subsequently liberated, and RISC is turned on to catalyze the guide strand's binding to target sequences. Target gene expression is suppressed because the attached mRNA is subsequently broken and destroyed by cellular nucleases. Unlike siRNA, shRNA is often delivered via a viral vector. ShRNAs are short, tightly hair-pin shaped RNAs that are produced by RNA polymerase III or a modified form of RNA polymerase II. Reduced efficiency of shRNA-mediated gene silencing in cells with low Dicer levels. In order to function, shRNAs must make direct contact with chromosomal DNA due to the promoter-dependent expression characteristic [149].

In addition, shRNAs have a nuclear effect, which complicates their application even further. siRNAs are produced by RNA polymerase II and III and are single-stranded RNAs. The primordial miRNA (pri-miRNA) has a hairpin loop structure and is produced after transcription. The Drosha-DGCR8 complex then cleaves the pre-miRNA into mature miRNA. The pre-miRNA is processed into the mature functional miRNA, a duplex consisting of roughly 22 nucleotides. So that the guide strand may continue to bind to the mRNA, the double-stranded miRNA gets integrated into RISC. In contrast to the complimentary binding displayed by siRNA, miRNA very weakly attaches to its target sequence. Multiple messenger RNAs (miRNAs) can attach to a single mRNA sequence. Depending on the degree of similarity between the target mRNA and the miRNA sequence, the mRNA may be degraded by endonucleolytic cleavage or its production may be suppressed via regulation of translation [145].

CRISPR/Cas9-based strategies

In order to specifically generate Cas9 protein and/or the sgRNA that targets genes crucial for cell viability (as depicted in Figure 2), the CRISPR/Cas9 gene therapy approach can employ cancer-specific promoters that regulate genes that are highly expressed in tumour cells. Prominent examples of such promoters include hTERT, Ran, Brms1, and Mcm5 [150-151]. The construction of CRISPR/Cas9 ribonucleoproteins in cells that do not have cancer can undermine the specificity of CRISPR/Cas9-based treatment, even while their activity is lowered in healthy tissues. The expression selectivity was greatly increased by establishing a 'logical AND gate gene circuit' in which two promoters regulated a single target gene. The transcription of the Cas9 gene is regulated by hUPII, a promoter specific to the bladder. However, the Cas9 protein is inactive until it is coupled to the sgRNA, whose expression is regulated in this model by the hTERT promoter that targets cancer cells. Only cells that develop into bladder cancer, satisfying both criteria, contain the complete ribonucleoprotein complex. The generated CRISPR/Cas9 complex initially targets the LacI gene encoding a suppressor protein in order to increase production of effector genes directed by the lac operator. As a direct outcome of this study, the hBAX, p21, and E-cadherin genes were put to use. They were found to trigger apoptosis, growth arrest, and decreased motility in bladder cancer cells when activated [152].

CRISPR/Cas9-based strategies

There is a dire need for cutting-edge therapeutic approaches in the fight against cancer. An exceptionally powerful treatment for cancer has been made available thanks to the clustered regularly interspaced short palindromic repeats (CRISPR)-associated nuclease 9 (CRISPR/Cas9) technology (Figure 3). CRISPR/Cas9 is a recently developed molecular scissor that is already being put to use in a wide variety of fields, from plant breeding to the treatment of neurological disorders and cancer. CRISPR is a part of prokaryotes' adaptive immune system [153]. Multiple changes to the genetic material of cells, including DNA and RNA, contribute to the development of cancer [154].

It is possible to defeat cancer by reversing these changes in the cancer cell genome. Numerous studies have shown that CRISPR/Cas9 can correct cancer-causing mutations in genes. In the human genome, lncRNAs are found in the protein-coding region, and there is much evidence and data supporting the use of the CRISPR/Cas9 system to edit these lncRNAs [155-156].

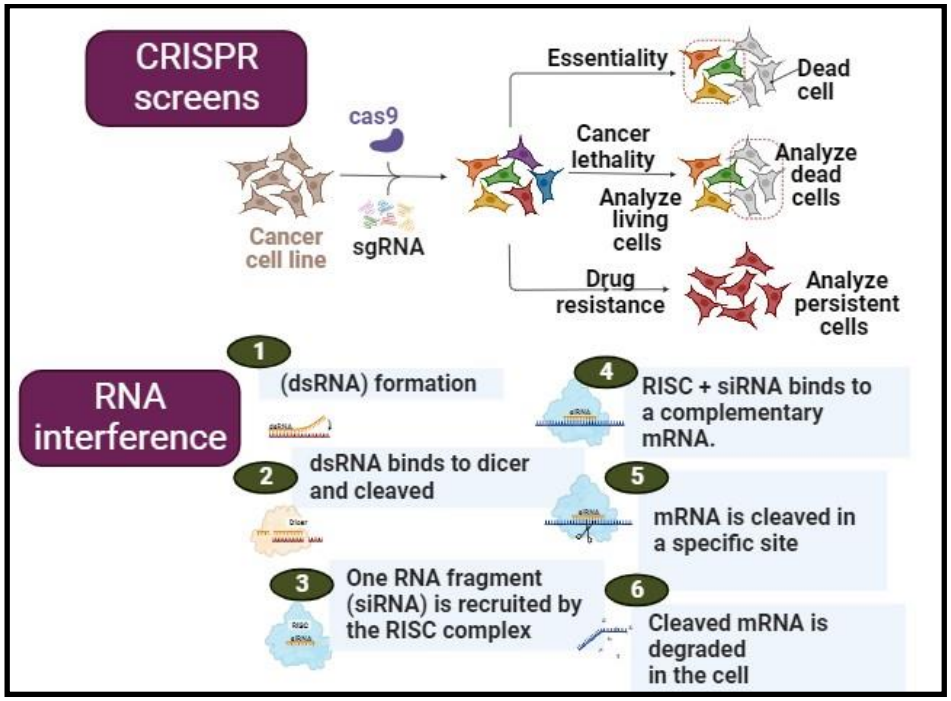


Figure 2. Molecular editing tools. CRISPR screens for cancer cell treatments, and RNAi for mRNA editing.

Structure and features of the CRISPR/Cas9 system

Ishino and his team originally discovered CRISPR in *Escherichia coli* (*E. coli*)during late 1980s, where they found a series of spacer-like sequences within the clustered repetitions, which they eventually dubbed CRISPR, followed by certain modifications by various research groups to report new types of CRISPR/Cas systems. Different processes lead to the classification of many different subtypes within each of the three main groups [157]. The CRISPR/Cas9 system is a type II CRISPR system found in Streptococcus pyogenes. Due to its high efficiency and pinpoint accuracy, it has quickly become the mammalian system of choice. CRISPR/Cas9 is the first synthetic CRISPR/Cas system for editing genomes because its single guide RNAs (sgRNAs) can be easily programmed with a recognition sequence of a tiny 20 nucleotides in length [158]. The sgRNA is made up of two pieces of CRISPR RNA, one of which has a sequence that is fully complementary to the target site and the other of which is only partially complementary [159]. The CRISPR/Cas9 system also includes the Cas9 nuclease, which is activated by the RNAs that direct it to specific places within a genome. Together, the sgRNA and Cas9 protein produce a more powerful complex that is able to identify the complementary DNA sequence at the target site, which is bookended on each side by the protospacer adjacent motif (PAM). N can be any of A, T, G, or C, and the PAM typically consists of NGG or NAG; the PAM aids in the initiation of DNA double-stranded breaks [160].

CRISPR and colon cancer

LncRNA dysregulation regulates colon cancer cell proliferation. When ZEB1-AS1 is overexpressed in colon cancer cells, miR-455-3p is taken up and the expression of p21-activated kinases 2 (PAK2) is increased. Limiting cell proliferation in SW480 colon cancer cells via upregulation of LINC01082 results in a smaller tumour. Inhibiting gene expression of the LINC01296 gene in colon cancer cells (SW480 and SW620) slowed their proliferation [161].

Targeting miR-21a in colon cancer cells led to a decrease in cell proliferation when LINC01082 was silenced [162]. The expression of taurine-upregulated gene 1 (TUG1) was shown to be elevated in both HCT116 and LOVO malignant colonocytes, correlating with the downregulation of p63 in these cells. Reduced expression of TUG1 suppresses the growth of HCT116 and LoVo cells [163].
Reducing tumor development in vivo and stopping colon cancer cell proliferation can be achieved by targeting TUG1 [164]. Tumors form when cell proliferation is improperly controlled, leading to the upregulation of oncogenes and downregulation of tumor suppressor genes [165], Because of its capacity to alter many genes, CRISPR/Cas9, a powerful genome editing tool, provides novel therapeutic options for cancer patients. Inhibiting tumor development by oncogene knockout is one function of the CRISPR/Cas9 system [165]. Cancer-causing mutations in the beta-catenin gene can be treated using a new kind of gene therapy by lowering the mutation rate [166]. By editing the HSV-1 genome with CRISPR/Cas9, Zhang et al. engineered HSV oncolytic viruses with the aim of curing colon cancer. Secretory mucin MUC5AC aids in the progression of drug-resistant colon cancer. In order to cure colorectal cancer, Pothuraju et al. used RNA interference and CRISPR/Cas9 modification in cell culture and mice models in vivo to knock down the MUC5AC gene. In order to treat colon cancer and keep an eye on angiogenesis, Chakraborty D et al. found that they needed to target NPY/Y2R. Angiogenesis was suppressed in mice treated with a Y2R antagonist by gene editing using CRISPR/Cas9[167]. HuR enhances its expression thanks to ELAVL1, an RNA-binding protein of HuR. Apoptosis was boosted when HuR was deleted using CRISPR/Cas9. Therapeutic targeting of HuR may be effective for colon cancer[168].

Current CRISPR delivery mechanisms

In order to engage directly with target cells, the CRISPR/Cas9 components must overcome a number of physical hurdles [169]. In addition, the Cas9 protein and the sgRNA need to reach the nucleus at the same moment for the gene editing mechanism to function properly (Figure 3). Therefore, the type of delivery method is crucial for implementing CRISPR/Cas9-based gene editing therapeutics (Figure 3). CRISPR/Cas9 components were supplied through microinjections, hydrodynamics, and electroporation; nevertheless, these methods performed poorly *in vivo*. Because of their immunogenic response, increased off-target effect, high cost, and limited capacity for loading pharmaceuticals, viral vectors have yet to make it into clinical use of treatments [170]. However, adenoviral vectors are incapable of enclosing a CRISPR/Cas9 DNA plasmid vector because to its excessive size (10,000 base pairs). Lipid nanoparticle carriers are one example of a non-viral vector that could be used to transport the huge and lengthy CRISPR/Cas9 DNA plasmids. These considerations have led to the development of a new field of research in which non-viral vectors are preferred to viral vectors for application. The non-viral vector systems include lipid nanoparticles and gold (Au) nanoparticles [170].
Nanocarriers produced from non-viral vectors are now feasible because to developments in nanotechnology (Figure 6), and they can be used to package cancer therapies that are unlikely to elicit an immune response, have adequate delivery of cargo capabilities, and are highly biologically compatible. Lipid-based delivery systems provide a number of advantages over other types of drug delivery systems, including their simplicity, low cost, and potential for large-scale production [170]. In terms of production method and physical characteristics, lipid nanoparticles can be broken down into a number of distinct categories, such as solid lipid nanoparticles,

nanostructured lipid carriers, niosomes, and liposomes. Solid lipid nanoparticles (SLNs) are composed of solid lipids and have a spherical shape with a size range of 50-1000 nm. Solid solution, lipid shell model, and drug-enriched shell are all viable options for encapsulating cancer medicines in SLNs' lipid matrix [171]. Anticancer medicines are distributed throughout the lipid matrix in the solid solution model. When the SLNs are cooled, the medication and lipids undergo a phase separation that results in the formation of the shell. Medicines precipitate in the lipid shell model right before the lipid recrystallizes. Since SLNs can carry both hydrophilic and hydrophobic drugs, they can deliver anticancer drugs with more precision [155].

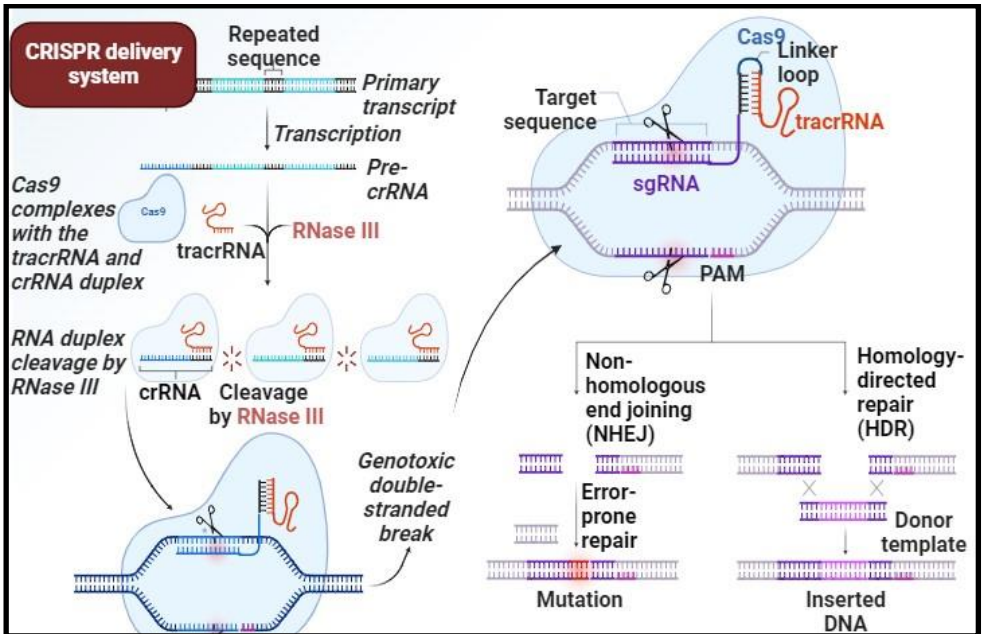


Figure 3. The mechanism of CRISPR delivery system and its action at target site.

Advancements in the CRISPER delivery systems

At cellular levels, proteins play the lead role in thephysiology, whereas RNA serves as a bridge between genes and their translated versions, the proteins[172-173]. The study of proteins and their roles in illness was formerly a primary focus of scientific inquiry, but with the advent of cutting-edge technological tools, researchers have moved their attention to genes. The ncRNAs are a class of RNAs that cannot encode proteins but can facilitate biological processes by interacting with other RNAs [174]. The lncRNA and short ncRNA (sncRNA) are two types of ncRNA that are distinguished by their respective nucleotide (nt) lengths (>200 nt in lncRNA and 200 nt in sncRNA). Since sncRNAs play such a crucial role in cancer, researchers have started paying more attention to miRNAs, siRNAs, and piRNAs[175]. Key regulators of cellular processes, ncRNAs are stabilized through interacting with other ncRNA subtypes, and occurrence of anymutations in ncRNAs may affect their functions lead to carcinogenesis[176]. Most functional ncRNAs originate from introns, which are the inactive byproducts of alternative splicing [177]. The most studied noncoding RNA (ncRNA) is a member of the sncRNA family; it is 22 nucleotides in length (nt) and plays a critical role in post-transcriptional gene silencing in mammals. MiRNAs regulate over 60% of the translation of proteins into a readable form. Cell fate decisions including proliferation, differentiation, and apoptosis are regulated by miRNAs, which play a role in cancer [178]. In addition, miRNA expression levels are not the same in normal and malignant cells. In many ways, miRNA acts as a mediator

of cell carcinogenesis, much like oncogenes and tumor suppressors [179-180]. Overgrowth of tumours can be caused by a malfunction in the miRNA processing machinery, which can be caused by both genetic and epigenetic alterations [181]. [181]. Polymerase II is thought to be responsible for the transcription of the vast majority of the discovered lncRNA, which can be found in the nucleolus or the cytoplasm [182]. Recently, research has focused on siRNA and miRNA as a potentially useful therapeutic class for treating cancer and other disorders. Targeting functional pathways that are overexpressed at a certain cellular level or in a tissue of interest, miRNAs can help alter the pace at which cells grow and survive. Furthermore, reintroducing some miRNAs into a particular cell type may result in potent therapeutic efficacy. Because miRNA has been identified as a promising therapeutic target, we should expect to see a proliferation of novel RNA-based medicinal solutions in the near future[183-184]. An further potential ncRNA inhibitor that shows promise in treating several disorders is siRNA. Because of its short half-life in the body, siRNA is often delivered to its target utilizing one of many alternative methods. There are now 20 clinical studies employing siRNA to treat various diseases; four of these are specifically targeting cancer[185].

Mechanism of ncRNAs therapeutic activity in different diseases and in cancer treatment

It is thought that lncRNA is engaged in cellular molecular processes, although our understanding of its mechanism is still somewhat restricted. The lncRNAs can be classified into the transcription regulation, post-transcriptional regulation, and others categories, according totheir specific roles [186]. Some lncRNAs have been shown to regulate gene transcription via transcriptional interference, which is a mechanism involved in transcriptional control. In this way, lncRNAs function as regulators of both chromatin remodeling and transcriptional interference. Translational regulation and splicing regulation are the two primary post-translational ways by which lncRNAs exert their effects. Another way transcriptional factor production may be regulated is through translational control and splicing regulation, either by binding to translational factors or ribosomes or by competing with splicing factors to govern translation [186-188]. Small nuclear factor 90-associated RNA (snaR) is an example of a lncRNA that exerts its effect via modulating the translation of mRNAs[188]. Recent studies have looked at the potential of siRNA and miRNA as a novel type of medicine for the treatment of multipledisorders including cancer and infections (Figure 4). siRNA inhibits mRNA translation by serving as a competitive template [189]. It inhibits the translation of the protein of interest by acting as an antisense molecule, which speeds up the decay of its mRNA [190]. Furthermore, certain lncRNA can increase mRNA stability by interacting directly or indirectly with miRNA [29]. Some antisense lncRNA, however, can stabilize mRNA by attaching to the miRNA's mRNA-binding site [92]. The miRNAs are small, non-protein-coding RNA molecules that bind complementarily to their mRNA targets, suppressing their expression by a combination of mRNA cleavage and translational repression[191]. Together, the RNase III enzyme Drosha and the nuclear protein DGCR8 break the longer pri-miRNA into the shorter pre-miRNA (70 nts). The miRNA matures into a dsRNA duplex with the target mRNA after being transported to the cytoplasm by Exportin 5 and subsequently cleaved by Dicer and TRBP [192].

The drug development industry has been radically altered by the revelation of ncRNAs role in translational processes. Cancer, diabetes, cardiovascular issues, metabolic syndromes, kidney issues, and infection are only some of the conditions that have been found to benefit from the use of ncRNA. Several therapeutic applications of

ncRNA delivery targeting cancer stem cells and metastatic tumours have been found to be effective [193]. By the end of 2018, twenty siRNA clinical trials investigating cancer and diabetes were commenced, and some of these had progressed to Phase II clinical trials [194]. There are currently four drugs based on RNAi for treating different cancers in clinical trials [195]. Bevasiranib, used to treat eye problems, was the first siRNA medication to reach Phase III clinical trials [196]. In vivo, siRNA-based therapies work by selectively repressing gene expression. This class of ncRNA therapeutics is more selective than others because, unlike miRNA therapeutics, siRNA treatments target only the gene of interest. Silencing of certain genes is achieved by introducing siRNA molecules that bind specifically to messenger RNA [197]. Chemically synthesised siRNA molecules have successfully completed phase I clinical trials in patients with solid tumours [197].

Endosome escaping for enhanced targeted delivery

For RNA therapies to be effective, it is essential to reach precisely at target and complex formation with desired mRNA, both of which require traversing extracellular and intracellular barriers. However, higher molecular weights bearing -ve charges, structural instability, and naked RNA inhibitors are unable to delivered at their targedsites within the biological system. One enzyme that aids in RNAi breakdown that is excreted by the kidneys is serum endonuclease in the living organism [198]. To avoid destruction by endosomes and boost RNAi efficacy, RNAi tools like siRNA, ASNs, aptamers, synthetic mRNAs, and NPs can be chemically modified (Figure 5). Chemical changes have been devised to boost RNAi in vivo stability and avoid many of the problems associated with siRNA distribution [199]. To make a stable phosphorothioate group, a sulphur atom can be substituted for the phosphate group's nonbridging oxygen atom during chemistry. Because of this, RNAi is more stable and has better pharmacokinetics (PK). Enhancing the hydrophobicity of RNAi improves its PK by raising affinities of the plasma proteins, and hence help accumulation of tissue RNAi. However, the miRNA affinity and nucleotide stability are both decreased by the introduction of the phosphorothioate group into ASNs [200]. PEI can be introduced to every other nitrogen atom of ASNs to generate a stable amide, which improves RNA stability and affinity by displacing the sugar phosphate backbone of RNA [201]. The use of NPs is yet another strategy for boosting the reliability and performance of RNAi. NPs can actively or passively transfer RNAi to a tumour site while protecting it from degradation [202]. Non-toxic, biodegradable, and immunogenic RNAi delivery systems that are able to evade endosomal degradation and deliver their RNAi payload safely and intracellularly to the cytoplasm are preferred [202]; thus, promoting RNAi silencing [203]. Viral vectors are highly effective gene delivery technologies, but their application in humans is restricted due to safety concerns. This has led to the development of non-viral delivery technologies as a serious contender. It has been shown in Phase I clinical studies that nonviral delivery systems using targeted polymers, nontargeted lipids, a biodegradable polymer matrix, and PEI can be effective [204]. Electrostatic interactions between positively charged molecules and the negative charge on siRNA are commonly used to protect it from being degraded in the endosomes. Mesoporous silica NPs functionalized with PEI and cyclodextrin were reported by one group. PEI creates an electrostatic interaction with siRNA, as stated by the authors, and cyclodextrin aids in reducing the toxicity of PEI [205]. Since lipid NPs are more biocompatible, they are less harmful. Noncovalent lipid/polymer complexes ('lipoplexes') are being researched as a potential siRNA delivery method with the goal of increasing serum stability and transfection efficiency. To lengthen the lifespan of the NPs in circulation,

these systems can be PEGylated using long polyethylene glycol (PEG) chains. Lipoplexes made from neutral, anionic, or PEGylated phospholipids are more stable and have a stronger therapeutic effect than lipoplexes made from cationic phospholipids [206]. Liposomal siRNA dispersion can be enhanced by using cell-penetrating peptides (CPPs) based on polyarginine, the release of which is triggered by the acidic TME via an acid-cleavable hydrazine linker. Newly developed polymeric NPs release their siRNA payload into acidic TME, where the silencing impact is maximised [207].

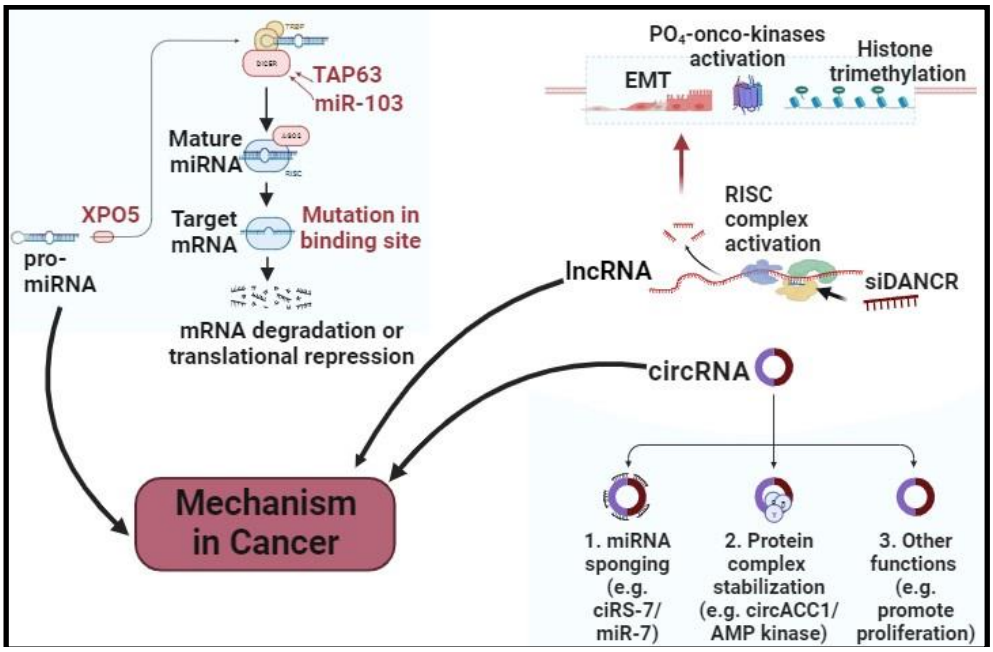


Figure 4. Mechanism of action of various ncRNAs (miRNA, lncRNA, circRNA, etc.) in cancer cell apoptosis.

Nanoparticles (NPs) to improve delivery efficiency

Due to their large surface area and weak quantum mechanical effects, nanoparticles (NPs) typically display unique magnetic, thermal, optical, and electrical characteristics [208]. NPs are increasingly being employed as drug carriers due to their ability to selectively deliver chemotherapeutics at target tumor tissues while sparing healthy organs (Figure 5). Biodegradable, long-lasting, immune-system-unaffected, cheap, simple to produce, and selective payload release are desirable qualities in an NP carrier [209]. In the guided bottom-up technique used to produce medically important NPs, external stimuli cause designed macromolecular components to interact with one another and self-assemble into forms that would not be conceivable without this guidance. The NPs can either contain the drug or have the drug bonded to its surface. Nanoplatform classes include liposomes, polymeric micelles, drug-conjugated polymers, and dendrimers comprise the basis for most drug delivery nanoparticles [210-211]. Drug-loaded nanoparticles can be delivered to disease areas by either passive targeting, active targeting, or physical targeting. Increased permeability and retention (EPR) is the mechanism through which NP-sized entities are selectively absorbed by tumor cells during passive targeting[212]. In active targeting, NPs are functionalized with ligands such as antibodies, proteins, and peptides [213], which interact with receptors overexpressed at the target site [214]. External sources or fields are utilised for physical targeting in treatments like

photothermal and magnetic hyperthermia to guide NPs to the desired place and control their release. For any particular targeting method, the drug release can be triggered by a shift in pH, temperature, or both. Understanding the combinatorial implications of size, shape, surface chemistry, patient-specific information, and other properties is vital for creating an effective NP. Since it would take too long and too many resources to optimise all of these qualities through tests, computer modelling is applied to limit the number of possible outcomes. Simulations have been used to model the quantum mechanical interactions between ligand receptors and NPs. Mesoscale modelling and Monte Carlo simulations are also frequently used when precise values are unknown [215]. Because of their accessibility, amenability to modification, biocompatibility, and stability, NPs have emerged as promising options for targeted drug delivery. These characteristics make it possible to encapsulate and deliver highly toxic drugs precisely to the tumour site. In addition to passive targeting via the EPR effect and active targeting via ligand-receptor interactions, next-generation photothermal and magnetic hybrid NPs permit time- and location-controlled drug delivery via the heat created by the particles. Physical targeted clinical trials have only just begun [216]. Nanoparticles (NPs) can form their own unique structures by self-assembly, from amphiphilic micelles and liposomes to rotaxanes, dendrimers, and metal-core particles. These particles' unique characteristics make them ideal carriers for a wide range of drugs and biological molecules, allowing them to be targeted to specific organs and tissues. Although micelles and liposomes are the most often used NPs, researchers are becoming increasingly curious about the potential of other particle forms for drug delivery. For example, compared to spherical structures, dendrimers have a larger surface area and can carry more cargo, while rotaxanes can release their payload in response to an external trigger. Metal-core particles are also useful for physical, physical-active, and passive targeting. Tight management of many nanoparticle features is required for effective medicine distribution to target locations without side effects [217]. Negative consequences can occur if the dimensions, shape, and/or surface charge are not optimal. Variations in the surrounding environment can also affect how well NP drugs are delivered. In vitro and in vivo testing is required to make sure NPs are safe for usage in humans and animals before they can be used in the clinic. Computational modeling has been helpful for NP design optimization because to the complexity and unpredictability of the medication delivery mechanism [218]. Due to their low level of predictability, computational models are not yet commonly employed in the pharmaceutics industry. The drug-to-patient scale is often overlooked in favor of smaller, more specialized processes in most models of medication administration. Rarely do we see models that span many time and space scales simultaneously. While multiscale modeling can be useful in guiding nanoparticle design, it would still need to be informed by prior studies and confirmed in the lab before being widely accepted. There is a need for enhanced and clinically verified multiscale modeling that incorporates individual data e.g. vascular network imaging, the genome, and family history. Several computational models have been experimentally tested outside of clinical settings to aid in the creation of more effective nanocarriers[219-220].

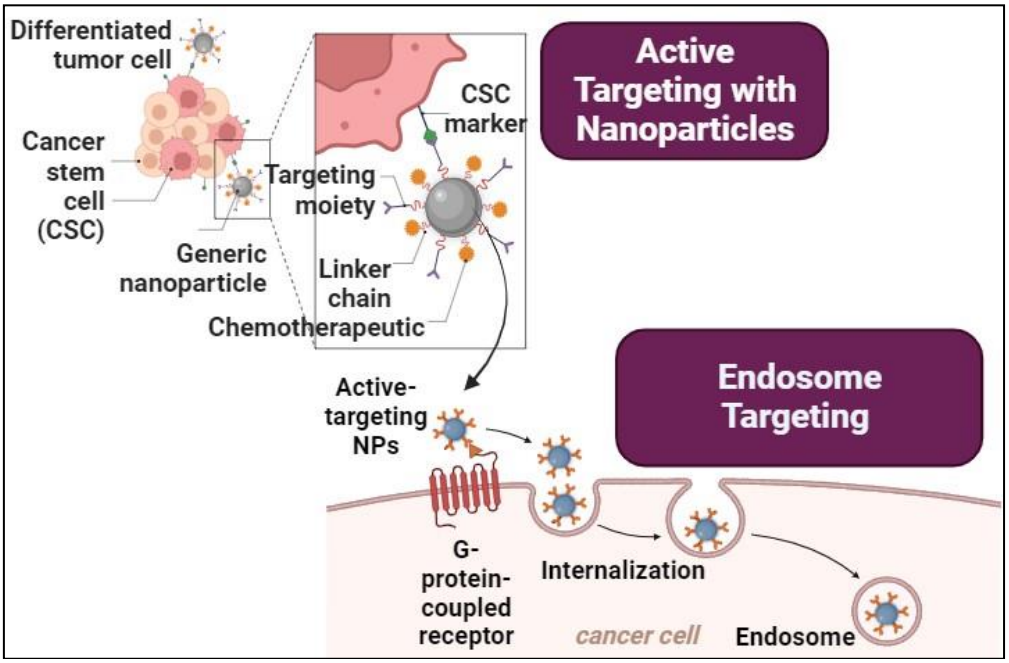


Figure 5. Mechanism of the action of NPs for targeted drug delivery at the target tumor tissues.

Combinatorial therapies involving ncRNAs with different cancer treatment regimen

Recently, much advancements in nucleic acid sequencing technologies have revealed human genome is transcribed into ncRNAs. Transcribed nucleotide sequences provide the fundamental structural elucidation of ncRNAs, which are involved in controlling the genes activities by base-pairing both at transcriptional and or translational levels. Then the folds produces in ncRNAs to achieve higher stability by acquiring secondary structures or tertiary structures is thought another way for producing an array of biological functions [221]. Human lncRNAs like maternally expressed gene 3 (MEG3), which has a cancer-suppressing impact via activating the p53 signalling pathway, are one such example [222]. Base complementarity allowed two distal motifs from an evolutionarily conserved area of MEG3 to engage and generate alternate, jointly linked pseudo-knot structures (kissing loops). Destroying these connections hampered MEG3 folding, interrupted p53 signaling that relied on MEG3, and eventually reduced MEG3's cancer-suppressive activity. Thus, maintaining kissing loops structures is a mean through which the anticancer impact of lncRNA MEG3 can be preserved[223]. The sncRNAs (18-200 nt) and lncRNAs (>200nt) are two broad categories based on transcript size. The small nucleolar RNAs (snoRNAs) along with the miRNAs constitute two exclusive examples of sncRNAs. Gene regulation, mRNA maturation, and protein synthesis are among processes in which ncRNAs have been shown to have a role. Also, by recognizing a wide variety of molecular targets, signaling pathways associated with ncRNAs can direct distinct cellular biological responses [176]. Tumor repressors like zinc finger protein 750 (ZNF750) have been reported to provide better outcomes for patients suffering SCC [224]. Meanwhile, the lncRNA terminal differentiation-induced ncRNA (TINCR) could be a cancer-suppressing target of this pathway. ZNF750 was found to reduce the malignant phenotype of SCC by upregulating the expression levels of the lncRNA TINCR [225]. Further worsening the SCC malignant phenotype both *in vitro* and *in vivo*, ChIRP findings depicted a close association of lncRNA colon cancer-associated

transcript-1 (CCAT1) with TP63 and SOX2 which are the master transcription factors (TFs) to activate the TP63/SOX2-CCAT1-EGFR signaling pathway [226]. Further research explained that ncRNAs activates either upstream or downstream sequences of genes to promote SCC development. There is growing evidence that ncRNAs may help to both the initiation and suppression of multiple types of cancers. Therefore, ncRNAs plays a central role in the regulation or modulation for the development, diagnosis, and therapies against certain diseases [227].

The anticancer therapeutic efficacy of chemotherapeutic medicines or siRNAs may be enhanced by combining them with miRNAs, and this strategy can be developed for cancer therapy. Human malignancies have been linked to several types of lncRNAs e.g., natural antisense transcripts (NATs), lincRNAs, and transcribed ultra-conserved regions (T-UCRs), and. Since many lncRNAs are mostly localized inside the nucleus, but shows their activity at distinct places as that miRNAs do. Specific and novel treatment regimen against cancers can be developed by acquiring the properties of such lncRNAs[228]. Resistance to anticancer medications is a severe problem in cancer treatment, sometimes lead to recurrence and even mortalities. Although the mechanism of chemosensitivity and chemoresistance is intricate, ncRNAs are becoming increasingly recognized as a means to circumvent it[229].
Some miRNAs have dual involvement in modulating the susceptibility of different cancers to different treatments, making them the most investigated ncRNAs that help producing resistance or sensitivity against chemotherapies. MiR-125 is one such example that is linked to treat cellular resistance in a variety of malignancies. By downregulating the levels of dihydrofolate reductase (DHFR) and thymidylate synthase (TS) enzymes make colon cancer resistant against methotrexate and osteosarcoma to Tomudex. The miRNA make breast cancer cells resistant against paclitaxel by downregulating pro-apoptotic Bcl-2 antagonist killer 1 (Bak1). An inverse correlation was found between 5-fluorouracil resistance with the expression levels of miR-125b in hepatocarcinoma [230].

Another group of ncRNAs implicated in treatment resistance in cancer is lncRNAs. Increased sensitivity to imatinib was shown in some cases when HOTAIR was used to inhibit the production of multidrug resistance-associated protein 1 (MRP1). After the blockage of the estrogen receptor (ER) pathway either by lowering the hormones expression or exposure to tamoxifen, the ligand-independent activities of the ER and resistance for tamoxifen in breast cancer cells are also reversed. Consequently, HOTAIR seems therapeutically potential target for treating individuals suffering multiple cancer types and are now resistant to standard chemotherapeutics [231].
Recently, it is reported that circRNAs actively take part in in chemoresistance. HSA_CIRC_0001258 activates chemoresistance in osteosarcoma by increasing GSTM2 expression via miR-744-3p activities. The release of miR-646 that downregulate the levels of CDK6, knockdown of HSA_CIRC_0081143 raised the sensitivity for cisplatin in gastric cancer cells both *in vivo* and *in vitro*. Another study, where miR-1183/PDPK1 pathway was targeted in where NSCLC, HSA_CIRC_0004015 found to modulate the tyrosine kinase inhibitor (TKI) resistance [232-233].

Tumor cells have been reported to resist for chemotherapies or even the sensitivity for radiotherapies are posed by the certain ncRNAs. Tumor cells turned immortal when exposed to certain radiations primarily due to their impaired DNA damage repair mechanism and predisposition for more rapid division [234]. Despite this, literature

also reported that radiation may increase tumor recurrence and metastasis by triggering epithelial-mesenchymal transition (EMT) and/or by enhancing the cancer stem cells (CSCs) population [235].

Radiotherapy response in different cancers, particularly NSCLC, tumors of the head and neck, SCC, and squamous cervical carcinoma, showed a strong correlation in expression patterns of a subset of miRNAs [236]. This suggests that these miRNAs may serve as biomarkers for radioresistance. Some miRNAs may be valuable prognostic biomarkers of radioresistance in breast cancer e.g. miR-139-5p target those genes which are highly authenticated in the prognosis of radiotherapy exposed cancer patients [237]. Additionally, there is a distinctive pattern of change in the expression of certain miRNAs post irradiation. To avoid unnecessary delays in switching to a different treatment regimen and to find precise reaction to irradiation, miRNAs can also be utilized as biomarkers [238]. Certain circulating miRNAs such as metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), HOTAIR, GAS5 and H19, and lncRNAs could be useful in examining radiotherapy resistance in cancer patients [239].

By regulating apoptosis, DNA damage repair, and the epigenetic reprogramming of target genes, ncRNAs can alter radiosensitivity. Huge number of miRNAs (miR-21, -125b, -148, -181a, and -196a) facilitate the down regulation of apoptosis associated genes like casp-3 (caspase-3), ICAM-2 (intercellular adhesion molecule-2), PRKCD (protein kinase C delta), ANXA1 (annexin A1), or DNMT3B (DNA methyltransferase 3b)in multiple cancers [240]. Some lncRNAs (lincRNA-p21, LOC285194, ANRIL, AK294004, lincRNA-ROR, & MALAT1), modulates the expression of apoptosis associated genes by direct binding with respective protein units or either the endogenous RNAs (ceRNAs) competitors [241-242]. Some lncRNAs may regulate DNA damage response and hence influence radioresistance. By modulating communication between Ku80 and DNA-dependent protein kinase catalytic subunit (DNA-PKcs), lncRNA LINP1 enables repair of DNA double-strand breaks. Inhibition of LINP1 increases the sensitivity of breast cancer cells for radiotherapies [243].

Precision medicine cannot function without therapeutic targeting strategies. Multiple preliminary research exploring anticancer techniques for targeting oncogenic ncRNAs have been started already. The antisense oligonucleotides (ASOs), locked nucleic acids (LNAs), and morpholino oligonucleotides (MOs) are the three approaches presented for therapeutic targets [244]. Specific complementarity between ASOs and their target regions facilitates RNase H-mediated demolition of the RNA. Specific complementarity and RNase H-mediated demolition of the target sequence are provided by LNAs, which are likewise single strand oligonucleotides comprising a stretch of DNA bordered by LNA nucleotides [245]. In contrast to ASOs and LNAs, MOs are 25 nt non-ionic analogs of DNA molecules attached to their target RNAs to speed up their demolition [246]. Oncogenic ncRNAs in cancer have been the focus of these approaches. For instance, when miR-10b ASOs were used in conjunction with modest doses of doxorubicin to treat breast cancer in animal models, tumor size reduction was much greater than when using doxorubicin alone. Furthermore, the researchers tested miR-10b LNAs and discovered that they increased the susceptibility of breast cancer to doxorubicin in animal models without negatively affecting normal tissue, which hints at a low toxicity profile for the administration of this LNA nanoparticle [247].

Although tiny ncRNAs have showed promise as therapeutic medicines in vitro, their poor bioavailability in vivo is a significant obstacle. This highlights the critical need for research into new methods of medicine delivery. Different type of carriers for tiny ncRNAs or related strategies are designed and studied extensively to address the common issues of shorter half-life of the molecules, off-target effects, and least transfection during the targeted delivery or release of RNAs [174].

The first category of nanoparticles (NPs)-based carriers includes polymeric SANs, LNPs made of lipids, inorganic NPs, and self-assembled oligonucleotides. Huang and colleagues successfully prepared calcium-phosphate-lipid NPs, and found superior to conventional carrier techniques for transporting siRNA into HCC cells. These lipid NPs carrying VEGF siRNAs showed a substantial antiangiogenic effect in HCC in vivo model by downregulating VEGF expression specifically in the tumour microenvironment [248]. Methods for improving the specificity and stability of small ncRNAs that precisely hit their target genes in tumors is by making conjugates of siRNAs to respective carrier molecules, modification of siRNAs using molecules of lipids and PEG molecules, and using self-assembled lipid NPs (SAmiRNAs). For the treatment of liver cancer, two research groups jointly developed GalN-conjugated siRNAs by chemically attaching specific siRNAs with (2-3)N-acetylgalactosamine (GalNAc) [249]. The asialoglycoprotein receptor (ASGPR) is particularly GalNAc-binding and is extensively expressed and surface-localized on membranes of hepatocytes. When ASGPR interacts with GalNAc ligand promotes clathrin-mediated endocytosis. Majority of GalNac-siRNAs molecules arriving at HCC lesions and forms RISC complexes to degrade target mRNAs [250].

Unfortunately, drug resistance is becoming a big problem in the medical science. A more standardized treatment for people with ER-positive breast cancer is endocrine therapy using tamoxifen and aromatase inhibitors. In endocrine therapy, resistance was observed created by enhanced growth factor signaling and altered expression levels with different functions of ERs. The significance of ncRNA in this endocrine therapy resistance has not yet been figured out.

The miRNAs and lncRNAs appeared as essential molecules in our understanding of the genetic and biochemical procedures that underlie breast cancer. A particular specified tumor treatment therapies are the only option regimen for patients with endocrine-resistant breast cancer can be expanded by the discovery of novel ncRNAs that may serve as therapeutic targets in these cancers[251].

The ncRNAs gained much interest as potential targets for new treatment methods. Due to the complexity of creating miRNA-based medicines, selected miRNA therapeutics has been found suitable for clinical trials. Avoiding harmful and off-target effects, along with stable and effective delivery methods, a significant level of determining optimal miRNA targets or group of targets to hit for a particular clinical state. Involvement of additional ncRNAs, such as lncRNAs and circRNAs, as possible druggable molecules in cancer has emerged recently [252].

Emerging technologies involving ncRNAs for cancer treatment

Genome engineering tools

Genome-engineering strategies like as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system are under use to down regulate

expression levels of lncRNAs (Figure 6). ZFNs allow for site specific or targeted alteration in the genome. ZFNs were used to insert RNA destabilizing elements (RDE) into the MALAT1 locus in tumor cells. In stable knockout clones of cells, its expression was almost completely reduced which suggests an entire loss of function model [253]. Alternative splicing was not affected by the inactivation. RDEs are incorporated into genomic loci like poly-A signals, where they function as termination elements and silence downstream sequences. RDE can also be used to silence pseudogenes [254].

Long ncRNA genes are resistant to the kinds of point mutations that can cause nonsense-mediated RNA decay in protein-coding genes. This suggests that they may operate via broad structures that necessitate a macro level response. Using the CRISPR/Cas9 system in conjunction with matched sgRNAs (single guide RNAs) makes this a reality. Steric silencing of lncRNA gene transcription by CRISPR interference (CRISPRi) is possible. A catalytically dead Cas9 (dCas9) protein, which lacks endonucleolytic function, and a guide RNA (gRNA) are the two main components of CRISPRi. The sequences on either template or non-template strands of DNA duplex in the promoter, or the -35 regions, are what gRNAs aim to silence. In eukaryotic cells, this is superior than direct inhibition of RNA polymerase. In order to enhance the epigenetic silencing of the genes, dCas9 can be merged to the Kruppel-associated box (KRAB), which is a repressor domain of that protein [255-256].

Nanobodies

Cancer-related RNA-protein networks are vulnerable to disruption by nanobodies (Figure 6). High-affinity and selective camelid heavy-chain antibodies (HcAbs) have them as a changeable region. Moreover, they are non-immunogenic proteins that have got structural stability and soluble antigen binding domains showing higher sequence similarities with human Immunoglobulin heavy chain V gene (VH). These aptamers dissociate cancer-specific RNA-RBP cellular networking by interfering with protein/nucleic acid or protein to protein interactions [257].

Synthetic nanobodies that can bind nucleic acids were created and a gene library was created. A nanobody cAbBC1rib3 specifically binds to the stRNA BC1 at nanomolar quantities. The nanobody can also bind to stRNAs that are unrelated to one other. However, it did not interact with negatively charged proteins or single- or double-stranded DNA/RNA. Nanobody incorporation or absence had no effect on thermal unfolding and refolding processes. Therefore, stRNA epitope recognition nanobodies can be developed. However, in subsequent efforts, their specificity should be enhanced [258].

Aptamers

Single stranded nucleic acids (ssDNA or RNA) having a higher affinity and specificity to their targets are called aptamers (Figure 6). Structural mimics to that of antibodies but composed of nucleic acid led to penetrate and transport tissues more effectively while causing less immune responses. They function primarily by identifying the secondary structure of lncRNAs and blocking the interaction between RNA and proteins (Figure 6). Aptamers are recognized and grown *in vitro* using the SELEX method, which allows for the incorporation of altered nucleic acids to generate nuclease-resistant RNA aptamers [259].

RNA decoy

RNA decoys can be produced by mimicking lncRNAs, which are linked to different proteins for sequestration. As a strategic tool, they might obstruct the formation of lncRNA-RBP complexes. An anti-HIV spoof that specifically targeting Tat protein has been successfully been developed by complexing Tat with TAR-RNA hairpin. While the real TAR-RNA residing inside nucleolus, this spoof is found in the nProteins may be bound and sequestered by RNA decoys, which would replicate the actions of lncRNAs. They could be employed to stop the lncRNA-RBP complexes from forming functionally. Researchers developed a spoof that imitates the Tat protein of the virus to fight HIV. The (TAR) RNA hairpin that the Tat protein carries is attached to it. This ruse is located in the nucleolus, as opposed to the nucleus, which contains the actual TAR RNA [260].

lncRNA regulatory elements or expression patterns

In tumor cells that have over expression of H19 levels, the diphtheria toxin-A gene was inserted into the H19 promoter region of the plasmid BC-819 (DTA-H19). Tumor size decrease was documented after intratumoral injection in a variety of cancer types [261].

Chemical modifications

The existence of numerous intracellular ribonucleases, RNAs seems somewhat unstable molecules and have been known for bearing poor pharmacological properties. RNA is hydrophilic molecule bearing negative charge on the overall molecule and have been shown catalytic activity of ribose sugar primarily because of 2′-OH group. Therefore, RNA-based therapies requires chemical modification to improve their stability without diminishing their biological efficacy (Figure 6). Improving the pharmacological actions of siRNAs and ASOs can be achieved by replacing the ribose's (2′-OH) group with a variety of substituent, including 2′-methoxy (2′-OMe), 2′-methoxyethoxy (2′-MOE), 2′-4′-O-methylene Bridge, locked nucleic acid (LNA), and 2′-fluoro (2′-F) [262].

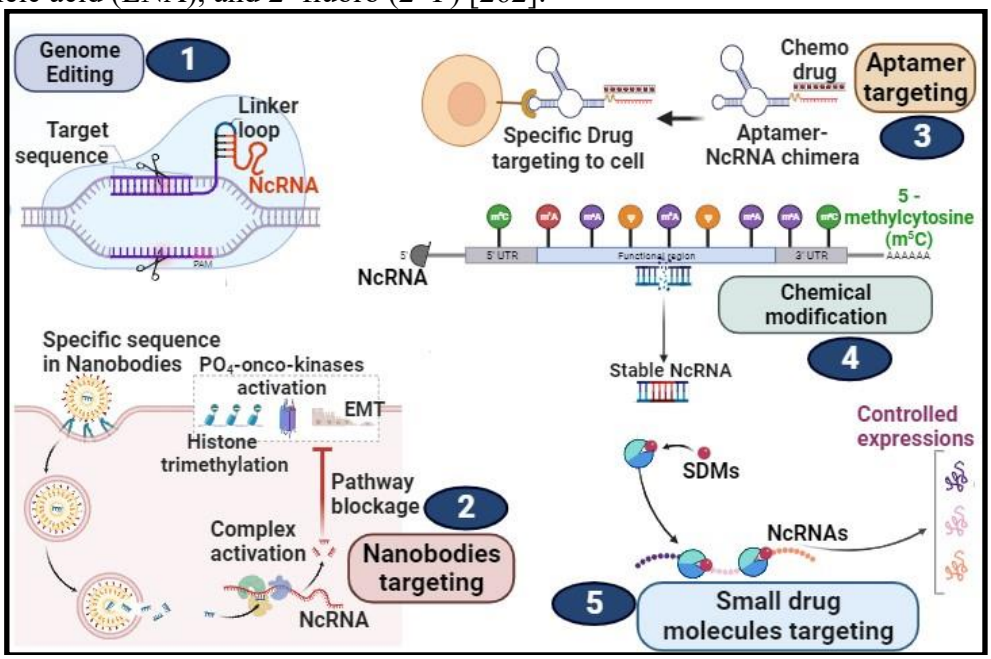


Figure 6. Various emerging techniques associated with ncRNAs for tumor treatment. (1) genome engineering tools, (2) nanobodies targeting, (3) aptamer targeting, (4) chemical modifications, (5) targeting by small drug molecules.

Small molecules

The connections between lncRNAs and RBPs can be disrupted by small molecules attaching to the RNAs by manipulating the structural orientation of the proteins or even blockage of the binding sites of the proteins or the RNAs (Figure 6). In depth interactive knowledge of lncRNA with proteins molecules is essential specifically physical association of both which can be identified through latest approaches[263]. *In vivo* RNA to proteins interactions using cross linking methods such as capture hybridization analysis of RNA targets (CHART) and RNA affinity purification (RAP) were used to link gene sequences for functional intergenic repeat element (FIRRE), XIST, MALAT1, and NEAT1 to their corresponding protein binding partners [264]. Quantification of lncRNA-protein interaction has been made possible by AlphaScreen technology introduced by PerkinElmer (Waltham, MA) found a solid relationship between HOTAIR and BDNF-AS, enhancer of zeste homolog 2 (EZH2). Moreover, the relationship was also confirmed for ellipticine which stimulates BDNF transcription [263]. It is confirmed that majority of enzymes offer different locations for the attachment of lncRNAs. The targeting of interactions of lncRNA with proteins may help modulate enzymatic activities involved in chromatin structural modification present in the non-catalytic domains [265].

Therapeutic targeting by systemic delivery mechanisms

Among lipid-based vectors, liposomes are useful for transporting nucleic acids. In an *in vivo* study, 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) nanoliposome that holds siRNA, the elevated lncRNA ceruloplasmin (NRCP) was down regulated in ovarian cancer, where a significant suppression and enhanced sensitivity to cisplatin in the tumors has been seen [262]. Surfaces of non-immunogenic or non-toxic polymeric vectors are modified to enhance structural stability, specificity for its particular tissue type and cellular intake. Dendrimers are specialized transporters for siRNAs. Moreover, using a modified poly amidoamine (PAMAM) delivery technique, an aptamer was designed to target nucleolin ligand on targeted cancerous cells, while shRNA plasmid was designed to knock-down B-cell lymphoma-extra large protein (Bcl-xL) in lung cancer. Transfection efficiency was dramatically increased in the modified vectors compared with non targeted vectors by either the covalent linkage or non-covalent aptamer attachment [266]. RNA can be delivered to specific cells or tissues using genetically modified viruses viz. adeno viruses, adeno associated viruses (AAVs), retro viruses, and herpes simplex viruses. Effective delivery of shRNAs *in vivo* and *ex vivo* has been demonstrated via viral vectors. They effectively and precisely inhibit the RNAs they are targeting. The proliferation and spreading of endometrial cancer cells were inhibited both *in vitro* and *in vivo* when lentiviral frame plasmids were used as vectors against HOTAIR [267-268].

Conclusion

The unknown history of DD, led to the development of potential therapeutic options by focusing primarily on small natural molecules or either their analogs that directly target proteins of interest either posing inhibitory or antagonistic effects. Early this century, human genome project was almost completely revealed with specific amount of genome transcribed strengthening our understanding that approximately 1% of the human genome codes for proteins. The “omics” (proteomics, genomics, metabolomics, etc.) era boost the increase in biopharmaceutical drugs safer for clinical application. Recently identified lncRNAs have crucial roles in biology and medicine, are involved in the extensive RNA-based regulatory networks that impact every facet of intracellular protein creation. These lncRNAs have multiple effects on cells due to

their intricate networks of interactions with proteins, other nucleic acids, and biological substances. The majority of cancer cells have compromised ncRNA functions. Furthermore, deregulation of these ncRNAs function and their connections to different biomolecules in cellular pathways have been connected to a number of cancer forms and other metabolic disorders and hence identifying significant novel targets for therapeutic intervention. Moreover, mutations that alter the sequence and downstream targets of a particular ncRNA are just one type of mutation that might cause disturbances in ncRNA networks. Advancements in the structural elucidation of tiny pharmacological molecules and the manufacturing methods of nucleic acid-based therapeutics (NBTs) will be a powerful tool for investigating specific regulatory ncRNAs and developing effective, efficient, and individually tailored treatments for human disease.

Summary

Drug discovery (DD) has an unknown history since the origin of mankind with the process of trial and error method. In traditional therapeutic systems, the focus always been paid to explore certain small natural molecules or either their analogs that may directly targeting proteins of interest either posing inhibitory or antagonistic effects. Early this century, it came to knowledge that about 75-80% of the human genome sequence has been transcribed, out of which only 1% of genome undergoes translation for proteins formation. In addition, the onset of the "omics" (proteomics, genomics, metabolomics, etc.) era, has boosted the increase in biopharmaceutical drugs approved by FDA/EMEA for clinical uses. It is evident now that these novel lncRNAs and their transcripts performs crucial cellular functions, and hence constitute diverse RNA-based regulatory networks to affect the expression levels of proteins at translational levels. Various ncRNAs make complex networking with certain other biomolecules such as nucleic acids and, proteins to pose multidimensional impacts on cell physiology. The underlying mechanism how ncRNAs work are continuously changing in different tumor cells. Moreover, how these ncRNAs functional dysregulation in cancer cell communicative and molecular pathways lead to multiple types of cancers by unveiling novel and innovative molecular targets. Moreover, the interruption in the ncRNA networking with various biomolecules is not under the threat of mutations for a complete gain or loss of functions, or either alteration in the nucleotides sequences, and downstream targets by certain ncRNAs is also important in this context. The innovative trends in the structural elucidation of small drug molecules combined with NBTs possibly be a futuristic tool to examine individual regulatory ncRNAs, to design highly precise, efficient, and personalized medicine for clinical use.

Keywords: ncRNAs, lncRNAs, Tumors, drug discovery, NBTs, personalized medicine.

Acknowledgements

We acknowledge this work to Professor Julia Li Zhong, for critically analyze and useful suggestion for compiling this work.

Author Contribution statement

MFN, KX, SS, AJ, AA, and ZM wrote the different segments of this draft. MR and MYW drawn the figures. MFN, KX, ZM, SHM, and MR critically revised the draft and language editing during post check. MFN, KX and ZM conceptualized this study.

Statement of no conflict of interests

The authors declared no any conflict of interest for the submission and publication of this draft as book chapter.

Scientific Ethics Declaration

It is hereby confirmed by the authors, we take the responsiblity for the scientific, moral, and if any legal aspects of this chapter, which is being published in Current Studies in Health and Life Sciences, 2023.

References

1. Geisler, S. and J. Coller, *RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts.* Nature reviews Molecular cell biology, 2013. **14**(11): p. 699-712.
2. Kogo, R., et al., *Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers.* Cancer research, 2011. **71**(20): p. 6320-6326.
3. Schmitt, A.M. and H.Y. Chang, *Long noncoding RNAs in cancer pathways.* Cancer cell, 2016. **29**(4): p. 452-463.
4. Charles Richard, J.L. and P.J.A. Eichhorn, *Platforms for investigating LncRNA functions.* SLAS TECHNOLOGY: Translating Life Sciences Innovation, 2018. **23**(6): p. 493-506.
5. Kornienko, A.E., et al., *Gene regulation by the act of long non-coding RNA transcription.* BMC biology, 2013. **11**: p. 1-14.
6. Wang, K.C. and H.Y. Chang, *Molecular mechanisms of long noncoding RNAs.* Molecular cell, 2011. **43**(6): p. 904-914.
7. Lau, E., *Zooming in on lncRNA functions.* Nature reviews genetics, 2014. **15**(9): p. 574-575.
8. Peng, W.-X., P. Koirala, and Y.-Y. Mo, *LncRNA-mediated regulation of cell signaling in cancer.* Oncogene, 2017. **36**(41): p. 5661-5667.
9. Hajjari, M. and A. Salavaty, *HOTAIR: an oncogenic long non-coding RNA in different cancers.* Cancer biology & medicine, 2015. **12**(1): p. 1.
10. Gutschner, T., M. Hämmerle, and S. Diederichs, *MALAT1—a paradigm for long noncoding RNA function in cancer.* Journal of molecular medicine, 2013. **91**: p. 791-801.
11. Pickard, M.R. and G.T. Williams, *Molecular and cellular mechanisms of action of tumour suppressor GAS5 LncRNA.* Genes, 2015. **6**(3): p. 484-499.
12. Lee, G.L., A. Dobi, and S. Srivastava, *Diagnostic performance of the PCA3 urine test.* Nature Reviews Urology, 2011. **8**(3): p. 123-124.
13. Matouk, I., et al., *The increasing complexity of the oncofetal h19 gene locus: functional dissection and therapeutic intervention.* International journal of molecular sciences, 2013. **14**(2): p. 4298-4316.
14. Statello, L., et al., *Gene regulation by long non-coding RNAs and its biological functions.* Nature reviews Molecular cell biology, 2021. **22**(2): p. 96-118.
15. Sonnenschein, C. and A.M. Soto, *Carcinogenesis explained within the context of a theory of organisms.* Progress in biophysics and molecular biology, 2016. **122**(1): p. 70-76.
16. Harvey, A.L., R. Edrada-Ebel, and R.J. Quinn, *The re-emergence of natural products for drug discovery in the genomics era.* Nature reviews drug discovery, 2015. **14**(2): p. 111-129.

17. Newman, D.J. and G.M. Cragg, *Natural products as sources of new drugs from 1981 to 2014.* Journal of natural products, 2016. **79**(3): p. 629-661.
18. Waltenberger, B., et al., *Natural products to counteract the epidemic of cardiovascular and metabolic disorders.* Molecules, 2016. **21**(6): p. 807.
19. Tintore, M., A. Vidal-Jordana, and J. Sastre-Garriga, *Treatment of multiple sclerosis—success from bench to bedside.* Nature Reviews Neurology, 2019. **15**(1): p. 53-58.
20. Newman, D.J., G.M. Cragg, and K.M. Snader, *Natural products as sources of new drugs over the period 1981− 2002.* Journal of natural products, 2003. **66**(7): p. 1022-1037.
21. Li-Weber, M., *New therapeutic aspects of flavones: the anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin.* Cancer treatment reviews, 2009. **35**(1): p. 57-68.
22. Fabricant, D.S. and N.R. Farnsworth, *The value of plants used in traditional medicine for drug discovery.* Environmental health perspectives, 2001. **109**(suppl 1): p. 69-75.
23. Alves, R.R. and I.M. Rosa, *Biodiversity, traditional medicine and public health: where do they meet?* Journal of ethnobiology and ethnomedicine, 2007. **3**: p. 1-9.
24. Kapranov, P., et al., *RNA maps reveal new RNA classes and a possible function for pervasive transcription.* Science, 2007. **316**(5830): p. 1484-1488.
25. Mercer, T.R., et al., *Specific expression of long noncoding RNAs in the mouse brain.* Proceedings of the National Academy of Sciences, 2008. **105**(2): p. 716-721.
26. Pauli, A., et al., *Systematic identification of long noncoding RNAs expressed during zebrafish embryogenesis.* Genome research, 2012. **22**(3): p. 577-591.
27. Ponting, C.P., P.L. Oliver, and W. Reik, *Evolution and functions of long noncoding RNAs.* Cell, 2009. **136**(4): p. 629-641.
28. Managadze, D., et al., *Negative correlation between expression level and evolutionary rate of long intergenic noncoding RNAs.* Genome biology and evolution, 2011. **3**: p. 1390-1404.
29. Salmena, L., et al., *A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?* cell, 2011. **146**(3): p. 353-358.
30. Chi, Y., et al., *Long non-coding RNA in the pathogenesis of cancers.* Cells, 2019. **8**(9): p. 1015.
31. Novikova, I.V., S.P. Hennelly, and K.Y. Sanbonmatsu, *Tackling structures of long noncoding RNAs.* International journal of molecular sciences, 2013. **14**(12): p. 23672-23684.
32. Ariel, F., et al., *Noncoding transcription by alternative RNA polymerases dynamically regulates an auxin-driven chromatin loop.* Molecular cell, 2014. **55**(3): p. 383-396.
33. Rutenberg-Schoenberg, M., A.N. Sexton, and M.D. Simon, *The properties of long noncoding RNAs that regulate chromatin.* Annual review of genomics and human genetics, 2016. **17**: p. 69-94.
34. Wierzbicki, A.T., T. Blevins, and S. Swiezewski, *Long noncoding RNAs in plants.* Annual Review of Plant Biology, 2021. **72**: p. 245-271.
35. M Kumar, M. and R. Goyal, *LncRNA as a therapeutic target for angiogenesis.* Current topics in medicinal chemistry, 2017. **17**(15): p. 1750-1757.
36. Amaral, P.P., et al., *lncRNAdb: a reference database for long noncoding RNAs.* Nucleic acids research, 2011. **39**(suppl_1): p. D146-D151.

37. Dinger, M.E., et al., *NRED: a database of long noncoding RNA expression.* Nucleic acids research, 2009. **37**(suppl_1): p. D122-D126.
38. Spizzo, R., et al., *Long non-coding RNAs and cancer: a new frontier of translational research?* Oncogene, 2012. **31**(43): p. 4577-4587.
39. Congrains, A., et al., *Genetic variants at the 9p21 locus contribute to atherosclerosis through modulation of ANRIL and CDKN2A/B.* Atherosclerosis, 2012. **220**(2): p. 449-455.
40. Johnson, R., *Long non-coding RNAs in Huntington's disease neurodegeneration.* Neurobiology of disease, 2012. **46**(2): p. 245-254.
41. van Poppel, H., et al., *The relationship between Prostate CAncer gene 3 (PCA3) and prostate cancer significance.* BJU international, 2012. **109**(3): p. 360-366.
42. Yang, Z., et al., *Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation.* Annals of surgical oncology, 2011. **18**: p. 1243-1250.
43. Zhang, Z., et al., *Evaluation of novel gene UCA1 as a tumor biomarker for the detection of bladder cancer.* Zhonghua yi xue za zhi, 2012. **92**(6): p. 384-387.
44. Mohammad, F., T. Mondal, and C. Kanduri, *Epigenetics of imprinted long non-coding RNAs.* Epigenetics, 2009. **4**(5): p. 277-286.
45. Yang, L., et al., *ncRNA-and Pc2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs.* Cell, 2011. **147**(4): p. 773-788.
46. Rinn, J.L., et al., *Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs.* cell, 2007. **129**(7): p. 1311-1323.
47. Tsai, M.-C., et al., *Long noncoding RNA as modular scaffold of histone modification complexes.* Science, 2010. **329**(5992): p. 689-693.
48. Huarte, M., et al., *A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response.* Cell, 2010. **142**(3): p. 409-419.
49. Feng, J., et al., *The Evf-2 noncoding RNA is transcribed from the Dlx-5/6 ultraconserved region and functions as a Dlx-2 transcriptional coactivator.* Genes & development, 2006. **20**(11): p. 1470-1484.
50. Clemson, C.M., et al., *An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles.* Molecular cell, 2009. **33**(6): p. 717-726.
51. Wahlestedt, C., *Targeting long non-coding RNA to therapeutically upregulate gene expression.* Nature reviews Drug discovery, 2013. **12**(6): p. 433-446.
52. Cesana, M., et al., *A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA.* Cell, 2011. **147**(2): p. 358-369.
53. Kim, T.-K., et al., *Widespread transcription at neuronal activity-regulated enhancers.* Nature, 2010. **465**(7295): p. 182-187.
54. Sahu, B., et al., *Dual role of FoxA1 in androgen receptor binding to chromatin, androgen signalling and prostate cancer.* The EMBO journal, 2011. **30**(19): p. 3962-3976.
55. Wang, D., et al., *Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA.* Nature, 2011. **474**(7351): p. 390-394.
56. Chen, Y.G., A.T. Satpathy, and H.Y. Chang, *Gene regulation in the immune system by long noncoding RNAs.* Nature immunology, 2017. **18**(9): p. 962-972.
57. de Goede, O., et al., *Long non-coding RNA gene regulation and trait associations across human tissues.* bioRxiv, 2019: p. 793091.

58. Romero-Barrios, N., et al., *Splicing regulation by long noncoding RNAs.* Nucleic acids research, 2018. **46**(5): p. 2169-2184.
59. Chen, R., et al., *Roles of lncRNAs and circRNAs in regulating skeletal muscle development.* Acta Physiologica, 2020. **228**(2): p. e13356.
60. Kretz, M., et al., *Control of somatic tissue differentiation by the long non-coding RNA TINCR.* Nature, 2013. **493**(7431): p. 231-235.
61. Augoff, K., et al., *miR-31 and its host gene lncRNA LOC554202 are regulated by promoter hypermethylation in triple-negative breast cancer.* Molecular cancer, 2012. **11**(1): p. 1-13.
62. Nojima, T. and N.J. Proudfoot, *Mechanisms of lncRNA biogenesis as revealed by nascent transcriptomics.* Nature Reviews Molecular Cell Biology, 2022. **23**(6): p. 389-406.
63. Yang, L., et al., *lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs.* Nature, 2013. **500**(7464): p. 598-602.
64. Xing, C., et al., *Role of lncRNA LUCAT1 in cancer.* Biomedicine & Pharmacotherapy, 2021. **134**: p. 111158.
65. Fire, A., et al., *Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans.* nature, 1998. **391**(6669): p. 806-811.
66. Moazed, D., *Small RNAs in transcriptional gene silencing and genome defence.* Nature, 2009. **457**(7228): p. 413-420.
67. Thomson, T. and H. Lin, *The biogenesis and function of PIWI proteins and piRNAs: progress and prospect.* Annual Review of Cell and Developmental, 2009. **25**: p. 355-376.
68. Fejes-Toth, K., et al., *Affymetrix ENCODE Transcriptome Project.* Cold Spring Harbor Laboratory ENCODE Transcriptome Project. Post-transcriptional processing generates a diversity of, 2009. **5**: p. 1028-32.
69. Carone, D.M., et al., *A new class of retroviral and satellite encoded small RNAs emanates from mammalian centromeres.* Chromosoma, 2009. **118**: p. 113-125.
70. Gary, D.J., N. Puri, and Y.-Y. Won, *Polymer-based siRNA delivery: perspectives on the fundamental and phenomenological distinctions from polymer-based DNA delivery.* Journal of Controlled Release, 2007. **121**(1-2): p. 64-73.
71. Pushparaj, P.N. and A.J. Melendez, *Short interfering RNA (siRNA) as a novel therapeutic.* Clinical and experimental pharmacology & physiology, 2006. **33**(5-6): p. 504-510.
72. Ji, A., et al., *Functional gene silencing mediated by chitosan/siRNA nanocomplexes.* Nanotechnology, 2009. **20**(40): p. 405103.
73. Urakami, T. and N. Oku, *Current status of siRNA delivery technology and siRNA drug development.* The Open Drug Delivery Journal, 2007. **1**(1).
74. Schiffelers, R.M., M.C. Woodle, and P. Scaria, *Pharmaceutical prospects for RNA interference.* Pharmaceutical research, 2004. **21**: p. 1-7.
75. Guo, J., et al., *Therapeutic targeting in the silent era: advances in non-viral siRNA delivery.* Molecular Biosystems, 2010. **6**(7): p. 1143-1161.
76. Ma, C., Y. Liu, and L. He, *MicroRNAs-powerful repression comes from small RNAs.* Science in China Series C: Life Sciences, 2009. **52**: p. 323-330.
77. Deiters, A., *Small molecule modifiers of the microRNA and RNA interference pathway.* The AAPS journal, 2010. **12**: p. 51-60.
78. Akhtar, S. and I.F. Benter, *Nonviral delivery of synthetic siRNAs in vivo.* The Journal of clinical investigation, 2007. **117**(12): p. 3623-3632.

79. Guo, P., et al., *Engineering RNA for targeted siRNA delivery and medical application.* Advanced drug delivery reviews, 2010. **62**(6): p. 650-666.
80. Li, X., et al., *A microRNA imparts robustness against environmental fluctuation during development.* Cell, 2009. **137**(2): p. 273-282.
81. van Rooij, E., A.L. Purcell, and A.A. Levin, *Developing microRNA therapeutics.* Circulation research, 2012. **110**(3): p. 496-507.
82. Esquela-Kerscher, A., et al., *The let-7 microRNA reduces tumor growth in mouse models of lung cancer.* Cell cycle, 2008. **7**(6): p. 759-764.
83. Trang, P., et al., *Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice.* Molecular Therapy, 2011. **19**(6): p. 1116-1122.
84. Taft, R.J., et al., *Non-coding RNAs: regulators of disease.* The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 2010. **220**(2): p. 126-139.
85. Medina, P.P. and F.J. Slack, *microRNAs and cancer: an overview.* Cell cycle, 2008. **7**(16): p. 2485-2492.
86. Asangani, I.A., et al., *MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer.* Oncogene, 2008. **27**(15): p. 2128-2136.
87. Yu, W., et al., *Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA.* Nature, 2008. **451**(7175): p. 202-206.
88. Pasmant, E., et al., *Characterization of a germ-line deletion, including the entire INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF.* Cancer research, 2007. **67**(8): p. 3963-3969.
89. Yap, K.L., et al., *Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a.* Molecular cell, 2010. **38**(5): p. 662-674.
90. Gupta, R.A., et al., *Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis.* nature, 2010. **464**(7291): p. 1071-1076.
91. Qureshi, I.A., J.S. Mattick, and M.F. Mehler, *Long non-coding RNAs in nervous system function and disease.* Brain research, 2010. **1338**: p. 20-35.
92. Faghihi, M.A., et al., *Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of β-secretase.* Nature medicine, 2008. **14**(7): p. 723-730.
93. Chandra Gupta, S. and Y. Nandan Tripathi, *Potential of long non-coding RNAs in cancer patients: from biomarkers to therapeutic targets.* International journal of cancer, 2017. **140**(9): p. 1955-1967.
94. Jemal, A., et al., *Global cancer statistics.* CA: a cancer journal for clinicians, 2011. **61**(2): p. 69-90.
95. Heidenreich, A., et al., *EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and local treatment with curative intent—update 2013.* European urology, 2014. **65**(1): p. 124-137.
96. Burzio, V.A., et al., *Expression of a family of noncoding mitochondrial RNAs distinguishes normal from cancer cells.* Proceedings of the National Academy of Sciences, 2009. **106**(23): p. 9430-9434.
97. Villegas, J., et al., *Expression of a novel non-coding mitochondrial RNA in human proliferating cells.* Nucleic acids research, 2007. **35**(21): p. 7336-7347.
98. Villota, C., et al., *Expression of mitochondrial non-coding RNAs (ncRNAs) is modulated by high risk human papillomavirus (HPV) oncogenes.* Journal of Biological Chemistry, 2012. **287**(25): p. 21303-21315.

99. Rivas, A., et al., *Determination of the differential expression of mitochondrial long non-coding RNAs as a noninvasive diagnosis of bladder cancer.* BMC urology, 2012. **12**(1): p. 1-8.
100. Berrondo, C., et al., *Expression of the long non-coding RNA HOTAIR correlates with disease progression in bladder cancer and is contained in bladder cancer patient urinary exosomes.* PloS one, 2016. **11**(1): p. e0147236.
101. Cao, L., et al., *Microarray profiling of bone marrow long non-coding RNA expression in Chinese pediatric acute myeloid leukemia patients.* Oncology Reports, 2016. **35**(2): p. 757-770.
102. Zhuang, W., et al., *Upregulation of lncRNA MEG3 promotes osteogenic differentiation of mesenchymal stem cells from multiple myeloma patients by targeting BMP4 transcription.* Stem cells, 2015. **33**(6): p. 1985-1997.
103. Tang, H., et al., *Salivary lncRNA as a potential marker for oral squamous cell carcinoma diagnosis.* Molecular medicine reports, 2013. **7**(3): p. 761-766.
104. Yao, Y., et al., *Knockdown of long non-coding RNA XIST exerts tumor-suppressive functions in human glioblastoma stem cells by up-regulating miR-152.* Cancer letters, 2015. **359**(1): p. 75-86.
105. Salvador, M.A., et al., *The histone deacetylase inhibitor abexinostat induces cancer stem cells differentiation in breast cancer with low Xist expression.* Clinical cancer research, 2013. **19**(23): p. 6520-6531.
106. Ren, C., et al., *Functions and mechanisms of long noncoding RNAs in ovarian cancer.* International Journal of Gynecologic Cancer, 2015. **25**(4).
107. Tantai, J., et al., *Combined identification of long non-coding RNA XIST and HIF1A-AS1 in serum as an effective screening for non-small cell lung cancer.* International journal of clinical and experimental pathology, 2015. **8**(7): p. 7887.
108. Weakley, S.M., et al., *Expression and function of a large non-coding RNA gene XIST in human cancer.* World journal of surgery, 2011. **35**: p. 1751-1756.
109. Geng, Y., et al., *Large intervening non-coding RNA HOTAIR is associated with hepatocellular carcinoma progression.* Journal of International Medical Research, 2011. **39**(6): p. 2119-2128.
110. Ma, M.-z., et al., *Long non-coding RNA HOTAIR, a c-Myc activated driver of malignancy, negatively regulates miRNA-130a in gallbladder cancer.* Molecular cancer, 2014. **13**: p. 1-14.
111. Hao, S. and Z. Shao, *HOTAIR is upregulated in acute myeloid leukemia and that indicates a poor prognosis.* International journal of clinical and experimental pathology, 2015. **8**(6): p. 7223.
112. Ono, H., et al., *Long noncoding RNA HOTAIR is relevant to cellular proliferation, invasiveness, and clinical relapse in small-cell lung cancer.* Cancer medicine, 2014. **3**(3): p. 632-642.
113. Wu, Z.-H., et al., *Long non-coding RNA HOTAIR is a powerful predictor of metastasis and poor prognosis and is associated with epithelial-mesenchymal transition in colon cancer.* Oncology reports, 2014. **32**(1): p. 395-402.
114. Deng, Q., et al., *Prognostic value of long non-coding RNA HOTAIR in various cancers.* PloS one, 2014. **9**(10): p. e110059.
115. Zhang, Z., et al., *Long non-coding RNA chromogenic in situ hybridisation signal pattern correlation with breast tumour pathology.* Journal of clinical pathology, 2016. **69**(1): p. 76-81.
116. Liu, B., et al., *Expression and mechanisms of long non-coding RNA genes MEG3 and ANRIL in gallbladder cancer.* Tumor Biology, 2016. **37**: p. 9875-9886.

117. Ye, Y., et al., *High expression of AFAP1-AS1 is associated with poor survival and short-term recurrence in pancreatic ductal adenocarcinoma.* Journal of translational medicine, 2015. **13**(1): p. 1-11.
118. Shen, Y., et al., *Prognostic and predictive values of long non-coding RNA LINC00472 in breast cancer.* Oncotarget, 2015. **6**(11): p. 8579.
119. Iguchi, T., et al., *A long noncoding RNA, lncRNA-ATB, is involved in the progression and prognosis of colorectal cancer.* Anticancer research, 2015. **35**(3): p. 1385-1388.
120. Jiang, Y.-Z., et al., *Transcriptome analysis of triple-negative breast cancer reveals an integrated mRNA-lncRNA signature with predictive and prognostic value.* Cancer research, 2016. **76**(8): p. 2105-2114.
121. He, C., et al., *Aberrant NEAT 1 expression is associated with clinical outcome in high grade glioma patients.* Apmis, 2016. **124**(3): p. 169-174.
122. Liu, Y.-R., et al., *Comprehensive transcriptome analysis identifies novel molecular subtypes and subtype-specific RNAs of triple-negative breast cancer.* Breast Cancer Research, 2016. **18**: p. 1-10.
123. Zhu, S., et al., *Reduced expression of the long non-coding RNA AI364715 in gastric cancer and its clinical significance.* Tumor Biology, 2015. **36**: p. 8041-8045.
124. Sun, W., et al., *Decreased expression of long noncoding RNA AC096655. 1-002 in gastric cancer and its clinical significance.* Tumor Biology, 2013. **34**: p. 2697-2701.
125. !!! INVALID CITATION !!! .
126. Malik, R., et al., *The lncRNA PCAT29 inhibits oncogenic phenotypes in prostate cancer.* Molecular Cancer Research, 2014. **12**(8): p. 1081-1087.
127. Shen, Z., et al., *Long non-coding RNA profiling in laryngeal squamous cell carcinoma and its clinical significance: potential biomarkers for LSCC.* PLoS One, 2014. **9**(9): p. e108237.
128. Ma, M.z., et al., *Long non-coding RNA-LET is a positive prognostic factor and exhibits tumor-suppressive activity in gallbladder cancer.* Molecular carcinogenesis, 2015. **54**(11): p. 1397-1406.
129. Benetatos, L., et al., *Promoter hypermethylation of the MEG3 (DLK1/MEG3) imprinted gene in multiple myeloma.* Clinical Lymphoma and Myeloma, 2008. **8**(3): p. 171-175.
130. Yuan, C., et al., *Aberrant expression of long noncoding RNA PVT1 and its diagnostic and prognostic significance in patients with gastric cancer.* Neoplasma, 2016. **63**(3): p. 442-449.
131. Shi, W.-h., et al., *Upregulation of the long noncoding RNA PCAT-1 correlates with advanced clinical stage and poor prognosis in esophageal squamous carcinoma.* Tumor Biology, 2015. **36**: p. 2501-2507.
132. Di Leva, G. and C.M. Croce, *miRNA profiling of cancer.* Current opinion in genetics & development, 2013. **23**(1): p. 3-11.
133. Gawronski, K.A. and J. Kim, *Single cell transcriptomics of noncoding RNAs and their cell-specificity.* Wiley Interdisciplinary Reviews: RNA, 2017. **8**(6): p. e1433.
134. Xiang, D., et al., *Superior performance of aptamer in tumor penetration over antibody: implication of aptamer-based theranostics in solid tumors.* Theranostics, 2015. **5**(10): p. 1083.
135. Li, H., et al., *Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects.* Signal transduction and targeted therapy, 2020. **5**(1): p. 1.

136. Yang, J., et al., *CRISPR/Cas9-mediated noncoding RNA editing in human cancers.* RNA biology, 2018. **15**(1): p. 35-43.
137. Zhang, H., et al., *Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer.* Molecular Cancer, 2021. **20**: p. 1-22.
138. Ashrafizadeh, M., et al., *Progress in delivery of siRNA-based therapeutics employing nano-vehicles for treatment of prostate cancer.* Bioengineering, 2020. **7**(3): p. 91.
139. Li, W., et al., *MicroRNA-34a: potent tumor suppressor, cancer stem cell inhibitor, and potential anticancer therapeutic.* Frontiers in cell and developmental biology, 2021. **9**: p. 640587.
140. Setten, R.L., J.J. Rossi, and S.-p. Han, *The current state and future directions of RNAi-based therapeutics.* Nature reviews Drug discovery, 2019. **18**(6): p. 421-446.
141. Wang, D., P.W. Tai, and G. Gao, *Adeno-associated virus vector as a platform for gene therapy delivery.* Nature reviews Drug discovery, 2019. **18**(5): p. 358-378.
142. Chen, Q., et al., *Exosomal non-coding RNAs-mediated crosstalk in the tumor microenvironment.* Frontiers in cell and developmental biology, 2021. **9**: p. 646864.
143. Li, W., et al., *Exosomal non-coding RNAs: Emerging roles in bilateral communication between cancer cells and macrophages.* Molecular Therapy, 2022. **30**(3): p. 1036-1053.
144. Uppaluri, K.R., et al., *Unlocking the potential of non-coding RNAs in cancer research and therapy.* Translational Oncology, 2023. **35**: p. 101730.
145. MacFarlane, L.-A. and P. R Murphy, *MicroRNA: biogenesis, function and role in cancer.* Current genomics, 2010. **11**(7): p. 537-561.
146. Fire, A.Z., *Gene silencing by double-stranded RNA (Nobel lecture).* Angewandte Chemie International Edition, 2007. **46**(37): p. 6966-6984.
147. Bora, R.S., et al., *RNA interference therapeutics for cancer: challenges and opportunities.* Molecular medicine reports, 2012. **6**(1): p. 9-15.
148. Costa, F.F., *Non-coding RNAs: lost in translation?* Gene, 2007. **386**(1-2): p. 1-10.
149. Rao, D.D., et al., *siRNA vs. shRNA: similarities and differences.* Advanced drug delivery reviews, 2009. **61**(9): p. 746-759.
150. Bowman, K.R., J.H. Kim, and C.S. Lim, *Narrowing the field: cancer-specific promoters for mitochondrially-targeted p53-BH3 fusion gene therapy in ovarian cancer.* Journal of Ovarian Research, 2019. **12**(1): p. 1-12.
151. Rama Ballesteros, A.R., et al., *Tissue Specific Promoters in Colorectal Cancer.* 2015.
152. Liu, Y., et al., *Synthesizing AND gate genetic circuits based on CRISPR-Cas9 for identification of bladder cancer cells.* Nature communications, 2014. **5**(1): p. 5393.
153. Jinek, M., et al., *RNA-programmed genome editing in human cells.* elife, 2013. **2**: p. e00471.
154. Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* cell, 2011. **144**(5): p. 646-674.
155. Fellmann, C., et al., *Cornerstones of CRISPR–Cas in drug discovery and therapy.* Nature reviews Drug discovery, 2017. **16**(2): p. 89-100.

156. Zhuo, C., et al., *Genomic editing of non-coding RNA genes with CRISPR/Cas9 ushers in a potential novel approach to study and treat schizophrenia.* Frontiers in molecular neuroscience, 2017. **10**: p. 28.
157. Makarova, K.S., et al., *Evolution and classification of the CRISPR–Cas systems.* Nature Reviews Microbiology, 2011. **9**(6): p. 467-477.
158. Gasiunas, G., et al., *Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria.* Proceedings of the National Academy of Sciences, 2012. **109**(39): p. E2579-E2586.
159. Brouns, S.J., et al., *Small CRISPR RNAs guide antiviral defense in prokaryotes.* Science, 2008. **321**(5891): p. 960-964.
160. Hsu, P.D., et al., *DNA targeting specificity of RNA-guided Cas9 nucleases.* Nature biotechnology, 2013. **31**(9): p. 827-832.
161. Chen, S. and X. Shen, *Long noncoding RNAs: functions and mechanisms in colon cancer.* Molecular cancer, 2020. **19**: p. 1-13.
162. Chen, G., et al., *A biodegradable nanocapsule delivers a Cas9 ribonucleoprotein complex for in vivo genome editing.* Nature nanotechnology, 2019. **14**(10): p. 974-980.
163. Zhai, H.-y., et al., *Overexpression of long non-coding RNA TUG1 promotes colon cancer progression.* Medical science monitor: international medical journal of experimental and clinical research, 2016. **22**: p. 3281.
164. Tian, L., et al., *Taurine up-regulated 1 accelerates tumorigenesis of colon cancer by regulating miR-26a-5p/MMP14/p38 MAPK/Hsp27 axis in vitro and in vivo.* Life sciences, 2019. **239**: p. 117035.
165. Skoulidis, F. and J.V. Heymach, *Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy.* Nature Reviews Cancer, 2019. **19**(9): p. 495-509.
166. Wu, X.-H., et al., *Expression and significance of hypoxia-inducible factor-1α and glucose transporter-1 in laryngeal carcinoma Corrigendum in/ol/6/1/287.* Oncology letters, 2013. **5**(1): p. 261-266.
167. Kanarek, N., et al., *Histidine catabolism is a major determinant of methotrexate sensitivity.* Nature, 2018. **559**(7715): p. 632-636.
168. Papaccio, F., et al., *Concise review: cancer cells, cancer stem cells, and mesenchymal stem cells: influence in cancer development.* Stem cells translational medicine, 2017. **6**(12): p. 2115-2125.
169. Xu, X., et al., *Delivery of CRISPR/Cas9 for therapeutic genome editing.* The journal of gene medicine, 2019. **21**(7): p. e3107.
170. Tong, S., et al., *Engineered materials for in vivo delivery of genome-editing machinery.* Nature Reviews Materials, 2019. **4**(11): p. 726-737.
171. Battaglia, L. and M. Gallarate, *Lipid nanoparticles: state of the art, new preparation methods and challenges in drug delivery.* Expert opinion on drug delivery, 2012. **9**(5): p. 497-508.
172. Wapinski, O. and H.Y. Chang, *Long noncoding RNAs and human disease.* Trends in cell biology, 2011. **21**(6): p. 354-361.
173. Mercer, T.R., M.E. Dinger, and J.S. Mattick, *Long non-coding RNAs: insights into functions.* Nature reviews genetics, 2009. **10**(3): p. 155-159.
174. Matsui, M. and D.R. Corey, *Non-coding RNAs as drug targets.* Nature reviews Drug discovery, 2017. **16**(3): p. 167-179.
175. Gutschner, T. and S. Diederichs, *The hallmarks of cancer: a long non-coding RNA point of view.* RNA biology, 2012. **9**(6): p. 703-719.
176. Anastasiadou, E., L.S. Jacob, and F.J. Slack, *Non-coding RNA networks in cancer.* Nature Reviews Cancer, 2018. **18**(1): p. 5-18.

177. Rossi, S. and G.A. Calin, *Bioinformatics, non-coding RNAs and its possible application in personalized medicine.* MicroRNA Cancer Regulation: Advanced Concepts, Bioinformatics and Systems Biology Tools, 2013: p. 21-37.
178. Mendell, J.T., *MicroRNAs: critical regulators of development, cellular physiology and malignancy.* Cell cycle, 2005. **4**(9): p. 1179-1184.
179. Hammond, S.M., *MicroRNAs as tumor suppressors.* Nature genetics, 2007. **39**(5): p. 582-583.
180. Nicoloso, M.S., et al., *MicroRNAs—the micro steering wheel of tumour metastases.* Nature Reviews Cancer, 2009. **9**(4): p. 293-302.
181. Esteller, M., *Non-coding RNAs in human disease.* Nature reviews genetics, 2011. **12**(12): p. 861-874.
182. Shi, X., et al., *Long non-coding RNAs: a new frontier in the study of human diseases.* Cancer letters, 2013. **339**(2): p. 159-166.
183. Yang, X., et al., *MDR1 siRNA loaded hyaluronic acid-based CD44 targeted nanoparticle systems circumvent paclitaxel resistance in ovarian cancer.* Scientific reports, 2015. **5**(1): p. 8509.
184. Ganesh, S., et al., *In vivo biodistribution of siRNA and cisplatin administered using CD44-targeted hyaluronic acid nanoparticles.* Journal of controlled release, 2013. **172**(3): p. 699-706.
185. Yang, X., et al., *Cluster of differentiation 44 targeted hyaluronic acid based nanoparticles for MDR1 siRNA delivery to overcome drug resistance in ovarian cancer.* Pharmaceutical research, 2015. **32**: p. 2097-2109.
186. Ma, L., V.B. Bajic, and Z. Zhang, *On the classification of long non-coding RNAs.* RNA biology, 2013. **10**(6): p. 924-933.
187. Lin, D., et al., *Translational control by a small RNA: dendritic BC1 RNA targets the eukaryotic initiation factor 4A helicase mechanism.* Molecular and cellular biology, 2008. **28**(9): p. 3008-3019.
188. Parrott, A.M., et al., *The evolution and expression of the snaR family of small non-coding RNAs.* Nucleic acids research, 2011. **39**(4): p. 1485-1500.
189. Beltran, M., et al., *A natural antisense transcript regulates Zeb2/Sip1 gene expression during Snail1-induced epithelial–mesenchymal transition.* Genes & development, 2008. **22**(6): p. 756-769.
190. Gong, C. and L.E. Maquat, *lncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3′ UTRs via Alu elements.* Nature, 2011. **470**(7333): p. 284-288.
191. Wang, V. and W. Wu, *MicroRNA-based therapeutics for cancer.* BioDrugs, 2009. **23**(1): p. 15-23.
192. Braicu, C., et al., *Clinical and pathological implications of miRNA in bladder cancer.* International journal of nanomedicine, 2015: p. 791-800.
193. Sakamoto, N., et al., *Non-coding RNAs are promising targets for stem cell-based cancer therapy.* Non-coding RNA Research, 2017. **2**(2): p. 83-87.
194. Chakraborty, C., et al., *Therapeutic miRNA and siRNA: moving from bench to clinic as next generation medicine.* Molecular Therapy-Nucleic Acids, 2017. **8**: p. 132-143.
195. Kim, H.J., et al., *Recent progress in development of siRNA delivery vehicles for cancer therapy.* Advanced drug delivery reviews, 2016. **104**: p. 61-77.
196. Lares, M.R., J.J. Rossi, and D.L. Ouellet, *RNAi and small interfering RNAs in human disease therapeutic applications.* Trends in biotechnology, 2010. **28**(11): p. 570-579.

197. Ozpolat, B., A.K. Sood, and G. Lopez-Berestein, *Liposomal siRNA nanocarriers for cancer therapy.* Advanced drug delivery reviews, 2014. **66**: p. 110-116.
198. Bumcrot, D., et al., *RNAi therapeutics: a potential new class of pharmaceutical drugs.* Nature chemical biology, 2006. **2**(12): p. 711-719.
199. Selvam, C., et al., *Therapeutic potential of chemically modified si RNA: Recent trends.* Chemical biology & drug design, 2017. **90**(5): p. 665-678.
200. Krützfeldt, J., et al., *Silencing of microRNAs in vivo with 'antagomirs'.* nature, 2005. **438**(7068): p. 685-689.
201. Oh, S.Y., Y. Ju, and H. Park, *A highly effective and long-lasting inhibition of miRNAs with PNA-based antisense oligonucleotides.* Molecules and cells, 2009. **28**: p. 341-345.
202. Weinstein, S. and D. Peer, *RNAi nanomedicines: challenges and opportunities within the immune system.* Nanotechnology, 2010. **21**(23): p. 232001.
203. Mukherjee, S., R.N. Ghosh, and F.R. Maxfield, *Endocytosis.* Physiological reviews, 1997. **77**(3): p. 759-803.
204. Zuckerman, J.E. and M.E. Davis, *Clinical experiences with systemically administered siRNA-based therapeutics in cancer.* Nature reviews Drug discovery, 2015. **14**(12): p. 843-856.
205. Shen, J., et al., *Cyclodextrin and polyethylenimine functionalized mesoporous silica nanoparticles for delivery of siRNA cancer therapeutics.* Theranostics, 2014. **4**(5): p. 487.
206. Ewe, A., et al., *Liposome-polyethylenimine complexes (DPPC-PEI lipopolyplexes) for therapeutic siRNA delivery in vivo.* Nanomedicine: Nanotechnology, Biology and Medicine, 2017. **13**(1): p. 209-218.
207. Xiang, B., et al., *Enhancing siRNA-based cancer therapy using a new pH-responsive activatable cell-penetrating peptide-modified liposomal system.* International Journal of Nanomedicine, 2017: p. 2385-2405.
208. Ochekpe, N.A., P.O. Olorunfemi, and N.C. Ngwuluka, *Nanotechnology and drug delivery part 1: background and applications.* Tropical journal of pharmaceutical research, 2009. **8**(3).
209. Yu, X., et al., *Design of nanoparticle-based carriers for targeted drug delivery.* Journal of nanomaterials, 2016. **2016**.
210. Svenson, S. and D.A. Tomalia, *Dendrimers in biomedical applications—reflections on the field.* Advanced drug delivery reviews, 2012. **64**: p. 102-115.
211. Torchilin, V., *Targeted polymeric micelles for delivery of poorly soluble drugs.* Cellular and Molecular Life Sciences CMLS, 2004. **61**: p. 2549-2559.
212. Yuan, F., et al., *Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size.* Cancer research, 1995. **55**(17): p. 3752-3756.
213. Bertrand, N., et al., *Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology.* Advanced drug delivery reviews, 2014. **66**: p. 2-25.
214. Ruoslahti, E., *Specialization of tumour vasculature.* Nature Reviews Cancer, 2002. **2**(2): p. 83-90.
215. Haddish-Berhane, N., J.L. Rickus, and K. Haghighi, *The role of multiscale computational approaches for rational design of conventional and nanoparticle oral drug delivery systems.* International journal of nanomedicine, 2007. **2**(3): p. 315-331.
216. Li, Y., M. Kröger, and W.K. Liu, *Endocytosis of PEGylated nanoparticles accompanied by structural and free energy changes of the grafted polyethylene glycol.* Biomaterials, 2014. **35**(30): p. 8467-8478.

217. Oupicky, D., et al., *Importance of lateral and steric stabilization of polyelectrolyte gene delivery vectors for extended systemic circulation.* Molecular Therapy, 2002. **5**(4): p. 463-472.
218. Kreuter, J., *Nanoparticles—a historical perspective.* International journal of pharmaceutics, 2007. **331**(1): p. 1-10.
219. Saha, R.N., et al., *Nanoparticulate drug delivery systems for cancer chemotherapy.* Molecular membrane biology, 2010. **27**(7): p. 215-231.
220. Oh, K.T., et al., *Polymeric nanovehicles for anticancer drugs with triggering release mechanisms.* Journal of Materials Chemistry, 2007. **17**(38): p. 3987-4001.
221. Sun, Q., Q. Hao, and K.V. Prasanth, *Nuclear long noncoding RNAs: key regulators of gene expression.* Trends in Genetics, 2018. **34**(2): p. 142-157.
222. Piccoli, M.-T., et al., *Inhibition of the cardiac fibroblast–enriched lncRNA Meg3 prevents cardiac fibrosis and diastolic dysfunction.* Circulation research, 2017. **121**(5): p. 575-583.
223. Uroda, T., et al., *Conserved pseudoknots in lncRNA MEG3 are essential for stimulation of the p53 pathway.* Molecular cell, 2019. **75**(5): p. 982-995. e9.
224. Pan, L., et al., *ZNF750 inhibited the malignant progression of oral squamous cell carcinoma by regulating tumor vascular microenvironment.* Biomedicine & Pharmacotherapy, 2018. **105**: p. 566-572.
225. Hazawa, M., et al., *ZNF750 is a lineage-specific tumour suppressor in squamous cell carcinoma.* Oncogene, 2017. **36**(16): p. 2243-2254.
226. Jiang, Y., et al., *Co-activation of super-enhancer-driven CCAT1 by TP63 and SOX2 promotes squamous cancer progression.* Nature communications, 2018. **9**(1): p. 3619.
227. Zhong, M.-E., et al., *LncRNA H19 regulates PI3K–Akt signal pathway by functioning as a ceRNA and predicts poor prognosis in colorectal cancer: integrative analysis of dysregulated ncRNA-associated ceRNA network.* Cancer cell international, 2019. **19**: p. 1-13.
228. Ling, H., M. Fabbri, and G.A. Calin, *MicroRNAs and other non-coding RNAs as targets for anticancer drug development.* Nature reviews Drug discovery, 2013. **12**(11): p. 847-865.
229. Diesch, J., et al., *A clinical-molecular update on azanucleoside-based therapy for the treatment of hematologic cancers.* Clinical epigenetics, 2016. **8**: p. 1-11.
230. Yu, X., et al., *CXCL12/CXCR4 axis induced miR-125b promotes invasion and confers 5-fluorouracil resistance through enhancing autophagy in colorectal cancer.* Scientific Reports, 2017. **7**(1): p. 42226.
231. Xue, X., et al., *LncRNA HOTAIR enhances ER signaling and confers tamoxifen resistance in breast cancer.* Oncogene, 2016. **35**(21): p. 2746-2755.
232. Xue, M., et al., *hsa_circ_0081143 promotes cisplatin resistance in gastric cancer by targeting miR-646/CDK6 pathway.* Cancer cell international, 2019. **19**: p. 1-11.
233. Zhou, Y., et al., *Circular RNA hsa circ 0004015 regulates the proliferation, invasion, and TKI drug resistance of non-small cell lung cancer by miR-1183/PDPK1 signaling pathway.* Biochemical and biophysical research communications, 2019. **508**(2): p. 527-535.
234. Delaney, G., et al., *The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines.* Cancer: Interdisciplinary International Journal of the American Cancer Society, 2005. **104**(6): p. 1129-1137.

235. Lee, S.Y., et al., *Induction of metastasis, cancer stem cell phenotype, and oncogenic metabolism in cancer cells by ionizing radiation.* Molecular cancer, 2017. **16**(1): p. 1-25.
236. Wang, X.-C., et al., *Expression and function of miRNA in postoperative radiotherapy sensitive and resistant patients of non-small cell lung cancer.* Lung cancer, 2011. **72**(1): p. 92-99.
237. Pajic, M., et al., *miR-139-5p modulates radiotherapy resistance in breast cancer by repressing multiple gene networks of DNA repair and ROS defense.* Cancer research, 2018. **78**(2): p. 501-515.
238. Niemoeller, O.M., et al., *MicroRNA expression profiles in human cancer cells after ionizing radiation.* Radiation oncology, 2011. **6**: p. 1-5.
239. Fayda, M., et al., *Do circulating long non-coding RNAs (lncRNAs)(LincRNA-p21, GAS 5, HOTAIR) predict the treatment response in patients with head and neck cancer treated with chemoradiotherapy?* Tumor Biology, 2016. **37**: p. 3969-3978.
240. Mueller, A.-K., et al., *MicroRNAs and their impact on radiotherapy for cancer.* Radiation research, 2016. **185**(6): p. 668-677.
241. Liu, Q., et al., *LncRNA loc285194 is a p53-regulated tumor suppressor.* Nucleic acids research, 2013. **41**(9): p. 4976-4987.
242. Yang, P., et al., *The long noncoding RNA-ROR promotes the resistance of radiotherapy for human colorectal cancer cells by targeting the p53/miR-145 pathway.* Journal of gastroenterology and hepatology, 2017. **32**(4): p. 837-845.
243. Zhang, Y., et al., *Long noncoding RNA LINP1 regulates repair of DNA double-strand breaks in triple-negative breast cancer.* Nature structural & molecular biology, 2016. **23**(6): p. 522-530.
244. Mah, J.K., *An overview of recent therapeutics advances for Duchenne muscular dystrophy.* Duchenne Muscular Dystrophy: Methods and Protocols, 2018: p. 3-17.
245. Østergaard, M.E. and P.J. Hrdlicka, *Pyrene-functionalized oligonucleotides and locked nucleic acids (LNAs): Tools for fundamental research, diagnostics, and nanotechnology.* Chemical Society Reviews, 2011. **40**(12): p. 5771-5788.
246. Inoue, S., et al., *Nanobiopolymer for direct targeting and inhibition of EGFR expression in triple negative breast cancer.* PLoS One, 2012. **7**(2): p. e31070.
247. El Fatimy, R., et al., *Genome editing reveals glioblastoma addiction to microRNA-10b.* Molecular Therapy, 2017. **25**(2): p. 368-378.
248. Huang, K.-W., et al., *Galactose derivative-modified nanoparticles for efficient siRNA delivery to hepatocellular carcinoma.* Biomacromolecules, 2018. **19**(6): p. 2330-2339.
249. Nair, J.K., et al., *Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing.* Journal of the American Chemical Society, 2014. **136**(49): p. 16958-16961.
250. Foster, D.J., et al., *Advanced siRNA designs further improve in vivo performance of GalNAc-siRNA conjugates.* Molecular Therapy, 2018. **26**(3): p. 708-717.
251. Hayes, E.L. and J.S. Lewis-Wambi, *Mechanisms of endocrine resistance in breast cancer: an overview of the proposed roles of noncoding RNA.* Breast Cancer Research, 2015. **17**(1): p. 1-13.
252. Vicentini, C., et al., *Current role of non-coding RNAs in the clinical setting.* Non-coding RNA Research, 2019. **4**(3): p. 82-85.
253. Miller, J.C., et al., *An improved zinc-finger nuclease architecture for highly specific genome editing.* Nature biotechnology, 2007. **25**(7): p. 778-785.

254. Gutschner, T., M. Baas, and S. Diederichs, *Noncoding RNA gene silencing through genomic integration of RNA destabilizing elements using zinc finger nucleases.* Genome research, 2011. **21**(11): p. 1944-1954.
255. Qi, L.S., et al., *Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression.* Cell, 2013. **152**(5): p. 1173-1183.
256. Gilbert, L.A., et al., *CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes.* Cell, 2013. **154**(2): p. 442-451.
257. Van Audenhove, I. and J. Gettemans, *Nanobodies as versatile tools to understand, diagnose, visualize and treat cancer.* EBioMedicine, 2016. **8**: p. 40-48.
258. Cawez, F., et al., *Combinatorial design of a nanobody that specifically targets structured RNAs.* Journal of molecular biology, 2018. **430**(11): p. 1652-1670.
259. Watrin, M., et al., *In vitro selection of RNA aptamers derived from a genomic human library against the TAR RNA element of HIV-1.* Biochemistry, 2009. **48**(26): p. 6278-6284.
260. Li, M.-J., et al., *Long-term inhibition of HIV-1 infection in primary hematopoietic cells by lentiviral vector delivery of a triple combination of anti-HIV shRNA, anti-CCR5 ribozyme, and a nucleolar-localizing TAR decoy.* Molecular Therapy, 2005. **12**(5): p. 900-909.
261. Mizrahi, A., et al., *Development of targeted therapy for ovarian cancer mediated by a plasmid expressing diphtheria toxin under the control of H19 regulatory sequences.* Journal of translational medicine, 2009. **7**(1): p. 1-11.
262. Slaby, O., R. Laga, and O. Sedlacek, *Therapeutic targeting of non-coding RNAs in cancer.* Biochemical journal, 2017. **474**(24): p. 4219-4251.
263. Pedram Fatemi, R., et al., *Screening for small-molecule modulators of long noncoding RNA-protein interactions using AlphaScreen.* Journal of biomolecular screening, 2015. **20**(9): p. 1132-1141.
264. West, J.A., et al., *The long noncoding RNAs NEAT1 and MALAT1 bind active chromatin sites.* Molecular cell, 2014. **55**(5): p. 791-802.
265. Fatemi, R.P., D. Velmeshev, and M.A. Faghihi, *De-repressing LncRNA-targeted genes to upregulate gene expression: focus on small molecule therapeutics.* Molecular Therapy-Nucleic Acids, 2014. **3**.
266. Ayatollahi, S., et al., *Aptamer-targeted delivery of Bcl-xL shRNA using alkyl modified PAMAM dendrimers into lung cancer cells.* The international journal of biochemistry & cell biology, 2017. **92**: p. 210-217.
267. Schott, J.W., et al., *Viral and synthetic RNA vector technologies and applications.* Molecular Therapy, 2016. **24**(9): p. 1513-1527.
268. Huang, J., et al., *Lentivirus-mediated RNA interference targeting the long noncoding RNA HOTAIR inhibits proliferation and invasion of endometrial carcinoma cells in vitro and in vivo.* International Journal of Gynecologic Cancer, 2014. **24**(4).

About The Authors

Dr. Muhammad Farrukh Nisar (Ph.D.)
Assistant Professor of Biochemistry, Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan.
Email: mfarrukhnisar@cuvas.edu.pk, ORCID: 0000-0001-8873-1227

Amir Javed (M.Phil.)
Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan.
Email: javedamir371@gmail.com, ORCID: 0009-0004-4342-8

Asif Ali (M.Phil.)
Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan.
Email: asifrahmani7476@gmail.com, ORCID: 0009-0002-7932-1642

Sania Shaheen (M.Phil.)
Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan.
Email: saniashaheen016@gmail.com, ORCID: 0009-0002-5231-6341

Dr. Muhammad Rafiq (Ph.D.)
Assistant Professor of Biochemistry, Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan.
Email: mrafiq@cuvas.edu.pk, ORCID: 0000-0001-9686-6551

Dr. Muhammad Yasir Waqas (Ph.D.)
Assistant Professor of Physiology, Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan.
Email: myasirwaqas@cuvas.edu.pk, ORCID: 0000-0001-6165-8285

Dr. Zahid Manzoor (Ph.D.)
Assistant Professor, Department of Pharmacology and Toxicology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan.
Email: zahidmanzoor@cuvas.edu.pk, ORCID: 0000-0002-2840-6788

Dr. Shaukat Hussain Munawar (Ph.D.)
Assistant Professor, Department of Pharmacology and Toxicology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan.
Email: shaukathussainmunawar@cuvas.edu.pk, ORCID: 0000-0003-2766-3835

Dr. Xu Kang (Ph.D., Post Doctorate)
Associate Professor, College of Pharmacy, Hubei University of Chinese Medicine, Wuhan, Hubei Province, China.
Email: kangxu05@hbtcm.edu.cn , ORCID: 0000-0002-8492-2058

To Cite This Chapter:
Nisar, M.F., X. Kang, A. Javed, A. Ali, S. Shaheen, M. Rafiq, M. Y. Waqas, Z. Manzoor, & S. H. Munawar. (2023). **non-coding RNAs: recent advancements in the development and biochemistry as novel anti-tumor drug molecules.** In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023* (pp. 240-282). ISRES Publishing

Section

III

VETERINARY AND ANIMAL SCIENCES

A GLOBAL BURDEN OF LUMPY SKIN DISEASE, OUTBREAKS, AND FUTURE CHALLENGES

Muhammad SAFDAR
Mehmet OZASLAN
Shafeeq ur REHMAN
Furqan SHAFQAT
Muhammad SHAN

Introduction

The symptoms of lumpy skin disease (LSD), which mostly affects cattle, include fever, lymphadenitis, mucosal irritation, cutaneous nodules, ocular discharge, and edema of subcutaneous cellular tissue. It is a disease that may be spread by different arthropods and results in large economic losses due to the exhaustion of cattle, damage to their hides, sterility, mastitis, and decreased milk output. Up to 20% of death has also been documented (Chihota et al., 2003; Shalaby et al., 2016). Other domestic animals and wildlife have been shown to have LSD both naturally occurring and experimentally infected (Authority, 2015; Usadov et al., 2018; Young et al., 1970). Many species have been shown to have LSD antibodies (Barnard, 1997; Hedger & Hamblin, 1983; Kayesh et al., 2020), although these might be responses to other capripoxviruses (Davies, 1982; Hamblin et al., 1990). A DNA virus that is a member of the poxviridae family is the main cause of lumpy skin disease (Tulman et al., 2001). Three viruses make up the capripoxvirus genus: Goat pox virus (GTPV), Lumpy skin disease virus (LSDV), and Sheep pox virus (SPPV). The LSDV was discovered as an etiological agent in the 1940s (Von Backstrom, 1945; Weiss, 1968). Before the 1980s, when it first appeared in the Middle East and other Near Eastern nations, LSD seemed to be exclusive to Africa (Wainwright et al., 2013). The illness was initially identified in 2014 in Azerbaijan (Zeynalova et al., 2016), and it then expanded to Europe in 2014 and 2016 before reaching Russia in 2017 (Authority, 2017).

LSDV is the cause of lumpy skin disease that includes the Poxviridae family and subfamily Chordopoxvirniae of the genus capripoxvirus. Knopvelsiekte, Pseudo-urticaria, Neethling viral sickness, and LSD are some of the terms used to describe the illness (Al-Salihi, 2014; E Tuppurainen et al., 2017). LSD is a transboundary, vector-borne, non-zoonotic illness that presently only affects ruminants, such as cattle and water buffaloes. Biting flies, mosquitoes, and ticks are the arthropod vectors that transfer illness (Bosch et al., 2017; Lubinga et al., 2013; Tuppurainen et al., 2011). The Lumpy skin disease is marked by swollen lymph nodes, high fever, and nodules on the skin that cause acute anorexia, decreased milk production, and infertility. Overall, it has an impact on the economic worth of animals since it will have an impact on their ability to produce meat and milk, high-quality hides, draught power, and reproductive effectiveness (abortion and infertility) (Rgbe, 2014). It is an illness that must be reported and has a terrible impact on international cattle commerce as well. Although the illness is indigenous to African nations, it has lately been discovered in hitherto undiscovered regions of the globe. Morris (1931) (Morris, 1930) noted that the first LSD case originated in Zambia and afterward spread to southern and northern African nations. Israel, Kuwait, Oman, and Yemen later were affected by it (Wainwright et al., 2013).

According to OIE, this illness is now widespread in a number of African, European, and Asian nations (Tuppurainen et al., 2015). Unknown factors may have contributed to the disease's spread to India, such as cattle crossing international borders or vectors traveling from nearby nations. LSD use has recently been recorded in nations that

border India, and Pakistan including Bangladesh and China. For appropriate planning of efficient disease management, a knowledge of the epidemiology of exotic illnesses becomes essential. The most recent LSD developments are summarised in this overview.

Several significant companies and sectors have indicated that LSD has a direct or indirect socioeconomic effect. The considerable drop in milk output is the first and most noticeable effect of LSD in the South Asian region, which is home to 21% of the world's dairy farm animals (Das et al., 2021). Arthropods, particularly blood-sucking insects (Annandale et al., 2014; Chihota et al., 2001; Irons et al., 2005; ESM Tuppurainen et al., 2017) contaminated feed, contaminated water, and direct transmission through saliva, nasal secretions, and semen in the later stages of the infection are the main vectors for the spread of LSDV. According to several studies, hard ticks may contribute to the spread of viruses (Lubinga et al., 2015; Tuppurainen et al., 2013). It has been shown that the virus and viral antigen responsible for lumpy skin disease is present in the saliva and other tick organs such as the midgut, haemocytes, and salivary glands (Lubinga et al., 2013; Lubinga, Clift, et al., 2014). Aedes aegypti is the only dipteran capable of completely transmitting the virus to susceptible cattle (Chihota et al., 2001).

Origin and genetics

The virus that causes lumpy skin disease (LSDV), which affects all domestic animals except dogs, is a member of the Poxviridae family of viruses. Two subfamilies make up the family: Entomopoxvirinae, which infects invertebrate hosts, and Chordopoxvirinae, which infects vertebrate hosts (Quinn et al., 2015). There are ten genera in the Chordopoxvirinae subfamily, including Capripoxvirus. This genus encompasses viruses from three different species, including LSDV, GTPV, and SPPV which infect cattle, goats, and sheep respectfully (King et al., 2012).

The LSDV is brick-shaped, encapsulated having complex symmetry. It also contains double-stranded DNA and reproduces in the cytoplasm of the host cell. It is 320 X 260 nm in size. The alike 2.4 kbp-long inverted terminal repeats that encircle the central coding region are seen throughout the 151 kbp-long LSDV genome, which comprises 156 putative genes. Sheeppox and goatpox virus homologs share 97% of the nucleotides in 30 structural and non-structural genes found in LSDV (Tulman et al., 2001; Tulman et al., 2002). When comparing SPPS, LSDV, and GTPV in Capri poxviruses, similar patterns have been seen. The later evolution of poxviruses leads to a reduction of their host range owing to gene loss. The LSDV 132, N2L, myxoma viruses M004.1 and M003.2, K7L, IL-1 receptor, F11L, and Vaccinia viruses unique gene are among the nine genes encoded by the terminal regions of the LSDV virus. Accumulated mutations in GTPV and SPPV are predicted to have a detrimental effect on the host range and virulence functions of the LSDV 132 gene. The three viruses' genome sequence length is unaffected by this disruption, but the lack of these genes in GTPV and SPPV implies that the host limitation to cows alone may be caused by this disruption (Biswas et al., 2020; Tulman et al., 2002).

Unlike other chordopoxviruses, the LSD virus contains 146 invariant genes that, when processed into proteins, regulate the production of proteins associated with stability and structural development, host range, mRNA synthesis, transcription, nucleotides metabolism, and DNA replication and virulence. The genes responsible for host range and viral virulence are different in the terminal region, and those that do exist have a lower level of amino acid similarity, on average only 43%, whether they are present

or not. The LSDV contains genes that are similar to epidermal growth factors like protein, IL-10, IL-1, binding proteins, the G protein-coupled CC chemokine receptor (GPCR), and more. Other genera of poxvirus include these genes often (Tulman et al., 2001).

The LSD clinical syndrome was first noted in Zambia in 1929. It was formerly assumed that it was the consequence of poisoning or a reaction to insect stings. Additional occurrences are documented from Zimbabwe (Southern Rhodesia), the Republic of South Africa, and Botswana (Bechuanaland) between 1943 and 1945. Up until 1949, a panzootic disease affected over 8 million cattle in South Africa, causing considerable economic losses (Diesel, 1949; Von Backstrom, 1945). Sudan in 1972, West Africa in 1974, and East Africa (Kenya) in 1957 were the first places where LSD was discovered and diagnosed. Between 1981 and 1986, outbreaks of epizootic LSD were also documented in Cameroon, Tanzania, Zimbabwe, Somalia, and Kenya with death rates of 20% in infected cattle(Brenner et al., 2006). Between 1929 and 1986, the illness was only present in a few sub-Saharan African nations (Davies, 1981). The United Arab Emirates (UAE) (2000), Lebanon (1993), Yemen (1995), Bahrain (2003), Kuwait (1991), Israel (2006), the World Organization for Animal Health (OIE), and Oman (2010) (Tuppurainen & Oura, 2012) have all reported finding LSD (Abdulqa et al., 2016; Coetzer & Tuppurainen, 2004).

In comparison to orthopoxvirus, mature capripoxvirions have bigger lateral bodies and a more oval appearance (Munz & Owen, 1966). Their typical dimensions are 320 x 260 nm (Ghaboussi, 1978). The LSD virus flourishes and multiplies to exceptionally high levels in a wide variety of cell cultures, comprising kidneys from lamb and calf, adrenal and thyroid glands, muscle, and testes. The onset of cytopathic effects during early isolation might take up to 11 days (Weiss & Geyer, 1959). There is only a single serotype of the LSD virus, that is hard to distinguish using conventional viral neutralization tests due to its serological similarity to the virus that causes sheep and goat pox (SGP) (Burdin, 1959).

Epidemiology

It is believed that the majority of LSD virus infections are transmitted via means of insects (MacOwan, 1959; Von Backstrom, 1945). The pox virus is very resilient and may live in infected tissue for at than 120 days, and likely for longer. The virus may also be discovered in the blood and nasal saliva, spit semen, and lacrimal secretions, which are regarded as the primary means of LSD transfer. Cross-protection between the LSD virus and sheep or goat pox viruses has been utilized in Kenya and the Middle East to immunize cattle against LSD. It is possible to recover the LSD virus from necrotic skin nodules that have been stored at -80°C for ten years and also from infected tissue culture that has been stored at 4°C for six months. When compared to thick-skinned local breeds like the Afrikaner and Afrikaner crossbreeds, the sickness often appears more strongly in imported Bostaurus breeds, such as Friesian cattle with necrotic skin nodules (Abdulqa et al., 2016). Even though all age groups are vulnerable, the clinical illness is more severe in young animals and cows that are lactating at their peak (Coetzer, 2004). A skin lesion harbouring the virus most likely appears at the injection site within 1-3 weeks following experimental infection by intradermal inoculation, but the incubation period in the field is between 2 and 5 weeks (Abdulqa et al., 2016; Haig, 1957). A significant, financially crippling, and reportable condition called lumpy skin disease reduced cow output as a result of widespread illnesses and persistent weakness. A thorough knowledge of the epidemiological elements of LSD relating to the pathogen, host, and environment may help with

preventative strategies. Particular focus was placed on exposing hosts and the virus to environments that were conducive to the disease's transmission and spread (Hailu et al., 2015). The disease's occurrence, geographic range, and method of transmission in big herds of cattle were all shown to result in significant economic losses (Salib & Osman, 2011; Tuppurainen & Oura, 2012).

Geographical Distribution

The geographic distribution of LSDV, GPV, and SPV are distinctive from one another, and over the last fifty years, SPV and GPV have been geographically limited to Africa and Asia, spreading from Africa to the north of the equator (Kitching et al., 1989). In 1929, LSD first appeared in nations in Sub-Saharan Africa, and over the next seventy years, it expanded to both the north and south. All of the sub-Saharan African nations, as well as Madagascar, are now included in the geographic scope of LSD. It is endemic to all of the African nations and may be found in a variety of ecological zones, including temperate, dry semi-arid, and desert regions (Davies, 1991).

Zambia was the first African country to report using LSD in 1929 (Gari et al., 2011). In 1989, LSD was discovered outside of Africa in Israel, Palestine, Jordan, Lebanon, Kuwait, Saudi Arabia, Iraq, Oman, Yemen, the United Arab Emirates, and Bahrain (Abutarbush et al., 2015; Al-Salihi & Hassan, 2015). In 2014, it was detected in Europe's middle and eastern regions (Sameea Yousefi et al., 2017; Tageldin et al., 2014). In 2016, LSD was proven in South East Europe, the Balkans, and the Caucasus.

Host range

Cattle and Asian water buffaloes (Bubals bubalis) are the principal natural hosts for LSDV, with all ages and sexes vulnerable to infection. Calves acquire serious lesions 24–48 hours before their dams (Elhaig et al., 2017). In breeds with thin skin, such as Friesians, particularly at cows' peak lactation, more severe illness symptoms have been seen (evik & Doan, 2017). Although mixed herds of cattle, buffalo, sheep, and goats are more often seen, no epidemiological data on the function of small ruminants as a reservoir for LSDV have been documented (Elhaig et al., 2017). In Egypt, LSDV affects local breeds of all ages, producing mild to severe illness, although severe instances have been discovered in young calves and alien breeds (Salib & Osman, 2011). Additionally, the development of disease outbreaks is greatly influenced by the separation of LSDV from naturally infected water buffaloes (Elhaig et al., 2017; Sharawi & Abd El-Rahim, 2011) Despite interaction with clinically infected cattle that was verified by viral isolation and PCR in a different investigation involving buffaloes, none of the buffaloes tested positive for LSDV by virus isolation and PCR, despite a modest rise in LSDV antibodies in their serum (Elhaig et al., 2017). Arabian Oryx (Oryx leucoryx) and spring box have both shown clinical indications of LSD (Antidorcas marsupialis). Additionally, giraffes (Giraffa camelopardalis) and impalas (Aepyceros melampus) have been experimentally infected, although wildlife does not play a large part in the epidemiology of the illness (Gortázar et al., 2022). Although sheep and goats may be experimentally infected with LSDV, the virus cannot be acquired naturally (Wolff et al., 2020).

Pathogenesis

After LSDV infection, the virus replicates, viremia develops, fever sets in, the virus localizes to the skin, and nodules form (Constable et al., 2016). After intradermal inoculation of the virus, the following incidents were experimentally observed:

Four to seven days post-infection (DPI), there is localized swelling at the inoculation site as 1-3 cm nodules or plaques. From 6 to 18 DPI, viral shedding manifests as oral and nasal discharge. The development of generalized cutaneous nodules and regional lymphadenopathy takes place between 7 to 19 DPI (Mulatu & Feyisa, 2018b). Virus found in semen 42 days after fever (Annandale et al., 2014). Different areas of the body often exhibit a unique structure termed "sit-fasts" (necrotic cores detached from the surrounding skin) (Gumbe, 2018), which may develop ulcers (Lubinga, Tuppurainen, et al., 2014; Namazi & Khodakaram Tafti, 2021).

The virus replicates intracellularly in pericytes, macrophages, fibroblasts, and endothelial cells, causing vasculitis and lymphangitis in infected tissues (Khan et al.; Namazi & Khodakaram Tafti, 2021).

Animals that are underweight, lactating cows, and young calves seem to have compromised humoral immunity, making them more vulnerable to natural infections (Babiuk et al., 2008). Animals that have survived a viral infection have exhibited lifetime immunity. Calves from infected mothers are immune to the clinical disease for roughly 6 months because they have acquired maternal antibodies (Tuppurainen et al., 2005). There are no known carriers of LSDV at this time, and infected animals recover from the virus (Namazi & Khodakaram Tafti, 2021; ESM Tuppurainen et al., 2017).

Transmission

Wild ruminants, water buffalo, and cattle are all susceptible to lumpy skin disease. The virus does not seem to infect sheep and goats, according to studies (El-Nahas et al., 2011; Lamien et al., 2011). LSDV may persist in the environment for a very long period at normal temperatures, especially in dried scabs. The virus lives for at least 18 days in air-dried hides, 35 days in desiccated crusts, and 33 days in necrotic skin nodules. (Mulatu & Feyisa, 2018b) the claim that heating the virus to 65°C for 30 minutes or 55°C for two hours would render it inactive. Since the virus may live for a long period in scabs or lesions, skin lesions are believed to be the main site of infection. Additionally, the virus is removed through milk, blood, saliva, lachrymal secretions, nasal, and semen (transmissible to suckling calves) (Namazi & Khodakaram Tafti, 2021).

Arthropods, particularly blood-sucking insects (Annandale et al., 2014; Chihota et al., 2001; Irons et al., 2005; ESM Tuppurainen et al., 2017) that pollute feed and water, as well as direct transfer in the latter stages of the infection through nasal secretions, saliva, and semen, are the main vectors for the spread of LSDV. According to some studies, there is no correlation between infection rates and cattle density, demonstrating that early on in the disease, direct viral transmission is less significant than indirect transmission, which is more important (Magori-Cohen et al., 2012; Namazi & Khodakaram Tafti, 2021).

The fact that most LSD outbreaks occur in the summer when arthropod activity is at its peak, may be evidence that several types of vectors, particularly blood-feeding

insects, are involved in the propagation of the virus (Kahana-Sutin et al., 2017; Namazi & Khodakaram Tafti, 2021; Sprygin et al., 2018).

Several studies have shown that hard ticks may play a part in the spread of viruses (Lubinga et al., 2015; Tuppurainen et al., 2013). The saliva and other tick organs, such as the hemocytes, salivary glands, and midgut, were identified to contain the virus and viral antigen that causes lumpy skin disease (Lubinga et al., 2013; Lubinga, Clift, et al., 2014). Moreover, molecular data were used to demonstrate that ticks mechanically and transstadially transmit the virus (Stubbs et al., 2012). The quick emergence of widespread epidemics, however, is not explained by their prolonged attachment to the host. As a result, it appears that ticks might be serving as viral reservoirs (Kahana-Sutin et al., 2017; Namazi & Khodakaram Tafti, 2021).

The only dipteran capable of fully transmitting the virus to vulnerable cattle is Aedes aegypti (Chihota et al., 2001). Anopheles stephensi Liston, Culicoides nubeculosus, or Culex quinquefasciatus Say mosquitoes could not transmit the virus (Chihota et al., 2003). Despite the presence of Stomoxys calcitrans in LSD outbreaks and its ability to spread the capripox virus to sheep and goats (Baldacchino et al., 2013; Yeruham et al., 1995), the transmission of LSDV to vulnerable animals has not been accomplished (Chihota et al., 2003). The finding of LSDV in Culicoides punctatus implies that this species may be a vector for viral transmission (Şevik & Doğan, 2017). Moreover, it is claimed that the ratio of biting insects to host populations is significantly associated with the chance of transmission (Gubbins et al., 2008; Namazi & Khodakaram Tafti, 2021).

In trials, both PCR and viral isolation revealed the persistence of the lumpy skin disease virus in bovine semen (Annandale et al., 2010; Givens, 2018; Irons et al., 2005). Additionally, semen contributed to the virus' spread among inseminated heifers (Annandale et al., 2014; Namazi & Khodakaram Tafti, 2021).

Clinical Signs and symptoms

Numerous researchers around the world have noted the following clinical signs, including viral excretion through nasopharyngeal and oropharyngeal release, viral localized on the skin, vasculitis, pyrexia, infected sperms, growth of abscesses harm the host's hide, decreased fertility of bull, destruction to livestock pelts, decreased milk yield and body mass, lymphangitis, and geographic swollen lymph viremia, virus multiplication, (Abutarbush et al., 2015; Annandale et al., 2014; Tasioudi et al., 2016; E Tuppurainen et al., 2017). Animals' different vital organs, particularly their digestive system, genital tract, snout, trachea, nasal mucosa, oral pad, abomasum, larynx, inner lips, testicles, spleen, liver, heart, renal, gingiva, udders, abomasum, uterine tubes, lungs, teats, and, are heavily infected (Al-Salihi & Hassan, 2015; Zeynalova et al., 2016).

While the incubation timespan for this illness is anywhere from 2-5 weeks in the wild, it has been shown to be as little as 7 days to a maximum of 14 days in lab circumstances. There are three clinical manifestations of lumpy skin disease: chronic, acute, and subacute. In the mild type of illness, intermittent fever is first noticed, then 1-2 skin nodules appear. Emaciation, epiphora, and agalactia are the most challenging clinical manifestations to manage. As the condition progresses, the skin may show signs of nodular lesions, erythema, and pruritis. Oro-nasal mucosae, pubic, and perineal lesions develop, causing severe pain (Salib & Osman, 2011). A large number of nodules appear all over the skin in large numbers in the severe form,

which lasts with about a week before they firm quickly and begin to be encapsulated by thin hemorrhagic rings. The nodules may scatter towards other mucosal membranes as they continue to scab over. The dermal layer and musculature are currently attached to these blister configurations, which have an apparent escalating deterioration template and the existence of epithelioid eosinophilic nodules, according to the histology of these lesions (Biswas et al., 2011). During 2 to 3 weeks, these exposed components dry up and harden, creating pain for moving animals. The development of fistulas, also known as "sit fasts," is caused by the ongoing degradation of these sites and the inadequate re-epithelialization that results. It has been observed that fly maggots or screwworm fly larvae often infest these wounds. Suppuration and subsequent chronic diseases further increase the risk of developing serious septic shock and ultimately dying from it (Abutarbush, 2017; Y. R. Khan et al., 2021). In certain situations, lymphadenitis can occur as well. Pneumonia is brought on by the necrosis material that continues to shed into the lungs when these clusters penetrate the nasal tract particularly. In severe circumstances, LSD is known to impair ovulation and result in fetal abortion in cattle. Painful patches around the genitalia in bull may cause infertility (Al-Salihi, 2014; Y. R. Khan et al., 2021).

Diagnosis Methods

Laboratory testing and field presumptive diagnosis are the foundation of diagnosis. Skin lesions with certain characteristics are diagnostic. Visceral nodules are seen in the lungs, musculoskeletal system, gut, uterine tubes, and teats during autopsy exams. Clinical and pathological tests show that the subcutaneous tissue and epidermis have been filled with cells. The area that is affected contains fibroblasts, lymphocytes, macrophages with few plasma cells, and neutrophils. The virus is isolated by allowing it to develop on the Vero kidney cells from an African green monkey and chorioallantoic membrane of chicken embryonated eggs. LSDV causes hemorrhagic pock sores and CAM to thicken. Additional evidence comes from observable cytopathological changes in muscle cell cultures, such as cellular rounding, shrinkage, and empty patches in the cell sheet, although definitive diagnosis needs OIE-recommended laboratory testing (LSDV is being identified by virus isolation, serological tests, real-time PCR or PCR). The presently used typical test for LSDV detection is the virus neutralization assay (Manual, 2010; Saiyad et al., 2022).

Laboratory diagnosis of LSDV is often performed using genomic techniques such LSDV specific real-time PCR 10 and Capri poxvirus generic gel-based PCR and immunological testing including enzyme-linked immunosorbent assay (ELISA) and virus neutralization test (VNT) (Sudhakar et al., 2020). Real-time PCR and conventional PCR are used to distinguish between LSDV vaccine isolates and wild-type pathogens.

Differential Diagnosis

Differential diagnoses of LSD in cattle are required. Clinical samples must be examined for the following diseases in cattle: bovine popular stomatitis virus (BPSV) causes bovine popular stomatitis, cowpox virus (CPXV) causes cowpox, pseudo-cowpox virus (PCPV) causes pseudo-cowpox, bovine pox virus (BPXV) causes buffalo pox, and bovine herpes virus type 2 causes pseudo-lumps on the injury (BHV-2) (Sudhakar et al., 2020). The response is confined in the epithelium, the lesions are delicate with a depressed apex, and the pseudo-LSD or Alleptron herpes virus infection is moderate. In addition to these disorders, parasitic, bacterial, fungal, and

inflammatory responses may also result in nodules that are often challenging to distinguish, especially in rare instances.

There have been notable LSD field epidemics in China, Hong Kong, Vietnam, Russia, and Turkiye due to the advent of vaccine-like recombinant LSDVs, which has crucial implications for LSD laboratory diagnosis, such as EEV-based DIVA tests and GPCR (Tuppurainen et al., 2020).

Treatment and Control

According to the OIE, immunization is the sole method of reducing LSD emergence and spread in endemic nations (Lu et al., 2021). Removing sick cattle out from the flock and administering supportive care such as anti-inflammatory medicines, vitamin injections, and antibiotics is standard practice. These treatments often lower the likelihood of pyrexia, additional infectious diseases, and inflammation, which increases the animal's appetite.

The cattle business is on the brink of collapse as a result of the recent breakout of illness in Pakistan. Here are some suggestions for preventing LSDV disorders in Pakistan. Along with removing diseased animals, outlawing the trade in live animals, and imposing travel restrictions, vaccination is the greatest method for controlling the spread of the virus in afflicted areas. NSAIDs may aid in avoiding secondary infections in addition to antibiotics. Testing for LSDV is required on bulls used for breeding. The disease's consequences may be lessened with the help of skilled nursing.

Economic losses of LSD

LSD is a new, very hazardous illness that is rapidly spreading in many nations, including Pakistan. In many wealthy and developing nations, LSD has resulted in significant economic losses. In neighboring regions (Pakistan, Turkiye, Ethiopia, etc.), strong fever and mastitis conditions caused abortion, a 10-85% milk reduction, a decline in growth and progress, compromised hides, a significant drop in infertility, the death of susceptible hosts, disordered eating behaviors, increased vaccination prices, and a decline in the quality and quantity of epithelium and hides that are unbearable including both large and small farmers (Jamil et al., 2022; Sajid et al., 2012).

The integrity of LSDV

Although LSDV can withstand extreme heat and drought, it cannot survive direct sunlight. According to reports, the virus takes 30 minutes to become inactive at 65 °C or when it comes into direct contact with lipophilic detergents, compared to 2 hours at 55 °C. There are several disinfectants that are very efficient against LSDV, including formalin, phenol, quaternary ammonium compounds, sodium hypochlorite, ether, chloroform, and iodine compounds (Alkhamis & VanderWaal, 2016; Mulatu & Feyisa, 2018a).

LSD preventive and therapeutic approaches

It is possible to employ a "homologous" strain of the LSDV vaccination that is live attenuated, such as the "Neethling strain." It is possible to employ a live attenuated vaccine strain of the "heterologous" goat pox or sheep pox virus (Manual, 2010). There should be a mass awareness campaign concerning the symptoms and clinical signs of LSD use and the productivity losses it causes. When unusual occurrences are found, they should be reported as quickly as possible to the veterinary authorities. It

is not advisable to bring animals suspected of having febrile nodular skin disease to farms. There should be an effort made to reduce the number of vectors in the affected areas. Unaffected animals can avoid infection by using mosquito repellent to eliminate mechanical transmission of LSD.

Due to the growing trade in various animal products and live animals, poverty in rural communities, a lack of diagnostic tools, and restricted availability of effective vaccinations in endemic areas, and other factors, LSDV is rapidly spreading across Pakistan. By implementing various control strategies, vector-borne illnesses may be managed. LSDV may be controlled with antibiotic medication. (Salib & Osman, 2011) on experiment bases LSDV can be controlled, they found that a weird mix of supportive treatment, antibacterial agents, anti-inflammatory drugs, and antiseptic treatments had positive outcomes. Within two to three weeks, mastitis, myiasis, pneumonia, diarrhea, and lameness may all improve (Salib & Osman, 2011).

Because LSDV therapy is highly expensive and not available to every farmer in the nation, adequate preventative measures should be taken to effectively manage the significant economic losses of milk reduction and skin damage, and hide the loss. In a different research, (Gari et al., 2010) found that immunization may lessen the loss of lactation and animal products owing to myiasis, fever, mortality, and miscarriage (Jamil et al., 2022).

Health And Economic Impact

LSD has a direct or indirect socioeconomic impact that has been reported by a number of important industries and sectors. In South Asia, where 21% of the world's dairy farm animals reside, the initial and most noticeable effect of LSD is the substantial decrease in milk production (Das et al., 2021). A Turkish analysis found that per lactation, an affected cow's average milk production decreased by 159L (Chihota et al., 2001). Despite the likelihood of secondary bacterial infection, meat from LSD-infected animals is still allowed to enter the food chain. Due to LSDV infection, it was observed that indigenous breeds and Friesian cattle in Ethiopia produced less meat annually by an estimated 1.2% and 6.2%, respectively (Radostits et al., 2007). Additionally, any scars, breaches, or lesions in the raw skin or hide of cattle may reduce the quality of leather, as was the case with animal skins that had been significantly impacted by LSD (Neamat-Allah, 2015). In 56% of cases, Bangladeshi leather is produced from cows, which is well acknowledged for its higher quality (Siddiky, 2017), accounting for 3.5% of the country's yearly exports (Şevik & Doğan, 2017). India is the tenth-largest exporter of leather products in the world, bringing in $8.500 billion yearly (Gari et al., 2011). Animals cannot be effectively used for draught because of pyrexia and lameness. Artificial insemination with diseased bull semen can spread LSD to breeding cattle, lowering the pregnancy rate (Das et al., 2021; E Tuppurainen et al., 2017).

The expenditures associated with trade restrictions, vaccination, quarantine and therapeutic interventions, feed, and labor, stamping out, maintaining farm biosecurity, and other factors are included in the indirect economic effect of LSD. Farm owners must pay an extra price for supplemental feed for diseased animals during their recovery time in addition to the extended period for fattening (Green, 1959). The cost of treating the infected cattle with broad-spectrum antibiotics and anti-inflammatory drugs as part of the LSD treatment in Jordan was calculated at US$ 35.04 (Paul et al., 2013). Sometimes it is necessary to eradicate a large population of diseased animals, as was the case in Greece (Hong, 2018) and Bulgaria, which experienced the worst

economic disaster with losses of almost US$ 8000 per herd (Dwivedi et al., 2019). The probability of LSD spreading quickly across the production and marketing routes is significant since it is a transboundary infectious disease (Tadesse Degu & Fesseha, 2020). Considering culling rates and the number of bulls in danger, a risk assessment study for LSD conducted on an Ethiopian bull market estimates a financial loss of US$6,67,785.6 (Abutarbush et al., 2016). It is not always logical to implement quarantine in a peripheral farming program in order to save money. Estimated quarantine costs in the USA were $145,000 (2010 US dollars) (Agianniotaki et al., 2017), which included personnel costs, cost of food, diagnostic tests costs, costs associated with throwing out test positives, and miscellaneous costs. Israel spent about $750,000 to contain the initial LSD outbreak by slaughtering all suspicious animals nearby and carrying out the ring vaccination (Casal et al., 2018; Das et al., 2021; Rossiter & Al Hammadi, 2009).

Future Recommendations

The disease has become a major concern to small-scale farmers. The disease was prevalent in greater Africa until the nineteenth century, then spread to the Russian Federation, Eastern Europe, the Middle East, and, more recently, Asia (Das et al., 2021; Saltykov et al., 2022). The scientific community has taken notice of LSD's repeated attacks on sensitive regions. It is to be understood that now is the crucial moment to plan for emergencies in order to prevent this transboundary disease from spreading abundantly. The focus should be placed on vector control, movement restriction, strict quarantine, advanced vaccination campaigns, appropriate veterinary treatment, and general management of farm sanitation facilities to prevent the invasion and spread of the disease (Das et al., 2021).

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

References

[1] C. Chihota, L. Rennie, R. Kitching, and P. Mellor, "Attempted mechanical transmission of lumpy skin disease virus by biting insects," *Medical and Veterinary Entomology,* vol. 17, no. 3, pp. 294-300, 2003.

[2] M. A. Shalaby *et al.*, "Recombinase polymerase amplification assay for rapid detection of lumpy skin disease virus," *BMC veterinary research,* vol. 12, no. 1, pp. 1-6, 2016.

[3] E. Young, P. Basson, and K. Weiss, "Experimental infection of game animals with lumpy skin disease virus (prototype strain Neethling)," 1970.

[4] T. Usadov *et al.*, "Lumpy skin disease virus, isolated in 2015 in Russia from cattle, is pathogenic for sheep at experimental infection," *Sel'skokhozyaĭstvennaya Biologiya,* vol. 53, no. 2, pp. 438-446, 2018.

[5] E. Authority, "Scientific opinion on the risks to plant health posed by Xylella fastidiosa in the EU territory, with the identification and evaluation of risk reduction options," *Efsa Journal,* vol. 13, no. 1, 2015.

[6] R. Hedger and C. Hamblin, "Neutralising antibodies to lumpy skin disease virus in African wildlife," *Comparative immunology, microbiology and infectious diseases,* vol. 6, no. 3, pp. 209-213, 1983.

[7] B. Barnard, "Antibodies against some viruses of domestic animals in southern African wild animals," 1997.

[8] M. E. H. Kayesh, M. T. Hussan, M. A. Hashem, M. Eliyas, and A. M. Anower, "Lumpy skin disease virus infection: An emerging threat to cattle health in Bangladesh," *Hosts and Viruses,* vol. 7, no. 4, p. 97, 2020.

[9] F. Davies, "Observations on the epidemiology of lumpy skin disease in Kenya," *Epidemiology & Infection,* vol. 88, no. 1, pp. 95-102, 1982.

[10] C. Hamblin, E. Anderson, M. Jago, T. Mlengeya, and K. Hirji, "Antibodies to some pathogenic agents in free-living wild species in Tanzania," *Epidemiology & Infection,* vol. 105, no. 3, pp. 585-594, 1990.

[11] E. Tulman, C. Afonso, Z. Lu, L. Zsak, G. Kutish, and D. Rock, "Genome of lumpy skin disease virus," *Journal of virology,* vol. 75, no. 15, pp. 7122-7130, 2001.

[12] K. Weiss, "Lumpy skin disease virus," in *Cytomegaloviruses. Rinderpest Virus. Lumpy Skin Disease Virus*: Springer, 1968, pp. 111-131.

[13] U. Von Backstrom, "Ngamiland cattle disease: preliminary report on a new disease, the etiological agent being probably of an infectious nature," *Journal of the South African Veterinary Association,* vol. 16, no. 1, pp. 29-35, 1945.

[14] S. Wainwright, A. El Idrissi, R. Mattioli, M. Tibbo, F. Njeumi, and E. Raizman, "Emergence of lumpy skin disease in the Eastern Mediterranean Basin countries," *FAO Empres Watch,* vol. 29, pp. 1-6, 2013.

[15] S. Zeynalova, K. Asadov, F. Guliyev, M. Vatani, and V. Aliyev, "Epizootology and molecular diagnosis of lumpy skin disease among livestock in Azerbaijan," *Frontiers in Microbiology,* p. 1022, 2016.

[16] E. F. S. Authority, "Lumpy skin disease: I. Data collection and analysis," *EFSA Journal,* vol. 15, no. 4, 2017.

[17] K. Al-Salihi, "Lumpy skin disease: Review of literature," *Mirror of research in veterinary sciences and animals,* vol. 3, no. 3, pp. 6-23, 2014.

[18] E. Tuppurainen, T. Alexandrov, and D. Beltrán-Alcrudo, "Lumpy skin disease-a manual for veterinarians," *FAO Animal Production and Health Manual,* no. 20, 2017.

[19] E. S. Tuppurainen *et al.*, "A potential role for ixodid (hard) tick vectors in the transmission of lumpy skin disease virus in cattle," *Transboundary and emerging diseases,* vol. 58, no. 2, pp. 93-104, 2011.

[20] J. Lubinga, E. Tuppurainen, W. Stoltsz, K. Ebersohn, J. Coetzer, and E. Venter, "Detection of lumpy skin disease virus in saliva of ticks fed on lumpy skin disease virus-infected cattle," *Experimental and applied acarology,* vol. 61, no. 1, pp. 129-138, 2013.

[21] J. Bosch, I. Iglesias, M. Muñoz, and A. De la Torre, "A cartographic tool for managing African swine fever in Eurasia: mapping wild boar distribution based on the quality of available habitats," *Transboundary and emerging diseases,* vol. 64, no. 6, pp. 1720-1733, 2017.

[22] H. Rgbe, "Lumpy skin disease (LSD): outbreak investigation, isolation and molecular detection of lumpy skin disease in selected areas of eastern Shewa, Ethiopia," Doctoral dissertation, AAU. 72. p, 2014.

[23] J. Morris, "Pseudo-urticaria," *Northern Rhodesia. Dept Anim Health Ann Rpt,* vol. 12, 1930.

[24] E. S. Tuppurainen, E. H. Venter, J. A. Coetzer, and L. Bell-Sakyi, "Lumpy skin disease: attempted propagation in tick cell lines and presence of viral DNA in field ticks collected from naturally-infected cattle," *Ticks and Tick-borne Diseases,* vol. 6, no. 2, pp. 134-140, 2015.

[25] M. Das *et al.*, "An updated review on lumpy skin disease: Perspective of southeast asian countries," *J. Adv. Biotechnol. Exp. Ther*, vol. 4, no. 3, pp. 322-333, 2021.
[26] C. H. Annandale, D. E. Holm, K. Ebersohn, and E. H. Venter, "Seminal transmission of lumpy skin disease virus in heifers," *Transboundary and emerging diseases*, vol. 61, no. 5, pp. 443-448, 2014.
[27] C. Chihota, L. Rennie, R. Kitching, and P. Mellor, "Mechanical transmission of lumpy skin disease virus by Aedes aegypti (Diptera: Culicidae)," *Epidemiology & Infection*, vol. 126, no. 2, pp. 317-321, 2001.
[28] P. Irons, E. Tuppurainen, and E. Venter, "Excretion of lumpy skin disease virus in bull semen," *Theriogenology*, vol. 63, no. 5, pp. 1290-1297, 2005.
[29] E. Tuppurainen *et al.*, "Capripoxvirus diseases: current status and opportunities for control," *Transboundary and emerging diseases*, vol. 64, no. 3, pp. 729-745, 2017.
[30] J. Lubinga, E. Tuppurainen, R. Mahlare, J. Coetzer, W. Stoltsz, and E. Venter, "Evidence of Transstadial and Mechanical Transmission of Lumpy Skin Disease Virus by A mblyomma hebraeum Ticks," *Transboundary and emerging diseases*, vol. 62, no. 2, pp. 174-182, 2015.
[31] E. S. Tuppurainen *et al.*, "Evidence of vertical transmission of lumpy skin disease virus in Rhipicephalus decoloratus ticks," *Ticks and tick-borne diseases*, vol. 4, no. 4, pp. 329-333, 2013.
[32] J. C. Lubinga *et al.*, "Demonstration of lumpy skin disease virus infection in Amblyomma hebraeum and Rhipicephalus appendiculatus ticks using immunohistochemistry," *Ticks and Tick-borne Diseases*, vol. 5, no. 2, pp. 113-120, 2014.
[33] P. J. Quinn, B. K. Markey, F. Leonard, E. FitzPatrick, and S. Fanning, "Concise review of veterinary microbiology," 2015.
[34] A. M. King, M. J. Adams, E. B. Carstens, and E. J. Lefkowitz, "Virus taxonomy: classification and nomenclature of viruses," in *Virus taxonomy: classification and nomenclature of viruses*, 2012, pp. 1327-1327.
[35] E. Tulman *et al.*, "The genomes of sheeppox and goatpox viruses," *Journal of virology*, vol. 76, no. 12, pp. 6054-6061, 2002.
[36] S. Biswas *et al.*, "Extended sequencing of vaccine and wild-type capripoxvirus isolates provides insights into genes modulating virulence and host range," *Transboundary and emerging diseases*, vol. 67, no. 1, pp. 80-97, 2020.
[37] A. Diesel, *The epizootology of" lumpy skin disease" in South Africa*. 1949.
[38] J. Brenner *et al.*, "Lumpy skin disease (LSD) in a large dairy herd in Israel, June 2006," *Israel Journal of Veterinary Medicine*, vol. 61, no. 3/4, p. 73, 2006.
[39] F. Davies, "Lumpy skin disease in virus diseases of food animals, Disease Monogtaphs. Vo 2 ed Ep. j Gibbs," ed: Academic press London, 1981.
[40] E. Tuppurainen and C. Oura, "lumpy skin disease: an emerging threat to Europe, the Middle East and Asia," *Transboundary and emerging diseases*, vol. 59, no. 1, pp. 40-48, 2012.
[41] J. Coetzer and E. Tuppurainen, "Lumpy skin disease," *Infectious diseases of livestock*, vol. 2, pp. 1268-1276, 2004.
[42] H. Abdulqa, H. Rahman, H. Dyary, and H. Othman, "Lumpy skin disease," *Reprod. Immunol. Open Access*, vol. 1, no. 25, pp. 2476-1974, 2016.
[43] E. Munz and N. Owen, "Electron microscopic studies on lumpy skin disease virus type" Neethling"," 1966.

[44] B. Ghaboussi, "Morphology and physical characteristics of sheep and goat pox viruses," *Archives of Razi Institute,* vol. 30, no. 1, pp. 107-115, 1978.
[45] K. Weiss and S. Geyer, "The effect of lactalbumin hydrolysate on the cytopathogenesis of lumpy skin disease virus in tissue culture," *Bull. epizoot. Dis. Afr,* vol. 7, p. 243, 1959.
[46] M. Burdin, "The use of histopathological examinations of skin material for the diagnosis of lumpy skin disease in Kenya," *Bull. Epizootic Dis. of Africa,* vol. 7, pp. 21-26, 1959.
[47] K. MacOwan, "Observations on the epizootiology of lumpy skin disease during the first year of its occurrence in Kenya," *Bull. Epizootic Dis. of Africa,* vol. 7, pp. 7-20, 1959.
[48] J. Coetzer, "Lumpy skin disease in Infectious disease of livestock. JAW Cloetzer and RC Tustin," ed: Oxford University Pro, 2004.
[49] D. Haig, "Lumpy skin disease," *Bull. Epizoot. Dis. Afr,* vol. 5, no. 9, pp. 421-430, 1957.
[50] B. Hailu, G. Alemayehu, and N. Seid, "Epidemiology, economic importance and control techniques of lumpy skin diseases," *Anim. Vet. Sci,* vol. 3, no. 2, pp. 58-66, 2015.
[51] F. A. Salib and A. H. Osman, "Incidence of lumpy skin disease among Egyptian cattle in Giza Governorate, Egypt," *Veterinary world,* vol. 4, no. 4, 2011.
[52] R. Kitching, P. Bhat, and D. Black, "The characterization of African strains of capripoxvirus," *Epidemiology & Infection,* vol. 102, no. 2, pp. 335-343, 1989.
[53] F. G. Davies, "Lumpy skin disease of cattle: a growing problem in Africa and the Near East," *World Animal Review,* vol. 68, no. 3, pp. 37-42, 1991.
[54] G. Gari, P. Bonnet, F. Roger, and A. Waret-Szkuta, "Epidemiological aspects and financial impact of lumpy skin disease in Ethiopia," *Preventive veterinary medicine,* vol. 102, no. 4, pp. 274-283, 2011.
[55] S. Abutarbush *et al.*, "Lumpy Skin Disease in J ordan: Disease Emergence, Clinical Signs, Complications and Preliminary-associated Economic Losses," *Transboundary and emerging diseases,* vol. 62, no. 5, pp. 549-554, 2015.
[56] K. Al-Salihi and I. Hassan, "Lumpy skin disease in Iraq: study of the disease emergence," *Transboundary and emerging diseases,* vol. 62, no. 5, pp. 457-462, 2015.
[57] P. Sameea Yousefi, K. Mardani, B. Dalir-Naghadeh, and G. Jalilzadeh-Amin, "Epidemiological study of lumpy skin disease outbreaks in North-western Iran," *Transboundary and emerging diseases,* vol. 64, no. 6, pp. 1782-1789, 2017.
[58] M. H. Tageldin *et al.*, "Lumpy skin disease of cattle: an emerging problem in the Sultanate of Oman," *Tropical animal health and production,* vol. 46, no. 1, pp. 241-246, 2014.
[59] M. M. Elhaig, A. Selim, and M. Mahmoud, "Lumpy skin disease in cattle: Frequency of occurrence in a dairy farm and a preliminary assessment of its possible impact on Egyptian buffaloes," *Onderstepoort Journal of Veterinary Research,* vol. 84, no. 1, pp. 1-6, 2017.
[60] S. Sharawi and I. Abd El-Rahim, "The utility of polymerase chain reaction for diagnosis of lumpy skin disease in cattle and water buffaloes in Egypt," *Revue Scientifique et Technique-OIE,* vol. 30, no. 3, p. 821, 2011.
[61] C. Gortázar, P. Barroso, R. Nova, and G. Cáceres, "The role of wildlife in the epidemiology and control of Foot-and-mouth-disease And Similar

Transboundary (FAST) animal diseases: a review," *Transboundary and Emerging Diseases,* vol. 69, no. 5, pp. 2462-2473, 2022.

[62] J. Wolff *et al.*, "Experimental infection and genetic characterization of two different capripox virus isolates in small ruminants," *Viruses,* vol. 12, no. 10, p. 1098, 2020.

[63] P. D. Constable, K. W. Hinchcliff, S. H. Done, and W. Grünberg, *Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats*. Elsevier Health Sciences, 2016.

[64] E. Mulatu and A. Feyisa, "Review: Lumpy skin disease," *J. Vet. Sci. Technol,* vol. 9, no. 535, pp. 1-8, 2018.

[65] A. Gumbe, "Review on lumpy skin disease and its economic impacts in Ethiopia," *J. Dairy Vet. Anim. Res,* vol. 7, no. 2, pp. 39-46, 2018.

[66] J. C. Lubinga, E. S. Tuppurainen, J. A. Coetzer, W. H. Stoltsz, and E. H. Venter, "Transovarial passage and transmission of LSDV by Amblyomma hebraeum, Rhipicephalus appendiculatus and Rhipicephalus decoloratus," *Experimental and applied acarology,* vol. 62, no. 1, pp. 67-75, 2014.

[67] F. Namazi and A. Khodakaram Tafti, "Lumpy skin disease, an emerging transboundary viral disease: A review," *Veterinary Medicine and Science,* vol. 7, no. 3, pp. 888-896, 2021.

[68] A. Khan, X. Du, R. Hussain, and O.-D. Kwon, "LUMPY SKIN DISEASE: A THREAT TO THE LIVESTOCK INDUSTRY-A REVIEW."

[69] S. Babiuk, T. Bowden, D. Boyle, D. B. Wallace, and R. Kitching, "Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle," *Transboundary and emerging diseases,* vol. 55, no. 7, pp. 263-272, 2008.

[70] E. S. Tuppurainen, E. Venter, and J. Coetzer, "The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques," *Onderstepoort Journal of Veterinary Research,* vol. 72, no. 2, pp. 153-164, 2005.

[71] E. El-Nahas, A. El-Habbaa, G. El-Bagoury, and M. Radwan, "Isolation and identification of lumpy skin disease virus from naturally infected buffaloes at Kaluobia, Egypt," *Global Veterinaria,* vol. 7, no. 3, pp. 234-237, 2011.

[72] C. E. Lamien *et al.*, "Use of the Capripoxvirus homologue of Vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: Development of a classical PCR method to differentiate Goat poxvirus from Sheep poxvirus," *Veterinary microbiology,* vol. 149, no. 1-2, pp. 30-39, 2011.

[73] R. Magori-Cohen *et al.*, "Mathematical modelling and evaluation of the different routes of transmission of lumpy skin disease virus," *Veterinary research,* vol. 43, no. 1, pp. 1-13, 2012.

[74] E. Kahana-Sutin, E. Klement, I. Lensky, and Y. Gottlieb, "High relative abundance of the stable fly Stomoxys calcitrans is associated with lumpy skin disease outbreaks in Israeli dairy farms," *Medical and Veterinary Entomology,* vol. 31, no. 2, pp. 150-160, 2017.

[75] A. Sprygin *et al.*, "Analysis and insights into recombination signals in lumpy skin disease virus recovered in the field," *PLoS One,* vol. 13, no. 12, p. e0207480, 2018.

[76] S. Stubbs, C. A. Oura, M. Henstock, T. R. Bowden, D. P. King, and E. S. Tuppurainen, "Validation of a high-throughput real-time polymerase chain reaction assay for the detection of capripoxviral DNA," *Journal of Virological Methods,* vol. 179, no. 2, pp. 419-422, 2012.

[77] I. Yeruham *et al.*, "Spread of lumpy skin disease in Israeli dairy herds," *Veterinary Record*, vol. 137, pp. 91-91, 1995.
[78] F. Baldacchino, V. Muenworn, M. Desquesnes, F. Desoli, T. Charoenviriyaphap, and G. Duvallet, "Transmission of pathogens by Stomoxys flies (Diptera, Muscidae): a review," *Parasite*, vol. 20, 2013.
[79] M. Şevik and M. Doğan, "Epidemiological and molecular studies on lumpy skin disease outbreaks in Turkiye during 2014–2015," *Transboundary and emerging diseases*, vol. 64, no. 4, pp. 1268-1279, 2017.
[80] S. Gubbins, S. Carpenter, M. Baylis, J. L. Wood, and P. S. Mellor, "Assessing the risk of bluetongue to UK livestock: uncertainty and sensitivity analyses of a temperature-dependent model for the basic reproduction number," *Journal of the Royal Society Interface*, vol. 5, no. 20, pp. 363-371, 2008.
[81] C. H. Annandale, P. C. Irons, V. P. Bagla, U. I. Osuagwuh, and E. H. Venter, "Sites of persistence of lumpy skin disease virus in the genital tract of experimentally infected bulls," *Reproduction in domestic animals*, vol. 45, no. 2, pp. 250-255, 2010.
[82] M. Givens, "Risks of disease transmission through semen in cattle," *Animal*, vol. 12, no. s1, pp. s165-s171, 2018.
[83] K. Tasioudi *et al.*, "Emergence of lumpy skin disease in Greece, 2015," *Transboundary and Emerging Diseases*, vol. 63, no. 3, pp. 260-265, 2016.
[84] P. Biswas, M. Rahman, A. Das, S. Ahmed, M. Giasuddin, and J. P. Christensen, "Risk for highly pathogenic avian influenza H5N1 virus infection in chickens in small-scale commercial farms, in a high-risk area, Bangladesh, 2008," *Transboundary and Emerging Diseases*, vol. 58, no. 6, pp. 519-525, 2011.
[85] S. M. Abutarbush, "Lumpy skin disease (knopvelsiekte, pseudo-urticaria, neethling virus disease, exanthema nodularis bovis)," in *Emerging and re-emerging infectious diseases of livestock*: Springer, 2017, pp. 309-326.
[86] Y. R. Khan *et al.*, "A review: surveillance of lumpy skin disease (LSD) a growing problem in Asia," *Microbial Pathogenesis*, vol. 158, p. 105050, 2021.
[87] S. Saiyad, H. Patel, and B. Bhanderi, "Lumpy skin disease (LSD): An overview," 2022.
[88] O. T. Manual, "Lumpy skin disease, Chapter 2.4. 14," ed, 2010.
[89] S. B. Sudhakar *et al.*, "Lumpy skin disease (LSD) outbreaks in cattle in Odisha state, India in August 2019: Epidemiological features and molecular studies," *Transboundary and Emerging Diseases*, vol. 67, no. 6, pp. 2408-2422, 2020.
[90] E. Tuppurainen *et al.*, "Field observations and experiences gained from the implementation of control measures against lumpy skin disease in South-East Europe between 2015 and 2017," *Preventive Veterinary Medicine*, vol. 181, p. 104600, 2020.
[91] G. Lu, J. Xie, J. Luo, R. Shao, K. Jia, and S. Li, "Lumpy skin disease outbreaks in China, since 3 August 2019," *Transboundary and Emerging Diseases*, vol. 68, no. 2, pp. 216-219, 2021.
[92] A. Sajid *et al.*, "Prevalence of goatpox disease in Punjab province of Pakistan," *J Anim Plt Sci*, vol. 22, no. 2 Suppl, pp. 28-32, 2012.
[93] M. Jamil *et al.*, "Lumpy Skin Disease: An insights in Pakistan," *Pakistan Journal of Medical & Health Sciences*, vol. 16, no. 06, pp. 824-824, 2022.
[94] E. Mulatu and A. Feyisa, "Journal of Veterinary Science & Technology," 2018.

[95] M. A. Alkhamis and K. VanderWaal, "Spatial and temporal epidemiology of lumpy skin disease in the Middle East, 2012–2015," *Frontiers in veterinary science*, vol. 3, p. 19, 2016.
[96] G. Gari, A. Waret-Szkuta, V. Grosbois, P. Jacquiet, and F. Roger, "Risk factors associated with observed clinical lumpy skin disease in Ethiopia," *Epidemiology & Infection*, vol. 138, no. 11, pp. 1657-1666, 2010.
[97] O. M. Radostits, C. Gay, K. W. Hinchcliff, and P. D. Constable, "A textbook of the diseases of cattle, horses, sheep, pigs and goats," *Veterinary medicine*, vol. 10, pp. 2045-2050, 2007.
[98] A. N. Neamat-Allah, "Immunological, hematological, biochemical, and histopathological studies on cows naturally infected with lumpy skin disease," *Veterinary world*, vol. 8, no. 9, p. 1131, 2015.
[99] M. Siddiky, "Dairying in South Asian region: opportunities, challenges and way forward," *SAARC Journal of Agriculture*, vol. 15, no. 1, pp. 173-187, 2017.
[100] H. Green, "Lumpy skin disease: its effect on hides and leather and a comparison on this respect with some other skin diseases," *Bull. Epizoot. Dis. Afr*, vol. 7, pp. 63-74, 1959.
[101] H. Paul, A. P. M. Antunes, A. D. Covington, P. Evans, and P. S. Phillips, "Bangladeshi leather industry: An overview of recent sustainable developments," *Journal of the Society of Leather Technologists and Chemists*, vol. 97, no. 1, pp. 25-32, 2013.
[102] S. C. Hong, "Developing the leather industry in Bangladesh," 2018.
[103] A. Dwivedi, D. Agrawal, and J. Madaan, "Sustainable manufacturing evaluation model focusing leather industries in India: a TISM approach," *Journal of Science and Technology Policy Management*, 2019.
[104] B. M. Tadesse Degu and H. Fesseha, "Epidemiological status and economic impact of lumpy skin disease-review," *Int J Recent Biotechnol*, vol. 8, pp. 1-15, 2020.
[105] S. Abutarbush *et al.*, "Adverse reactions to field vaccination against lumpy skin disease in Jordan," *Transboundary and emerging diseases*, vol. 63, no. 2, pp. e213-e219, 2016.
[106] E. I. Agianniotaki *et al.*, "Lumpy skin disease outbreaks in Greece during 2015–16, implementation of emergency immunization and genetic differentiation between field isolates and vaccine virus strains," *Veterinary microbiology*, vol. 201, pp. 78-84, 2017.
[107] J. Casal *et al.*, "Economic cost of lumpy skin disease outbreaks in three Balkan countries: Albania, Bulgaria and the Former Yugoslav Republic of Macedonia (2016-2017)," *Transboundary and emerging diseases*, vol. 65, no. 6, pp. 1680-1688, 2018.
[108] P. B. Rossiter and N. Al Hammadi, "Living with transboundary animal diseases (TADs)," *Tropical animal health and production*, vol. 41, no. 7, pp. 999-1004, 2009.
[109] Y. V. Saltykov, A. A. Kolosova, and V. A. Feodorova, "UPDATE OF LUMPY SKIN DISEASE: EMERGENCE IN ASIAN PART OF EURASIA," *Acta Veterinaria*, vol. 72, no. 3, 2022.

About The Authors

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkiye. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS),

Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 68 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.
E-mail: msafdar@cuvas.edu.pk, ORCID: 0000 0002 3720 2090

Prof. Dr. Mehmet OZASLAN received his PhD in 1995 at the Institute of Natural Sciences at Cukurova University, Turkiye. He is a professor of molecular biology and genetics. His research interests include cancer genetics, molecular virology, molecular genetics, microbiology, genetic mutations, etc. He has published more than 200 research articles in national and international well-reputed journals. He has also written many book chapters as well as edited books. He is an editor and editor-in-chief of many well-reputed national and international journals.
E-mail: ozaslanmd@gantep.edu.tr, ORCID: 0000 0001 9380 4902

Shafeeq ur Rehman graduated from the Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur. He continues to work on FMD and PPR viruses as his area of interest. He is still working as a Research Assistant in FMDV and PPRV project in Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan.
E-mail: shafeequrrehmanbhutta@gmail.com, ORCID: 0000-0003-3571-8226

Furqan Shafqat graduated from Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in medical biotechnology from University of Wollongong Australia.
E-mail: furqanshafqat007@gmail.com, ORCID: 0000-0002-2563-913X

Muhammad Shan graduated from Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan. He has a bachelor's degree in Applied Microbiology. He continues his post graduate study in Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur. He continues to work on antimicrobial resistance, virology, molecular biology, and immunology as his area of interest especially FMD and PPR viruses as his area of interest. He is working as a Research Assistant in NRPU project in Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan.
E-mail: muhammadshanofficial@gmail.com, ORCID: 0009-0000-0839-847X

To Cite This Chapter:
Safdar, M et al., (2023). **A global burden of lumpy skin disease, outbreaks, and future challenges**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 284-300). ISRES Publishing

INTEGRATING GENOMICS AND PRECISION MEDICINE

Hafiz Ishfaq AHMAD
Hira HAMEED
Mohsin ABBAS
Abdul JABAR

Introduction

Genomics and precision medicine sciences have gained great traction in recent years and are set to transform the healthcare system completely (Fernandes et al., 2017). Understanding the whole genetic makeup of an individual has been made possible through the study of genomics, which has shed light on the hereditary causes of many disorders. Simultaneously, precision medicine emphasizes customizing medical interventions to the distinct features of every individual patient (Fernando Carrasco-Ramiro et al., 2017). There is great potential for enhancing disease prevention, diagnosis, and treatment by integrating genomes and precision medicine, eventually improving patient outcomes (Panahiazar et al., 2014).

Our knowledge of human genetics has been completely changed by genomics, which has illuminated the intricate relationship between genetic variants and disease vulnerability (Goodrich et al., 2017). Researchers have successfully utilized sophisticated sequencing technologies and bioinformatics techniques to decode the genetic code and detect genetic variants, including single nucleotide polymorphisms (SNPs), copy number variations (CNVs), and structural rearrangements (Nakatochi et al., 2021). These genetic variations can influence an individual's predisposition to diseases, their response to therapies, and their likelihood of experiencing adverse drug reactions (Golomb & Evans, 2008).

On the other hand, precision medicine recognizes that each patient is unique, with distinct genetic profiles, environmental exposures, and lifestyle factors (Schoettler & Strek, 2020). It emphasizes the need to move away from a one-size-fits-all approach to healthcare and instead tailor medical interventions to the specific needs of each individual (Juengst et al., 2020). By considering an individual's genetic information alongside other relevant clinical data, precision medicine aims to optimize treatment decisions, maximize therapeutic efficacy, and minimize adverse effects (Enck et al., 2013).

Integrating genomics and precision medicine represents a paradigm shift in healthcare (Pritchard et al., 2017). By combining the wealth of genetic information provided by genomics with the individualized approach of precision medicine, healthcare providers can make more informed decisions and deliver personalized, targeted treatments (Griffin, 2022). This approach can transform clinical practice, enabling clinicians to identify the most appropriate treatment options based on an individual's genetic profile, predict treatment response, and tailor interventions to maximize efficacy and minimize harm (Pashayan et al., 2020; Scheen, 2016).

Moreover, integrating genomics and precision medicine extends beyond the clinic and has profound implications for research and drug development (Seyhan & Carini, 2019). By studying patients' genomic profiles, researchers can identify biomarkers that can serve as indicators of disease presence, progression, or response to treatment (Crowley et al., 2013). This knowledge enables the development of targeted therapies

and identifying specific patient populations most likely to benefit from these treatments (Y. Wang et al., 2020).

Despite the immense potential, integrating genomics and precision medicine is challenging (Dupont et al., 2021). The interpretation and validation of genomic data, the ethical and legal considerations surrounding genetic information, the privacy and security of patient data, and the need for robust infrastructure and workforce training are all crucial factors that must be addressed for successful implementation (Wright et al., 2013).

1. Genomics: Understanding the Genetic Landscape

Genomics, a field of study within genetics, is focused on understanding the complete set of genetic information encoded within an organism's DNA. It provides a comprehensive view of an individual's genetic landscape, encompassing all the genes, non-coding regions, and variations that make each person unique (Keller, 2011).

The human genome, consisting of approximately 3 billion base pairs, is a blueprint for building and maintaining a living organism (Passarge, 2007). Genomics seeks to unravel the intricacies of this blueprint by employing advanced technologies and analytical tools to sequence and interpret the DNA sequence (Wong et al., 2011).

Sequencing techniques, such as next-generation sequencing (NGS), have revolutionized the field of genomics by enabling the rapid and cost-effective determination of an individual's genetic code. NGS platforms can generate vast amounts of genomic data, revealing the order of nucleotide bases (adenine, thymine, cytosine, and guanine) that make up an individual's DNA (Satam et al., 2023).

Analyzing the genomic data allows scientists to identify genetic variations that contribute to human diversity and influence disease susceptibility and treatment response (Tam et al., 2019). These variations include single nucleotide polymorphisms (SNPs) and single-base differences in the DNA sequence between individuals (Tebbutt et al., 2007). SNPs can impact gene function, protein production, and cellular processes, affecting an individual's predisposition to certain diseases (Chorley et al., 2008).

In addition to SNPs, genomic analysis reveals other genetic variations, such as insertions, deletions, and rearrangements in the DNA sequence (Zhang et al., 2009). Copy number variations (CNVs), for example, involve duplications or deletions of specific segments of DNA, potentially leading to altered gene dosage and functional consequences (Pös et al., 2021). Structural variations, such as translocations or inversions, can disrupt the normal structure of chromosomes and have significant health implications (Spielmann et al., 2018).

Understanding the genetic landscape through genomics is not limited to studying the individual's DNA but also encompasses the interactions between genes and the environment (Fowler et al., 2011). Genomics allows researchers to investigate how genetic variations interact with environmental factors, lifestyle choices, and other variables to influence disease risk, treatment outcomes, and overall health (Suhre & Gieger, 2012).

Moreover, genomics plays a pivotal role in uncovering the genetic basis of rare genetic disorders and complex diseases (Maroilley & Tarailo-Graovac, 2019). By comparing the genomic profiles of affected individuals with healthy controls, scientists can identify disease-causing genetic variants and gain insights into the molecular

mechanisms underlying these conditions (Wei et al., 2021). This knowledge is instrumental in improving diagnostic accuracy, developing targeted therapies, and advancing personalized medicine approaches (Fernando Carrasco-Ramiro et al., 2017).

Genomics continually evolves, driven by technological advancements and data analysis methods (Hudson, 2008). As genomic sequencing becomes more accessible and affordable, its applications in healthcare, research, and personalized medicine continue to expand (Tawfik et al., 2023). Genomics holds the promise of unlocking deeper insights into the genetic basis of diseases, enabling the development of more effective treatments, and ultimately improving the health and well-being of individuals worldwide (Rexroad et al., 2019).

2. Precision Medicine: Tailoring Healthcare to the Individual

Precision medicine refers to the more accurate characterization of diseases through genetic and other approaches, enabling more precise targeting of disease subgroups with innovative medications. Two well-known instances include cancer and cystic fibrosis (Ashley, 2016). Precision medicine is defined formally as a systematic approach to healthcare that involves a set of decision rules specifically designed for each decision point, which establish a connection between the patient's current data and a recommended course of action. A range of treatment or care-related choices, encompassing judgments on pharmaceutical selection, dosage determination, timing of administration, endorsement of specific dietary or exercise regimens, and other potential alternatives, may be considered viable courses of action (Kosorok & Laber, 2019). With the support of both scientific and political viewpoints, the word "precision medicine" has gained tremendous popularity in recent years (Behrens, 2008; Khoury, 2015; Robinson, 2012). The fundamental idea behind precision medicine, which involves individualized treatment based on a person's genes, lifestyle, and environment, is not novel: transfusion patients and donors have been matched for over a century. However, developments in genetics and the expanding accessibility of health data provide a chance to turn precise personalized patient care into a practical reality (R. Hodson, 2016). Precision medicine represents an organic progression that integrates several research disciplines with clinical practice, establishing a comprehensive knowledge base that may effectively inform personalized patient treatment strategies (Bahcall, 2015). Precision medicine offers the potential to improve health outcomes by considering individuals' unique variations in genes, environment, and lifestyle. In the forthcoming decade, precision medicine is poised to significantly transform the healthcare landscape through its advancements in several crucial domains. These include the expansion of extensive cohorts, the integration of artificial intelligence (AI), the routine incorporation of clinical genomes, the incorporation of phenomics and environmental factors, and the delivery of valuable outcomes across diverse populations (Denny & Collins, 2021).

3. The Power of Integration: Genomics and Precision Medicine

Precision medicine has the potential to augment the field of medicine significantly. Nevertheless, implementing the required innovations will need significant time, potentially spanning several years. The utilization of genetics in medical decision-making has already been observed and is expected to increase in significance. To expedite these advancements, significant modifications are necessitated inside the framework and methodologies for data collection, storage, and exchange (S. J. Aronson & H. L. Rehm, 2015). The Human Genome Project (HGP), successfully

concluded in 2001, marked a significant milestone in advancing our understanding of medicine. Other programs similar to the Human Genome Project (HGP) are underway, encompassing a range of a few hundred to several million genomes. These programs vary in their specific objectives, ranging from targeted targets to a more comprehensive approach. P4 medicine, encompassing the principles of predictiveness, preventiveness, personalization, and participation, has emerged as a field that presents novel concepts, challenges, and prospects (F. Carrasco-Ramiro et al., 2017). In recent decades, genetic medicine has experienced rapid growth, emerging as a prominent discipline within acute and chronic healthcare. The rapid growth in this field has resulted in a deficiency in genomics knowledge and preparedness among nurses and healthcare professionals. The field of genomic medicine can potentially enhance the precision of evaluating, diagnosing, and treating certain acute care conditions (Kessler, 2018).

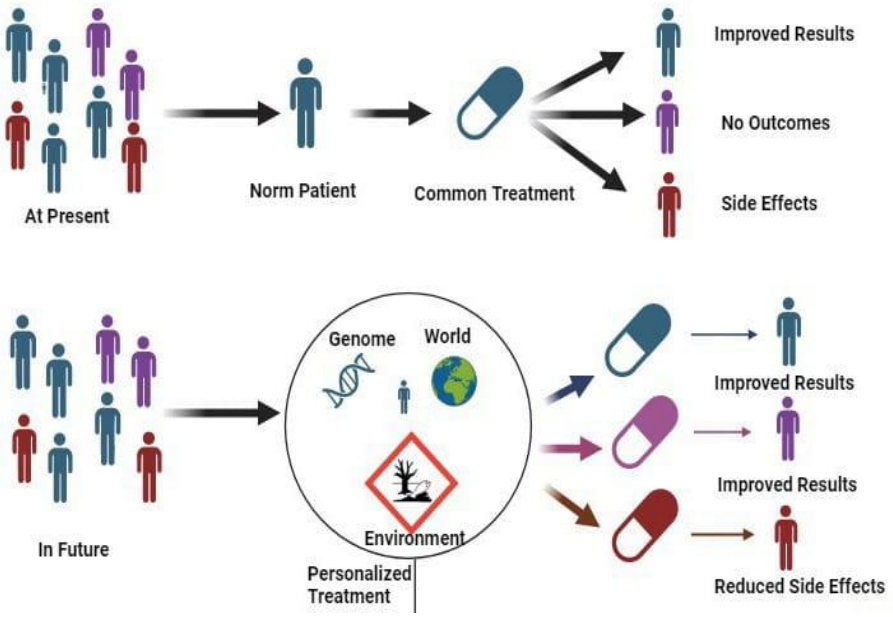


Figure 1. Effect of precision medicine on patients

4. Key Components of Integrating Genomics and Precision Medicine

Integrating genomics and precision medicine is the confluence of genetics, genomes, clinical data, and healthcare to deliver individualized and more effective medical treatment (Gordon, 2007). Comprehensive Genomic Profiling (CGP) is a strong and sophisticated molecular diagnostic method in genomics and precision medicine (Čerina et al., 2022). It includes systematically studying a patient's DNA to discover various genetic abnormalities, including mutations, copy number variations, rearrangements, and other genomic changes (Milbury et al., 2022). CGP attempts to give a thorough knowledge of the genetic composition of a patient's tumor or illness, which may be significant for diagnosis, prognosis, and treatment decision-making in numerous medical settings, notably in cancer care (Mateo et al., 2022). CGP can identify genetic abnormalities, including point mutations, insertions/deletions (indels), gene amplifications, and fusions (Guerrero et al., 2019). CGP may give useful prognostic information, helping doctors forecast the course of the medical condition and patient outcomes (Fumagalli & Barberis, 2021). Precision medicine depends

significantly on computational approaches and bioinformatics tools to process, analyze, and interpret large-scale genetic and clinical data (Gómez-López et al., 2019).

Table 1. Bioinformatics tools and their applications in precision medicine (Gómez-López et al., 2019)

Sr	Tools	Applications
1	**Genomic Sequencing** Next-Generation Sequencing (NGS)	This high-throughput technology is used for whole genome sequencing (WGS), whole exome sequencing (WES), RNA sequencing (RNA-seq), and other applications to generate genomic data.
2	**Variant Callers** Tools like GATK, Samtools, and FreeBayes **Variant Annotation** Tools like ANNOVAR and Variant Effect Predictor (VEP)	Provide information about the functional impact of genetic variants.
3	**Pharmacogenomics** PharmGKB	A database that collects information on drug-gene interactions, helping to predict how an individual may respond to a specific medication based on their genetic profile.
4	**SV callers** Tools like DELLY and Manta detect structural variations	Detect structural variations, such as translocations, inversions, and copy number variations (CNVs).
5	**Pathway Analysis** Ingenuity Pathway Analysis (IPA)	Helps identify biological pathways and networks affected by genomic variations, aiding in the understanding of disease mechanisms.
6	**Variant Interpretation** ClinVar	A public database that aggregates clinical interpretations of genetic variants, providing information on their significance in disease
7	**Clinical Decision Support Systems** IBM Watson for Genomics	Uses AI and machine learning to analyze genomic and clinical data to support healthcare professionals in making treatment decisions.
8	**Drug Target Prediction** DGIdb	A database that provides information on potential drug targets and their interactions with genomic alterations.
9	**Tumor Mutational Burden (TMB) Analysis** MSI Sensor and other TMB calculators	Calculate the TMB score, which can help determine eligibility for immunotherapy.

10	**Immune Profiling** CIBERSORT	Estimates the composition of immune cell populations in tumor samples based on gene expression data.
11	**Survival Analysis** Kaplan-Meier Plotter	Analyzes gene expression data to assess the impact of specific genes on patient survival.
12	**Data Integration** cBioPortal	Integrates genomic and clinical data to visualize genetic alterations in the context of pathways and patient outcomes.
13	**Data Privacy and Security** Secure Data Repositories	Systems like Genomic Data Commons (GDC) and the European Genome-phenome Archive (EGA) ensure the secure storage and sharing of genomic data while protecting patient privacy.
14	**Machine Learning and Artificial Intelligence (AI)** Deep Learning Models Random Forest, Support Vector Machines	Utilized for image analysis, clinical data prediction, and patient stratification. Used for various classification and prediction tasks.
15	**Cloud Computing Platforms**	Provide scalable resources for processing and analyzing large genomic datasets.

Artificial intelligence (AI) is experiencing significant growth as it focuses on developing computer systems that can perform tasks often associated with human intelligence. The rapid development of artificial intelligence (AI) software and technology, particularly neural network algorithms and graphics processing units (GPUs) utilized for training, has sparked a burgeoning interest in the field of medical AI applications (Dias & Torkamani, 2019). Since the beginning of scientific exploration, investigating the etiology of chronic, acute, infectious, and rare diseases has been a central area of inquiry within human health research. The increasing recognition of the intricate nature of these disorders has prompted us to acknowledge the necessity of accurately diagnosing and effectively treating individuals affected by them (Ahmed, 2022; Ahmed et al., 2023). Numerous methodologies have been employed in artificial intelligence (AI) research over several decades. One way involves simulating the human brain by representing it as a network of artificial neurons. These neurons receive input signals, analyze them, and transmit new signals to subsequent neurons (Chang et al., 2019). Physicians have always relied on their professional knowledge, judgment, and analytical skills in conjunction with rudimentary tools and constrained resources. The advent of digital health has brought about a societal shift wherein technical advancements have made enhanced medical processes accessible not just to healthcare professionals and their patients. Technologies such as genetics, biotechnology, wearable sensors, and artificial intelligence (AI) are increasingly being directed toward three main trajectories. Three key developments have emerged in the healthcare field. Firstly, there has been a shift towards prioritizing people as the central focus of care. Secondly, a substantial volume of data has been generated, necessitating the utilization of advanced analytics. Lastly, the foundation of precision medicine has been established (Mesko, 2017). The

temporal aspects of cancer detection, accurate cancer diagnosis, and determination of tumor stage are crucial factors that indicate tumor aggressiveness. These factors substantially influence clinical decision-making and ultimately affect patient outcomes. Throughout a limited timeframe, artificial intelligence (AI) has achieved significant advancements in the crucial domain of cancer. These advancements frequently exhibit comparable performance to human specialists while offering the supplementary advantages of scalability and automation (Bhinder et al., 2021).

5. Clinical Applications of Genomics and Precision Medicine

Genomics and precision medicine are revolutionizing healthcare by customizing medical care to individual genetic profiles, enabling better diagnosis, treatment, and prevention of diseases (Juengst et al., 2016; Strianese et al., 2020). This includes cancer genomic profiling, identifying hereditary disorders, tracking disease outbreaks, identifying medication resistance, and developing more potent treatments (Ahmed et al., 2020; McCarthy et al., 2013). Metagenomics analyzes the microbiome's genetic composition, and clinical trials use genomic data for patient selection and drug efficacy assessment (Zhao et al., 2021). Personalized treatment selection is a healthcare approach that tailors medical treatment to each patient's unique features, considering genetics, molecular profiles, lifestyle, and environmental factors (Pruis et al., 2022). This method aids in treatment decisions, targeting medications, monitoring and modifying therapy, and offering recommendations for illness prevention and health promotion (Kaplan & Stone, 2013). Examples include cancer tumour genetic profiling, cardiovascular disorders, psychiatric drugs, and rare genetic illnesses (Ginsburg & McCarthy, 2001). Risk assessment and predictive medicine are two key concepts in personalized medicine (Ainiwan et al., 2022). Predictive medicine uses genetic, molecular, clinical, and lifestyle data to predict a person's likelihood of developing a specific illness (Biesecker, 2013). Risk assessment systematically evaluates an individual's risk factors, such as genetics, environmental factors, and lifestyle choices (Reuben et al., 2017). This helps healthcare professionals suggest personalized preventative measures and informs shared decision-making between patients and healthcare professionals (Ozanne et al., 2014). Both approaches are essential for early interventions and personalized healthcare strategies (Simmons et al., 2012). Proactive disease prevention and early treatments can reduce illness incidence, severity, and healthcare expenditures (Sánchez-Hernández et al., 2020). Healthcare professionals evaluate risk factors, modify lifestyles, and promote vaccinations (Edelman & Kudzma, 2021). Regular health examinations aid in the early detection and monitoring of chronic illnesses (Health et al., 2006; Nami et al., 2022). Health promotion and education promote healthy habits (Yajima et al., 2001). Early treatment and medication are recommended for high-risk patients (Bhatt et al., 2004). Precision medicine and genetics can determine genetic vulnerability, enabling tailored preventative actions (Strianese et al., 2020). Community health programs provide access to healthcare and education. These initiatives not only improve individual health but also help healthcare systems remain sustainable (Brooks et al., 2017).

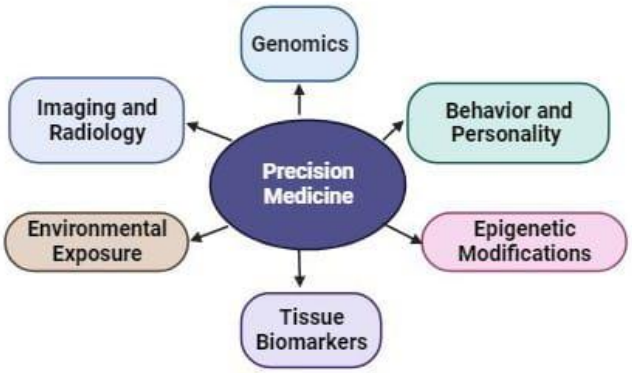


Figure 2. Clinical Applications of Precision Medicines in Healthcare

7. Genomics and Precision Medicine in Research and Drug Development

Drug development remains to be expensive and sluggish (Denny et al., 2018). Precision medicine highlights a unique interaction between genomes and drug development, one that gives both insights into the processes and possible treatment choices of a specific patient's ailment The application of genomics to guide drug research and development processes has prompted both enthusiasm and skepticism (Dugger et al., 2018). microRNA expression will not only help our knowledge of the illness but will also lead to the development of novel molecular biomarkers and therapeutic targets (Reid, 2015).

A complete mapping of the treatment-linked biomarkers will enable the identification of targets and histologies that may react to a certain medicine to plan better clinical trials (Movva et al., 2015). The core purpose of precision medicine is to identify biomarkers and therapeutic targets. N-glycoproteins represent a captivating group of proteins that hold promise as potential cancer biomarkers and therapeutic targets for small molecules, antibodies, and cellular interventions (Rolland et al., 2017). The dysregulation of many metabolites, including amino acids, lipids, nucleotides, and glycoses, is closely associated with chronic kidney disease (CKD). These metabolites hold promise as possible biomarkers for further investigation (Y.-N. Wang et al., 2019). The capacity to control NK cells for therapeutic reasons could rely on a fuller understanding of the biology of these cells and their relationship with autoimmune (Gianchecchi et al., 2021). Identifying the drug target is crucial to the success of mechanism-based drug development (Santos et al., 2017). Three primary strategies are commonly employed to maximize drugs' therapeutic efficacy while mitigating their adverse side effects. Three primary strategies can be employed to enhance drug delivery efficacy. Firstly, developing efficient drug delivery systems (DDSs) enables the targeted delivery of drug molecules to specific regions of interest. Secondly, the addition of permissible salts can facilitate the electrostatic propulsion of ionic drugs towards less polar target regions, thereby enhancing their solubility. Lastly, the elimination of surplus drug molecules that have been adsorbed onto cell membranes can be achieved through the utilization of biocompatible methods (Kundu et al., 2019).

8. Challenges in Integrating Genomics and Precision Medicine

Precision medicine is currently not effective. The inherent heterogeneity of individual tumours, especially between primary and metastatic tumours, and the heterogeneous character of cancer are recognised as major challenges in cancer therapies (Seoane &

De Mattos-Arruda, 2014). Data portals, such as the University of California, Santa Cruz (UCSC) Cancer Browser (now retired), cBioPortal, the International Cancer Genomics Consortium (ICGC) Data Portal, and the GDC Data Portal, possess a specialized backend infrastructure and serve as a proficient means to access and explore resource datasets that are centrally hosted. Currently, novel methodologies, including whole-genome sequencing11, DNA methylation whole-genome bisulfite sequencing12, and ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing13), are being employed to generate datasets for cancer genomics (Goldman et al., 2020). Structural variants (SVs) substantially impact inherited retinal diseases (IRD). Despite significant advancements in SV detection with the advent of genome sequencing, it is widely considered that the contribution of SVs to inherited retinal diseases (IRDs) is more than initially anticipated. To enhance the identification of gene-disruptive structural variations (SVs), a comprehensive analysis was conducted on short-read genome sequencing data (de Bruijn et al., 2023). NHS genetics facilities examined Scotland's Genomics England 100,000 Genomes Project diagnostic efficacy to assess genome sequencing for uncommon, hereditary diseases. With negative past genetic testing, four regional programmes recruited 999 people from 394 families in 200 unusual phenotypic categories (Hocking et al., 2023). Neurotherapies for diagnosis and treatment are consistently being made accessible to the general people beyond the confines of conventional healthcare environments. Illustrative instances encompass electroencephalography (EEG) neurofeedback, single-photon emission computerised tomography (SPECT) imaging employed for neuropsychiatric assessment, and the utilization of brain stimulation for off-label or experimental purposes [93]. The complexity of effectively engaging with genetic data is compounded by the discrepancy between our current capacity to generate such data and our limited understanding of its implications (Horton & Lucassen, 2023).

9. Future of Precision Medicine

Precision medicine is a method of healthcare that considers a patient's unique genetic, environmental, and lifestyle characteristics (Franssen et al., 2019; Williams et al., 2019). Advances in genomic medicine allow for better diagnosis and treatment choices (Juengst et al., 2020; Savoia et al., 2017). Pharmacogenomics and machine learning help identify trends and predict illness risks (Ho et al., 2019). Precision medicine includes preventive care and targeted therapies for rare diseases (Dzau et al., 2015). The integration of precision medicine into healthcare promises to generate more accurate diagnoses, anticipate disease susceptibility before symptoms manifest, and devise personalized treatment strategies that optimize both safety and efficacy (Johnson, Wei, Weeraratne, Frisse, Misulis, Rhee, Zhao, & Snowdon, 2021).

Summary

The integration of genomics and precision medicine has the potential to revolutionize healthcare by improving disease prevention, diagnosis, and treatment. Genomics has provided valuable insights into the genetic basis of diseases, allowing researchers to identify genetic variations that influence disease susceptibility and treatment response. Precision medicine recognizes that each patient is unique and aims to tailor medical interventions to the specific needs of each individual. By combining the wealth of genetic information provided by genomics with the individualized approach of precision medicine, healthcare providers can make more informed decisions and deliver personalized, targeted treatments. This approach can transform clinical practice, enabling clinicians to identify the most appropriate treatment options based on an individual's genetic profile, predict treatment response, and maximize efficacy

while minimizing harm. Furthermore, integrating genomics and precision medicine has profound research and drug development implications. By studying patients' genomic profiles, researchers can identify biomarkers that can serve as indicators of disease presence, progression, or response to treatment, leading to the development of targeted therapies and the identification of specific patient populations who are most likely to benefit from these treatments. However, some challenges need to be addressed for successful implementation, such as the interpretation and validation of genomic data, ethical and legal considerations surrounding genetic information, privacy and security of patient data, and the need for robust infrastructure and workforce training. Overall, the integration of genomics and precision medicine represents a paradigm shift in healthcare that has the potential to improve patient outcomes greatly.

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

Conflict of Interest

The authors declare no conflict of interest.

References

Ahmed, Z. (2022). "Precision medicine with multi-omics strategies, deep phenotyping, and predictive analysis." Progress in molecular biology and translational science **190**(1): 101-125.

Ahmed, Z., K. Mohamed, S. Zeeshan and X. Dong (2020). "Artificial intelligence with multi-functional machine learning platform development for better healthcare and precision medicine." Database **2020**: baaa010.

Ahmed, Z., S. Zeeshan and D. Lee (2023). "Artificial intelligence for personalized and predictive genomics data analysis." Frontiers in genetics **14**: 1162869.

Ainiwan, M., Q. Wang, G. Yesitayi and X. Ma (2022). "Identification of FERMT1 and SGCD as key marker in acute aortic dissection from the perspective of predictive, preventive, and personalized medicine." EPMA Journal **13**(4): 597-614.

Aronson, S. J. and H. L. Rehm (2015). "Building the foundation for genomics in precision medicine." Nature **526**(7573): 336-342.

Ashley, E. A. (2016). "Towards precision medicine." Nature Reviews Genetics **17**(9): 507-522.

Bahcall, O. (2015). "Precision medicine." Nature **526**(7573): 335-335.

Behrens, M. K. (2008). "Priorities for personalized medicine."

Bhatt, D. L., M. T. Roe, E. D. Peterson, Y. Li, A. Y. Chen, R. A. Harrington, A. B. Greenbaum, P. B. Berger, C. P. Cannon and D. J. Cohen (2004). "Utilization of early invasive management strategies for high-risk patients with non–ST-segment elevation acute coronary syndromes: results from the CRUSADE Quality Improvement Initiative." Jama **292**(17): 2096-2104.

Bhinder, B., C. Gilvary, N. S. Madhukar and O. Elemento (2021). "Artificial intelligence in cancer research and precision medicine." Cancer discovery **11**(4): 900-915.

Biesecker, L. G. (2013). "Hypothesis-generating research and predictive medicine." Genome research **23**(7): 1051-1053.

Brooks, D., M. Douglas, N. Aggarwal, S. Prabhakaran, K. Holden and D. Mack (2017). "Developing a framework for integrating health equity into the learning health system." Learning health systems **1**(3): e10029.

Carrasco-Ramiro, F., R. Peiró-Pastor and B. Aguado (2017). "Human genomics projects and precision medicine." Gene therapy **24**(9): 551-561.

Carrasco-Ramiro, F., R. Peiró-Pastor and B. Aguado (2017). "Human genomics projects and precision medicine." Gene Ther **24**(9): 551-561.

Čerina, D., V. Matković, K. Katić, I. B. Lovasić, R. Šeparović, I. Canjko, Ž. Bajić and E. Vrdoljak (2022). "Comprehensive Genomic Profiling in the Management of Ovarian Cancer—National Results from Croatia." Journal of Personalized Medicine **12**(7): 1176.

Chang, H. Y., C. K. Jung, J. I. Woo, S. Lee, J. Cho, S. W. Kim and T.-Y. Kwak (2019). "Artificial intelligence in pathology." Journal of pathology and translational medicine **53**(1): 1-12.

Chorley, B. N., X. Wang, M. R. Campbell, G. S. Pittman, M. A. Noureddine and D. A. Bell (2008). "Discovery and verification of functional single nucleotide polymorphisms in regulatory genomic regions: current and developing technologies." Mutation Research/Reviews in Mutation Research **659**(1-2): 147-157.

Crowley, E., F. Di Nicolantonio, F. Loupakis and A. Bardelli (2013). "Liquid biopsy: monitoring cancer-genetics in the blood." Nature reviews Clinical oncology **10**(8): 472-484.

de Bruijn, S. E., K. Rodenburg, J. Corominas, T. Ben-Yosef, J. Reurink, H. Kremer, L. Whelan, A. S. Plomp, W. Berger and G. J. Farrar (2023). "Optical genome mapping and revisiting short-read genome sequencing data reveal previously overlooked structural variants disrupting retinal disease− associated genes." Genetics in Medicine **25**(3): 100345.

Denny, J. C. and F. S. Collins (2021). "Precision medicine in 2030—seven ways to transform healthcare." Cell **184**(6): 1415-1419.

Denny, J. C., S. L. Van Driest, W. Q. Wei and D. M. Roden (2018). "The influence of big (clinical) data and genomics on precision medicine and drug development." Clinical Pharmacology & Therapeutics **103**(3): 409-418.

Dias, R. and A. Torkamani (2019). "Artificial intelligence in clinical and genomic diagnostics." Genome Medicine **11**(1): 70.

Dugger, S. A., A. Platt and D. B. Goldstein (2018). "Drug development in the era of precision medicine." Nature reviews Drug discovery **17**(3): 183-196.

Dupont, C. A., K. Riegel, M. Pompaiah, H. Juhl and K. Rajalingam (2021). "Druggable genome and precision medicine in cancer: current challenges." The FEBS journal **288**(21): 6142-6158.

Dzau, V. J., G. S. Ginsburg, K. Van Nuys, D. Agus and D. Goldman (2015). "Aligning incentives to fulfil the promise of personalised medicine." The Lancet **385**(9982): 2118-2119.

Edelman, C. and E. C. Kudzma (2021). Health promotion throughout the life span-e-book, Elsevier Health Sciences.

Enck, P., U. Bingel, M. Schedlowski and W. Rief (2013). "The placebo response in medicine: minimize, maximize or personalize?" Nature reviews Drug discovery **12**(3): 191-204.

Fernandes, B. S., L. M. Williams, J. Steiner, M. Leboyer, A. F. Carvalho and M. Berk (2017). "The new field of 'precision psychiatry'." BMC medicine **15**(1): 1-7.

Fowler, J. H., J. E. Settle and N. A. Christakis (2011). "Correlated genotypes in friendship networks." Proceedings of the National Academy of Sciences **108**(5): 1993-1997.

Franssen, F. M., P. Alter, N. Bar, B. J. Benedikter, S. Iurato, D. Maier, M. Maxheim, F. K. Roessler, M. A. Spruit and C. F. Vogelmeier (2019). "Personalized medicine for patients with COPD: where are we?" International journal of chronic obstructive pulmonary disease: 1465-1484.

Fumagalli, C. and M. Barberis (2021). Diagnostic and predictive biomarkers in lung cancer, MDPI. **13:** 2577.

Gianchecchi, E., D. V. Delfino and A. Fierabracci (2021). "Natural killer cells: potential biomarkers and therapeutic target in autoimmune diseases?" Frontiers in immunology **12**: 616853.

Ginsburg, G. S. and J. J. McCarthy (2001). "Personalized medicine: revolutionizing drug discovery and patient care." TRENDS in Biotechnology **19**(12): 491-496.

Goldman, M. J., B. Craft, M. Hastie, K. Repečka, F. McDade, A. Kamath, A. Banerjee, Y. Luo, D. Rogers and A. N. Brooks (2020). "Visualizing and interpreting cancer genomics data via the Xena platform." Nature biotechnology **38**(6): 675-678.

Golomb, B. A. and M. A. Evans (2008). "Statin adverse effects: a review of the literature and evidence for a mitochondrial mechanism." American Journal of Cardiovascular Drugs **8**: 373-418.

Gómez-López, G., J. Dopazo, J. C. Cigudosa, A. Valencia and F. Al-Shahrour (2019). "Precision medicine needs pioneering clinical bioinformaticians." Briefings in bioinformatics **20**(3): 752-766.

Goodrich, J. K., E. R. Davenport, A. G. Clark and R. E. Ley (2017). "The relationship between the human genome and microbiome comes into view." Annual review of genetics **51**: 413-433.

Gordon, E. (2007). "Integrating genomics and neuromarkers for the era of brain-related personalized medicine."

Griffin, S. (2022). "Diabetes precision medicine: plenty of potential, pitfalls and perils but not yet ready for prime time." Diabetologia **65**(11): 1913-1921.

Guerrero, A., N. Herranz, B. Sun, V. Wagner, S. Gallage, R. Guiho, K. Wolter, J. Pombo, E. E. Irvine and A. J. Innes (2019). "Cardiac glycosides are broad-spectrum senolytics." Nature metabolism **1**(11): 1074-1088.

Health, W. H. O. R., W. H. Organization, W. H. O. C. Diseases and H. Promotion (2006). Comprehensive cervical cancer control: a guide to essential practice, World Health Organization.

Ho, D. S. W., W. Schierding, M. Wake, R. Saffery and J. O'Sullivan (2019). "Machine learning SNP based prediction for precision medicine." Frontiers in genetics **10**: 267.

Hocking, L. J., C. Andrews, C. Armstrong, M. Ansari, D. Baty, J. Berg, T. Bradley, C. Clark, A. Diamond and J. Doherty (2023). "Genome sequencing with gene panel-based analysis for rare inherited conditions in a publicly funded healthcare system: implications for future testing." European Journal of Human Genetics **31**(2): 231-238.

Hodson, R. (2016). "Precision medicine." Nature **537**(7619): S49-S49.

Horton, R. and A. Lucassen (2023). "Ethical considerations in research with genomic data." The New Bioethics **29**(1): 37-51.

Hudson, M. E. (2008). "Sequencing breakthroughs for genomic ecology and evolutionary biology." Molecular ecology resources **8**(1): 3-17.

Johnson, K. B., W. Q. Wei, D. Weeraratne, M. E. Frisse, K. Misulis, K. Rhee, J. Zhao and J. L. Snowdon (2021). "Precision medicine, AI, and the future of personalized health care." Clinical and translational science **14**(1): 86-93.

Juengst, E., M. L. McGowan, J. R. Fishman and R. A. Settersten Jr (2016). "From "personalized" to "precision" medicine: the ethical and social implications of rhetorical reform in genomic medicine." Hastings Center Report **46**(5): 21-33.

Juengst, E. T., M. A. Flatt and R. A. Settersten (2020). Personalized genomic medicine and the rhetoric of empowerment. The Ethical Challenges of Emerging Medical Technologies, Routledge**:** 177-183.

Kaplan, R. M. and A. A. Stone (2013). "Bringing the laboratory and clinic to the community: mobile technologies for health promotion and disease prevention." Annual review of psychology **64**: 471-498.

Keller, E. F. (2011). "Genes, genomes, and genomics." Biological Theory **6**: 132-140.

Kessler, C. (2018). "Genomics and Precision Medicine: Implications for Critical Care." AACN Adv Crit Care **29**(1): 28-35.

Khoury, M. J. (2015). "Planning for the future of epidemiology in the era of big data and precision medicine." American journal of epidemiology **182**(12): 977-979.

Kosorok, M. R. and E. B. Laber (2019). "Precision Medicine." Annual Review of Statistics and Its Application **6**(1): 263-286.

Kundu, P., S. Das and N. Chattopadhyay (2019). "Managing efficacy and toxicity of drugs: Targeted delivery and excretion." International Journal of Pharmaceutics **565**: 378-390.

Maroilley, T. and M. Tarailo-Graovac (2019). "Uncovering missing heritability in rare diseases." Genes **10**(4): 275.

Mateo, J., L. Steuten, P. Aftimos, F. André, M. Davies, E. Garralda, J. Geissler, D. Husereau, I. Martinez-Lopez and N. Normanno (2022). "Delivering precision oncology to patients with cancer." Nature Medicine **28**(4): 658-665.

McCarthy, J. J., H. L. McLeod and G. S. Ginsburg (2013). "Genomic medicine: a decade of successes, challenges, and opportunities." Science translational medicine **5**(189): 189sr184-189sr184.

Mesko, B. (2017). The role of artificial intelligence in precision medicine, Taylor & Francis. **2:** 239-241.

Milbury, C. A., J. Creeden, W.-K. Yip, D. L. Smith, V. Pattani, K. Maxwell, B. Sawchyn, O. Gjoerup, W. Meng and J. Skoletsky (2022). "Clinical and analytical validation of FoundationOne® CDx, a comprehensive genomic profiling assay for solid tumors." PLoS One **17**(3): e0264138.

Movva, S., W. Wen, W. Chen, S. Z. Millis, Z. Gatalica, S. Reddy, M. von Mehren and B. A. Van Tine (2015). "Multi-platform profiling of over 2000 sarcomas: identification of biomarkers and novel therapeutic targets." Oncotarget **6**(14): 12234-12247.

Nakatochi, M., I. Kushima and N. Ozaki (2021). "Implications of germline copy-number variations in psychiatric disorders: review of large-scale genetic studies." Journal of Human Genetics **66**(1): 25-37.

Nami, M., R. Thatcher, N. Kashou, D. Lopes, M. Lobo, J. F. Bolanos, K. Morris, M. Sadri, T. Bustos and G. E. Sanchez (2022). "A proposed brain-, spine-, and mental-health screening methodology (NEUROSCREEN) for healthcare systems: Position of the society for brain mapping and therapeutics." Journal of Alzheimer's Disease **86**(1): 21-42.

Ozanne, E. M., R. Howe, Z. Omer and L. J. Esserman (2014). "Development of a personalized decision aid for breast cancer risk reduction and management." BMC medical informatics and decision making **14**(1): 1-8.

Panahiazar, M., V. Taslimitehrani, A. Jadhav and J. Pathak (2014). Empowering personalized medicine with big data and semantic web technology: promises, challenges, and use cases. 2014 ieee international conference on big data (big data), IEEE.

Pashayan, N., A. C. Antoniou, U. Ivanus, L. J. Esserman, D. F. Easton, D. French, G. Sroczynski, P. Hall, J. Cuzick and D. G. Evans (2020). "Personalized early detection and prevention of breast cancer: ENVISION consensus statement." Nature Reviews Clinical Oncology **17**(11): 687-705.

Passarge, E. (2007). Color atlas of genetics.

Pös, O., J. Radvanszky, G. Buglyó, Z. Pös, D. Rusnakova, B. Nagy and T. Szemes (2021). "DNA copy number variation: Main characteristics, evolutionary significance, and pathological aspects." biomedical journal **44**(5): 548-559.

Pritchard, D. E., F. Moeckel, M. S. Villa, L. T. Housman, C. A. McCarty and H. L. McLeod (2017). "Strategies for integrating personalized medicine into healthcare practice." Personalized medicine **14**(2): 141-152.

Pruis, M. A., F. H. Groenendijk, K. S. Badloe, A. van Puffelen, D. Robbrecht, W. N. Dinjens, S. Sleijfer, A.-M. C. Dingemans, J. H. von der Thüsen and P. Roepman (2022). "Personalised selection of experimental treatment in patients with advanced solid cancer is feasible using whole-genome sequencing." British journal of cancer **127**(4): 776-783.

Reid, G. (2015). "MicroRNAs in mesothelioma: from tumour suppressors and biomarkers to therapeutic targets." Journal of thoracic disease **7**(6): 1031.

Reuben, D. B., P. Gazarian, N. Alexander, K. Araujo, D. Baker, J. F. Bean, C. Boult, P. Charpentier, P. Duncan and N. Latham (2017). "The strategies to reduce injuries and develop confidence in elders intervention: falls risk factor assessment and management, patient engagement, and nurse co-management." Journal of the American Geriatrics Society **65**(12): 2733-2739.

Rexroad, C., J. Vallet, L. K. Matukumalli, J. Reecy, D. Bickhart, H. Blackburn, M. Boggess, H. Cheng, A. Clutter and N. Cockett (2019). "Genome to phenome: improving animal health, production, and well-being–a new USDA blueprint for animal genome research 2018–2027." Frontiers in genetics **10**: 327.

Robinson, P. N. (2012). "Deep phenotyping for precision medicine." Human mutation **33**(5): 777-780.

Rolland, D. C., V. Basrur, Y.-K. Jeon, C. McNeil-Schwalm, D. Fermin, K. P. Conlon, Y. Zhou, S. Y. Ng, C.-C. Tsou and N. A. Brown (2017). "Functional proteogenomics reveals biomarkers and therapeutic targets in lymphomas." Proceedings of the National Academy of Sciences **114**(25): 6581-6586.

Sánchez-Hernández, J. G., N. Rebollo, A. Martin-Suarez, M. V. Calvo and F. Muñoz (2020). "A 3-year prospective study of a multidisciplinary early proactive therapeutic drug monitoring programme of infliximab treatments in inflammatory bowel disease." British Journal of Clinical Pharmacology **86**(6): 1165-1175.

Santos, R., O. Ursu, A. Gaulton, A. P. Bento, R. S. Donadi, C. G. Bologa, A. Karlsson, B. Al-Lazikani, A. Hersey and T. I. Oprea (2017). "A comprehensive map of molecular drug targets." Nature reviews Drug discovery **16**(1): 19-34.

Satam, H., K. Joshi, U. Mangrolia, S. Waghoo, G. Zaidi, S. Rawool, R. P. Thakare, S. Banday, A. K. Mishra and G. Das (2023). "Next-generation sequencing technology: Current trends and advancements." Biology **12**(7): 997.

Savoia, C., M. Volpe, G. Grassi, C. Borghi, E. Agabiti Rosei and R. M. Touyz (2017). "Personalized medicine—a modern approach for the diagnosis and management of hypertension." Clinical science **131**(22): 2671-2685.

Scheen, A. J. (2016). "Precision medicine: the future in diabetes care?" Diabetes Research and Clinical Practice **117**: 12-21.

Schoettler, N. and M. E. Strek (2020). "Recent advances in severe asthma: from phenotypes to personalized medicine." Chest **157**(3): 516-528.

Seoane, J. and L. De Mattos-Arruda (2014). "The challenge of intratumour heterogeneity in precision medicine." Journal of internal medicine **276**(1): 41-51.

Seyhan, A. A. and C. Carini (2019). "Are innovation and new technologies in precision medicine paving a new era in patients centric care?" Journal of translational medicine **17**: 1-28.

Simmons, L. A., M. A. Dinan, T. J. Robinson and R. Snyderman (2012). "Personalized medicine is more than genomic medicine: confusion over terminology impedes progress towards personalized healthcare." Personalized medicine **9**(1): 85-91.

Spielmann, M., D. G. Lupiáñez and S. Mundlos (2018). "Structural variation in the 3D genome." Nature Reviews Genetics **19**(7): 453-467.

Strianese, O., F. Rizzo, M. Ciccarelli, G. Galasso, Y. D'Agostino, A. Salvati, C. Del Giudice, P. Tesorio and M. R. Rusciano (2020). "Precision and personalized medicine: how genomic approach improves the management of cardiovascular and neurodegenerative disease." Genes **11**(7): 747.

Suhre, K. and C. Gieger (2012). "Genetic variation in metabolic phenotypes: study designs and applications." Nature reviews genetics **13**(11): 759-769.

Tam, V., N. Patel, M. Turcotte, Y. Bossé, G. Paré and D. Meyre (2019). "Benefits and limitations of genome-wide association studies." Nature Reviews Genetics **20**(8): 467-484.

Tawfik, S. M., A. A. Elhosseiny, A. A. Galal, M. B. William, E. Qansuwa, R. M. Elbaz and M. Salama (2023). "Health inequity in genomic personalized medicine in underrepresented populations: a look at the current evidence." Functional & Integrative Genomics **23**(1): 54.

Tebbutt, S. J., A. James and P. D. Paré (2007). "Single-nucleotide polymorphisms and lung disease: clinical implications." Chest **131**(4): 1216-1223.

Wang, Y.-N., S.-X. Ma, Y.-Y. Chen, L. Chen, B.-L. Liu, Q.-Q. Liu and Y.-Y. Zhao (2019). "Chronic kidney disease: biomarker diagnosis to therapeutic targets." Clinica Chimica Acta **499**: 54-63.

Wang, Y., S. Zhang, F. Li, Y. Zhou, Y. Zhang, Z. Wang, R. Zhang, J. Zhu, Y. Ren and Y. Tan (2020). "Therapeutic target database 2020: enriched resource for facilitating research and early development of targeted therapeutics." Nucleic acids research **48**(D1): D1031-D1041.

Wei, C.-Y., J.-H. Yang, E.-C. Yeh, M.-F. Tsai, H.-J. Kao, C.-Z. Lo, L.-P. Chang, W.-J. Lin, F.-J. Hsieh and S. Belsare (2021). "Genetic profiles of 103,106 individuals in the Taiwan Biobank provide insights into the health and history of Han Chinese." NPJ genomic medicine **6**(1): 10.

Williams, J. R., V. M. Yeh, M. A. Bruce, C. Szetela, F. Ukoli, C. H. Wilkins and S. Kripalani (2019). "Precision medicine: familiarity, perceived health drivers, and

genetic testing considerations across health literacy levels in a diverse sample." Journal of genetic counseling **28**(1): 59-69.

Wong, K. M., T. J. Hudson and J. D. McPherson (2011). "Unraveling the genetics of cancer: genome sequencing and beyond." Annual review of genomics and human genetics **12**: 407-430.

Wright, G. E., P. G. Koornhof, A. A. Adeyemo and N. Tiffin (2013). "Ethical and legal implications of whole genome and whole exome sequencing in African populations." BMC Medical Ethics **14**: 1-15.

Yajima, S., T. Takano, K. Nakamura and M. Watanabe (2001). "Effectiveness of a community leaders' programme to promote healthy lifestyles in Tokyo, Japan." Health promotion international **16**(3): 235-243.

Zhang, F., C. M. Carvalho and J. R. Lupski (2009). "Complex human chromosomal and genomic rearrangements." Trends in Genetics **25**(7): 298-307.

Zhao, Z., K. Fei, H. Bai, Z. Wang, J. Duan and J. Wang (2021). "Metagenome association study of the gut microbiome revealed biomarkers linked to chemotherapy outcomes in locally advanced and advanced lung cancer." Thoracic cancer **12**(1): 66-78.

About The Authors

Dr. Hafiz Ishfaq Ahmad is working as an Assistant Professor in the Department of Animal Breeding and Genetics, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, and holds D.V.M. (UVAS), MPhil (UVAS), Ph.D. (China) & Post. Doc. (GIABR, China). Dr. Ahmad is a researcher in a multidisciplinary field, has engaged in several research domains in a range of organisms, but the primary focus on different aspects of genetic diversity and evolution. The current research activities include molecular evolution, sequencing analyses, the genetic and molecular basis of emerging antimicrobial resistance and metagenomic populations, genome annotation, and diversity analysis; the role of the gene conversion in the evolution of organisms; applications of polymorphisms; patterns of diversity and linkage disequilibrium in autosomal genes. His published work includes 2 books and 10 book chapters, 75 published research articles with a total of 300 IF, 15 oral presentations at International/National professional conferences and 20 published abstracts. He is working as an academic editor in 5 international journals, He is also reviewer for 15 scientific journals.
E-mail: ishfaq.ahmad@iub.edu.pk , ORCID: 0000-0002-7512-3254

Ms. Hira Hameed received her bachelor in 2022 at the Department of Microbiology, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur. Her research interests include cancer genetics, molecular virology, molecular genetics, microbiology, genetic mutations, etc.
E-mail: hirahameed560@gmail.com , ORCID: 0009-0009-5117-0353

Mr. Mohsin Abbas received his bachelor in 2022 at the Department of Microbiology, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur. His research interests include cancer genetics, molecular virology, molecular genetics, microbiology, genetic mutations, etc.
E-mail: mohsingabol3@gmail.com , ORCID: 0009-0001-6299-3496

Mr. Abdul Jabbar received his master in 2021 at the Department of Clinical Medicine, Faculty of Veterinary and Animal Sciences, University of Veterinary and Animal Sciences, Lahore. His research interests include cancer genetics, molecular virology, molecular genetics, microbiology, genetic mutations, etc.
E-mail: vet.drabduljabbar@gmail.com , ORCID: 0000-0003-4528-8389

To Cite This Chapter:
Ahmad, HI., Hameed H., Abbas M., & Jabbar A. (2023). **Integrating Genomics and Precision Medicine**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 301-317). ISRES Publishing

BIOTECHNOLOGY IN VETERINARY MEDICINE AND LIVESTOCK PRODUCTION

Riaz Ahmed GUL
Hira ANJUM
Khalid MEHMOOD

Introduction

Technically application of biological system for the production of different natural substance like biogas, antibiotics, hormone, enzyme and organic acid is called as biotechnology (Soetan and Abatan, 2008). In biotechnology we use animal deliberately to complete different chemical process of biological nature for the betterment of human (Olasupo, 2005). We can also define biotechnology as it is the advance method to construct or improve the product, for a specific purpose by modification of organism and plant is called as biotechnology (Steinberg and Raso, 1998: De, 2005: Liew and Hair-Bejo, 2015: Andayesh and Elhami, 2019: Yusuf et al., 2019). There is dramatic increase of world population and there is deficiency of food for human consumption as well, efforts are required to increase in animal production both in quantity wise as well as quality wise as well (Smidt and Niemann, 1999). Biotechnology have made successful effort in the field of Veterinary both in production as well as in veterinary medicine.

Today there is a lot of need in advancement in biotechnology and it is due to the following factors like:

- Environment friendly agricultural practices
- To eradicate genetic base diseases
- Increase host resistant
- Improvement of medicinal product for human consumption
- Protection of benefiting animal
- Safety of human
- To improve nutritional values of human animal feed.

Reference: Yusuf et al., 2019

These are the following landmarks in veterinary sciences which help us to increase livestock production in order to meet the current need of human.

- Cryopreservation of semen
- Artificial insemination
- Implementation of embryo transfer (ET)
- Gender preselected livestock
- Cloning of gene
- Advance veterinary diagnostic procedure
- Generation of functional gene transcripts
- Analysis of genome by molecular-genetic method
- Production of transgenic animal
- Cloning of embryonic, fetal and adult cells
- Worldwide exchange of genetic material through using frozen semen and embryo
- Integrated breeding programs by the help of biotechnological tools

Reference: Smidt and Niemann, 1999: Borroto, 2009: Mohammad Shafiee and Mohammad Shafiee, 2020: Yusuf et al., 2019)

Role of biotechnology in production

Cryopreservation of embryos and germ cells

Preservation of semen by the help of cryopreservation method is a routine practice at farm for artificial insemination purpose. This technique is also useful in the transfer of morulae, blastula and embryo to animal. Bovine morulae and blastocyst and be frozen, transferred and can be thawed again and used without appreciable loss in viability. For cryopreservation of embryo or germ layer these are cooled down to – 30 centigrade to -40 centigrade. This temperature work in all bovine caprine, ovine to some extent in equine embryo as well.

In situ ovum pick up from ovaries

For in vitro fertilization, it is necessary to collect oocyte from the from slaughter house animal as well as from living female as well. Short generation interval and exploitation of female gamete pool are very important goals in modern dairy farming. For the aspiration of small and pre-ovulatory follicle, ultrasound guided technology is being effectively used in cattle. By this technique we can collect developmentally competent oocyte for fertilization and allows repeated recovery of oocyte from the same animal without formation of scar. While laparoscopical method and transcutaneous ultra sound guided aspiration of follicle are traumatic and cause the formation of adhesions of ovaries and oviduct which can lead to or cause impairment of reproductive life span of oocyte donor. Trans-vaginal ultrasound aspiration does not have these issues and more follicle can be visualized and aspirated by the ultrasound guided technology.

Gender pre-selection at farm

Gender pre-selection is very important goal at farm for betterment of livestock production. Preference for both male and female is obvious as for the fattening purpose male animal is selected and for the purpose of pedigree breeding and for milk production female is selected. PCR method is being used to know the specific sequence to diagnose that either it is male or female embryo. Based on sex determining characteristic various efforts have been made in past to separate the sperm. By the help of flow cytometry, sperm can be shorted one the basis of minimal difference between X and Y chromosome bearing spermatozoa.

Cloning

The term clone means generating and genetically identical offspring. Cloning sheep and cloning human is a proactive question. It also reflects some of the public perception regarding the cloning of animal. By the help of micro surgical embryo bisection, isolation and proliferation or aggregation of single blastomers and nuclear transfer identical twin can be obtained in animal. In nuclear transfer we do enucleation of a recipient metaphase-II oocyte and similarly transfer of donor nucleus adhering cytoplasm. The nuclear programing is done which includes changes in nuclear lamin epitopes, nuclear morphology and protein synthesis these programing largely depends on species and procedure used for nuclear transfer. Most important benefit of nuclear would be that we can use bovine oocyte as a universal recipient as enucleated bovine oocyte can successfully reprogram somatic nuclei from different mammalian species. Most of the offspring obtain by nuclear transfer were normal both in size and healthy. However, abnormalities or drawbacks of nuclear transfer in the form of excessive fetal

growth, prolonged gestation and some other abnormities like joint problem are observed.

Genome analysis and gene mapping

There is a lot of need of molecular study in the field of livestock. Work on gene mapping is going on worldwide like in EU in the form of project like BOVMAP in bovine and PIGMAP for swine. In research, it is shown that approximately 400 genes are mapped and sequenced in bovine and fewer gene in other species as well. By the help of gene mapping, we can devise new breeding strategies, that would be a best way to achieve particular goals in short time then it would be possible by population genetics. By the help of gene mapping, we can also avoid unwanted side effect that would from selection. It would be a more effective and great achievement as undesirable side effect due to conventional selection is one of the major issue or drawbacks in animal breeding. Nowadays, DUMPS, BLAD and MHS DNA diagnostic technique are being used for elimination of genetic disorders. These techniques are also used in breeding for example kappa kasein variant in the milk of cattle.

Gene transfer

Biotechnology plays very important role in producing transgenic animal to chive the desired goal. Transgenic animal is those animals carrying recombinant DNA molecules in their genome introduced intentionally by human intervention.in gene transfer we identify desired gene, isolate and amplified, transfer to the recipient, testing of offspring for foreign gene integration, testing for expression of gene, testing of transfer of that gene to their offspring and propagation of transgenic animal. In gene transfer we do micro-injection into the pronucleus of fertilized oocyte, electroporation and by the help of retroviral vector. Micro-injection into the pro-nucleus of fertilized oocyte is still most common method which is used for the gene transfer. By the help of we can enhance the growth of animal in farm animal, improve the disease resistance and for the generation special trait in animal like milk production. Recently several proteins have been expressed in animal for the production of desired product from animal for human consumption to meet the need of human like Antithrombin-III and tissue plasminogen activator. Gene transfers also played a very important role to produce transgenic animal that provide us medicinal milk that can be used for the treatment of patient as well.

Artificial insemination

Artificial insemination is one of the very important advancements of biotechnology and it's one of the very widely spread biotechnology that is most commonly used specially in cattle production (Jacquelyn, 2008: Said et al., 2020). Artificial insemination procedure is highly successful, reliable and economical while successful rate of insemination is dependent on validity of sperm and tie of insemination as well (Jindal and Sharma, 2010).

Embryo splitting

It is a procedure in which we split embryo by the help of artificial microsurgical procedure to produce twins (Said et al., 2020). In this procedure we morale or blastocyst stage into equal halves with the help of inverted microscope that is connect with a micromanipulator and a surgical knife before transferring it into surrogate mother (Said et al., 2020).

Role of biotechnology in veterinary medicine

Molecular biology in Veterinary medicine

In order to understand disease at their molecular level, recombinant DNV technology offers a rational approach to medical personals for example in sickle cell anemia and cystic fibrosis. Human protein can be produced in abundance for therapeutic purpose due to the biotechnology. By the help of biotechnology, we are able to produce insulin, growth hormone and recombinant factor-VIII etc. for diagnostic test and for vaccine like hepatitis proteins can be produced by the help of biotechnology. Several other proteins which are produced with the help of biotechnology and being used in human are given in the table number 1.

Table 1. Medicinal product or protein produced with help of biotechnology being used for therapeutic purpose

Serial No.	Protein	Therapeutic function
1.	Insulin	Diabetes
2.	Somatostatin	Growth disorder
3.	Somatotropin	Growth disorder
4.	Factor VIII	Hemophilia
5.	Factor XI	Christmas disease
6.	Interferon beta	Leukemia and cancer
7.	Interferon y	Cancers, rheumatoid arthritis
8.	Granulocyte colony	Cancers
9.	Tumor necrosis factor	Cancers
10.	Epidermal growth factor	Ulcers
11.	Fibroblast growth factor	Ulcers
12.	Erythropoietin	Anemia
13.	Tissue plasminogen activator	Heart attack
14.	Lung surfactant protein	Respiratory distress
15.	Serum	Plasma supplement
16.	Albumen	Plasma supplement
17.	Relaxin	Aid in Child birth

(Reference: Soetan and Abatan, 2008)

Vaccines

In helminths and protozoan, we are unable to produce vaccine due to the antigen which produce or induce protective response and in obtaining sufficient quantities of vaccine trials. Molecular biology has bought lighter in order to understand the process of ageing. No RNA viruses can be detected in body fluid of infected animal and it is due to the development of reverse transcriptase polymerase chain reaction (RT-PCR). Several RT-PCR methods are developed and used in the study of new castle disease. PPR and PPRV are the disease which are caused by morbilly virus belonging to the paramyxoviridae family, and now these viruses can be differentiated with the help of RT- PCR and this happened due to the development of RT-PCR.

Gene Therapy

Gene therapy is basically another form of gene cloning. Gene therapy is used to control and due the disease which are inherited from parents. In gene therapy, we provide patient with a correct copy of the defective gene. In gene therapy, we add

functioning gene into a cell to correct a metabolic abnormality or inserted in cell to introduce a new function. This a major outbreak in the field of molecular biology. By the help of this technique, we have a good approach to treat cancer and other genetic disease of human as well.

Polymerase Chain Reaction

This is a very sensitive and specific technique for the amplification of a DNA or RNA segment via in vitro method. This is a most versatile technique in the field of agriculture, medical and veterinary (Okonko et al., 2006: Kosareva and Dinova, 2019). PCR have very important application in the field of gene cloning, biological studies, diagnostic and forensic. Recently PCR technique have helped us to identify the specific gene sequence in animal which act as a biomarker for the identification of desirable an undesirable trait of animal. This is very important application at farm level to increase the milk production at farm level. WE can trace offspring with the help of DNA finger Printing.

Bioinformatics in Veterinary medicine

It is the use of information technology in the field of biotechnology for the storage of data, data warehousing and sequencing analysis. It is one of the latest additions to the scientific world and it is a bridge between biology and information technology. Bioinformatics is the application of biochemistry and mathematic to understand the living system (Wambura, 2006). Bioinformatics tools are used in the detection of Newcastle disease in veterinary research area. Another researcher also used use bioinformatics to examine the genome of Newcastle disease virus in order to know that which sequence of virus produce false negative result (Aldous and Alexander, 2001).

According to a researcher, the aims and objective of bioinformatics are:

- To set the data in a way that allow scientist to arrange new and old data as they produced
- To produce tools or method which help in the analysis of data
- Application of tools for the interpretation of biological data to obtain a result in a biologically meaningful manner Reference: (Soetan and Abatan, 2008)

We can use bioinformatics in veterinary research to generate biological fata for research (Kaikabo and Kalshingi, 2007)

Diagnosis in veterinary medicine

Various biotechnology application has been introduced in veterinary for the diagnosis of veterinary disease like Newcastle disease virus (NDV). Present day technology is focusing on the customary procedure to expend immunological system and sub-atomic system from present day technology (Yusuf et al., 2019). It is also reported that the advancement in sub-atomic science has open a huge no of opportunities, method which are very helpful in veterinary indicative research center (Schmitt and Henderson, 2005: Itodo et al., 2018: Pagar et al., 2019).

Table 2. Modern advancement in biotechnology

Serial No.	Program	Introduction and Purpose	Reference

1.	Cows' genomics program	Scientist have started working on genomics of cow. In his study they worked on the phenotypic information, breeds, genome, SNPs of 5 high milk yielding cattle by using advance chips as well. The purpose of this study was to identify high milk producing genome and it would be great work in getting more milk production and early selection heifer at dairy.	(Yusuf et al., 2019)
2.	Work on bovine tuberculosis	With the help of Bill and Melinda Gates foundation work on Bovine tuberculosis has started and a team is planned. This program concentrates on bTB reconnaissance for bTB predominance, bTB control program through BCG inoculation. Foundation of storehouse and preparing of youthful research.	(Yusuf et al., 2019)
3.	Canine Health Research program	They have started canine research program to address serious issue of canine upkeep and support as for as wellbeing to counteract zoonotic contamination.	(Yusuf et al., 2019)
4.	*Brucella* fee village program	This program is formed to eradicate *Brucella* at small level first.	(Yusuf et al., 2019)

Table 3. Major constraints and ethical factor in applying biotechnological technique

Problem	Factor or hurdles	Reference
Constraints	• Lack of complete database • Biodiversity • Absence of mechanism between different institutions • Lack of policy and commitments	(Soetan and Abatan, 2008)
Ethical issues	• Beneficence • Risk prevention • Justice • Is it right to do • Do we have right to "play God"! • Who defines normality	(Said et al., 2020)

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

Acknowledgments

The authors concede the support of the Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan, during the write-up.

Conflict of Interest

The authors declare no conflict of interest.

Reference

1. Aldous EW, Alexander DJ (2001). Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). Avian.pathol.30: 1 1 7-1 28.
2. Andayesh, R. and Elhami, S., 2019. Application of modified sawdust for solid phase extraction, preconcentration and determination of trace lead in water samples. *Asian Journal of Green Chemistry*, *3*(4), pp.536-549.
3. Borroto, C.G., 2009. Biotechnology and its application to veterinary science. *Compendium of technical items presented to the International Committee or to Regional Commissions of the OIE, 2008*, pp.231-250.
4. De, M., 2005. Recent trends in biotechnology. *Current Science*, *88*(7), pp.1030-1031.
5. Itodo, A.U., Itodo, O.M., Iornumbe, E. and Fayomi, M.O., 2018. Sorptive chelation of metals by inorganic functionalized organic WOx–EDA nanowires: adsorbent characterization and isotherm studies. *Progress in Chemical and Biochemical Research*, *1*(1), pp.50-59.
6. Jacquelyn, G., 2008. *Microbiology: Principles and Explorations: International Student Version*. J. Wiley & Sons.
7. Jindal, S.K. and Sharma, M.C., 2010. *Biotechnology in animal health and production*. New India Publishing.
8. Kaikabo AA, Kalshingi HA (2007). Concepts of bioinformatics and its applications in veterinary research and vaccines development. Nigerian Vet. J.28(2): 39-46
9. Kosareva, O.N. and Dinova, G.E., 2019. Seasonal development of introduced apple-tree varieties under arid conditions of Western Kazakhstan. *EurAsian Journal of BioSciences*, *13*(2).
10. Liew, P.S. and Hair-Bejo, M., 2015. Farming of plant-based veterinary vaccines and their applications for disease prevention in animals. *Advances in virology*, *2015*.
11. Mohammad Shafiee, A.H. and Mohammad Shafiee, M.R., 2020. Determination of Clozapine by Air Assisted Dispersive Liquid-Liquid Microextraction Based on Solidification of Organic Droplet Followed by HPLC in Human Serum. *Advanced Journal of Chemistry-Section A*, *3*(2), pp.111-121.
12. Okonko, I.O., Olabode, O.P. and Okeleji, O.S., 2006. The role of biotechnology in the socio-economic advancement and national development: An Overview. *African Journal of Biotechnology*, *5*(23).
13. Olasupo, N.A., 2005. Food biotechnology and fortification. In *Proc. of the workshop on molecular biology techniques (theory and practicals) organized by Danifol Biotechnology Consult, March 23rd-25th*.
14. Pagar, T., Ghotekar, S., Pagar, K., Pansambal, S. and Oza, R., 2019. A review on bio-synthesized Co3O4 nanoparticles using plant extracts and their diverse applications. *Journal of Chemical Reviews*, *1*(4), pp.260-270.

15. Said, S., Agung, P.P., Putra, W.P.B. and Kaiin, E.M., 2020, April. The role of biotechnology in animal production. In *IOP Conference Series: Earth and Environmental Science* (Vol. 492, No. 1, p. 012035). IOP Publishing.
16. Schmitt, B. and Henderson, L., 2005. Diagnostic tools for animal diseases. *Revue scientifique et technique (International Office of Epizootics)*, *24*(1), pp.243-250.
17. Smidt, D. and Niemann, H., 1999. Biotechnology in genetics and reproduction. *Livestock Production Science*, *59*(2-3), pp.207-221.
18. Soetan, K.O. and Abatan, M.O., 2008. Biotechnology a key tool to breakthrough in medical and veterinary research. *Biotechnology and Molecular Biology Reviews*, *3*(4), pp.88-94.
19. Steinberg, F.M. and Raso, J., 1998. Biotech pharmaceuticals and biotherapy: an overview. *J Pharm Sci*, *1*(2), pp.48-59.
20. Wambura PN (2006). Use of virus suspensions without RNA extraction as RT-PCR templates for detection of Newcastle disease virus. African journal of Biotechnology. Vol. 5 (1 9): 1 722-1 724
21. Yusuf, Y., Nguyen, P.T., Lydia, E.L., Shankar, K. and Rahim, R., 2019. Biotechnology in Veterinary Medicine. *Journal of Environmental Treatment Techniques*, *7*(Special issue), pp.1157-1160.
22. Yusuf, Y., Nguyen, P.T., Lydia, E.L., Shankar, K. and Rahim, R., 2019. Biotechnology in Veterinary Medicine. *Journal of Environmental Treatment Techniques*, *7*(Special issue), pp.1157-1160

About The Authors

Dr. Riaz Ahmed GUL earned his Doctor of Veterinary Medicine (DVM) from Faculty of Veterinary and Animal Sciences (FV&AS), The Islamia University of Bahawalpur (IUB), Pakistan. He is currently enrolled in MPhil in Clinical medicine and Surgery (CMS) in Department of Clinical Medicine and Surgery department at Faculty of Veterinary and Animal Sciences (FV&AS), The Islamia University of Bahawalpur (IUB), Pakistan.
E-mail: rrriaz3032@gmail.com, ORCID: 0000 0001 5556 0572

Dr. Hira ANJUM earned his Doctor of Veterinary Medicine (DVM) from Faculty of Veterinary and Animal Sciences (FV&AS), The Islamia University of Bahawalpur (IUB), Pakistan. She is currently enrolled in MPhil in Clinical medicine at Medicine Section, Department of Clinical Sciences, College of Veterinary and Animal Sciences, Jhang, Pakistan
E-mail: hira.anjum37@gmail.com, ORCID: 0009 0006 3024 2395

Dr. Khalid MEHMOOD earned his Doctor of Philosophy (PhD) from Huazhong Agricultural University, Pakistan. He is currently working as Assistant Professor, in the Department of Clinical medicine, Faculty of Veterinary and Animal Sciences (FV&AS), The Islamia University of Bahawalpur (IUB), Pakistan.
E-mail: khalid.mehmood@iub.edu.pk, ORCID: 0000-0003-4720-8792

To Cite This Chapter:
Riaz, AG, et al. (2023). **Biotechnology in Veterinary Medicine and Livestock Production**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 318-325). ISRES Publishing

HOW COMPOSTING CAN REVOLUTIONIZE POULTRY WASTE MANAGEMENT: A COMPREHENSIVE GUIDE

Muhammad Tahir KHAN

Poultry waste

Due to the development of environmentally controlled housing technology during the past three decades, the poultry industry has experienced tremendous expansion. However, the chicken industry's quick expansion has led to some environmental issues. Several hundred tonnes of chicken waste are created every day, including dead birds, litter, and manure (Bolan et al. 2010). This trash must be regularly and promptly disposed of during a typical production cycle. Any delay in taking action to address these poultry wastes will increase costs and might have an adverse impact on the environment (Coufal et al. 2006; CAST, 2008).

Traditional disposal methods

The most common means of carcass disposal over the past few decades have been burial, incineration, landfills, rendering, on-farm freezers, or other preservation methods (CAST, 2008). On-farm burial, in which chicken carcasses are placed in an open trench or pit made in the ground and then covered with soil, is acknowledged as the most practical and affordable method of handling mortality losses (Wilkinson, 2011). However, nuisance complaints are raised when carcasses are buried (CAST, 2008; Bonhotal et al. 2014). The safest way to dispose of corpses is via incineration, and handling poultry carcasses won't affect the water quality. Additionally, this practice reduces the possibility of human exposure to pathogenic bacteria. Organic waste is burned in incinerators at high temperatures, producing carbon dioxide, carbon monoxide, nitrous oxide, calcium oxide, and water (CAST, 2008; Favoino and Hogg 2008). Although, the end product of burning or incineration is relatively bio-secure, there are several logistical and environmental problems with this option (Malone, 2006), most notably with regard to emissions (Bonhotal et al. 2014). Municipal landfills have been used extensively to dispose of poultry carcasses. However, disposal by landfill may pose a potential hazard to animal, poultry, and human health (Wilkinson, 2011). There may be an additional charge (tipping fee) if infected carcasses are bagged before shipment or immediately covered at the dump site (CAST, 2008).

Recycling chicken carcasses through rendering is environmentally friendly (NABC, 2004). A safe animal feed ingredient is created during the cooking stage of the rendering process, which also kills pathogenic bacteria (Nutsch and Spire 2004; NRA, 2006). The risks of disease transmission during routine pickup and the investment and operation costs of the rendering plants, however, are significant concerns with this disposal option (CAST, 2008; Bonhotal et al., 2014). For short-term storage of poultry carcasses, on-farm freezers or other preservation techniques have been utilized. Carcasses are kept in the freezers until they are picked up by the integrator and brought to a rendering factory. It is a great strategy for guaranteeing surface and ground water protection. It is an excellent method to ensure protection of surface and ground water. However, costs associated with the on-farm refrigeration and transportation need careful consideration (CAST, 2008). According to Crews et al. (1994), on-farm refrigeration is significantly more expensive than composting, burial, or incineration. Therefore, alternative carcass disposal methods with potential benefits are crucial to the running of any chicken farm. The logical solution to this issue is composting of litter and dead birds (Kelleher et al. 2002; Kumar et al. 2007).

Composting

According to Wilkinson (2011), composting is an aerobic biodegradation process in which helpful aerobic microorganisms that are naturally present in poultry litter decrease and turn organic waste (dead birds and litter) into a valuable end product. Composting has been become the most common method of carcass disposal (CAST, 2008). Many of the issues with air and water quality related to incineration and burial are addressed by on-farm composting (Ahmed et al. 2012). Additionally, this procedure eliminates the expense of routine carcass pick-up and delivery to rendering facilities (CAST, 2008). Pathogenic bacteria, fungi, viruses, and parasites are also killed by the heat produced during the process (Lu et al. 2003; Kumar et al. 2007; Seekins, 2011; Wilkinson et al. 2011; Ahmed et al. 2012; Bonhotal et al. 2014; Miller et al. 2016). According to earlier studies (Murphy, 1990; Senne et al. 1994), two-stage composting eliminates poultry-related bacterial pathogens, viruses linked to egg drop syndrome (EDS-76), and highly pathogenic avian influenza (HPAI). Conner et al. (1991) did not detect any enteric bacteria in poultry waste after a two-stage (primary and secondary) composting process was finished. These findings suggest that two-stage composting has the ability to efficiently remove possible pathogens from chicken waste (Imbeah, 1998; Vinodkumar et al. 2014) and to reduce disease outbreaks (Bonhotal et al. 2008).

Factors affecting composting

Moisture, carbon to nitrogen ratio (C:N), temperature, oxygen, porosity, and bulking agent all have an impact on the composting of carcasses. For composting to be successful, these components must be present in the proper quantity or ratio (Ritz and Worley, 2005).

Moisture

The most important element in determining whether a carcass composting process will be anaerobic or aerobic is the moisture content (Ritz and Worley 2005; Collins, 2009). To assist the formation of necessary enzymes in the composting process, an ideal moisture content is crucial (Kalbasi et al. 2005). In actuality, these enzymes hasten the composting process by dissolving big organic molecules into smaller ones (Mukhtar et al. 2004). The optimal aerobic moisture content, according to Keener et al. (2000) and Franco (2002), is between 50 and 60% (Collins, 2009; Seekins, 2011; Wilkinson, 2011). A moisture content of less than 40% will slow down the process due to reduced microbial activity (Morse *et al.* 2001; Ritz and Worley 2005). By adding a calculated amount of water to the compost pile, low moisture issues can be fixed (Collins, 2009). In contrast, if the moisture content is close to or above 70%, a compost pile may not heat up or may start to smell (Mukhtar et al. 2004), which can lead to problems, most notably with emissions (Kube, 2002; Looper, 2002). Turning the piles while adding more dry litter, straw, or other bulking agents to absorb the extra moisture can solve high moisture problems (Mukhtar et al. 2004; Kalbasi et al. 2005; Collins, 2009). A hand-squeeze method is typically used to roughly estimate the moisture content of the compost pile (Seekins, 2011). According to Ritz and Worley (2005), a tiny amount of well-watered compost will not drip water when compressed into a ball and will maintain its shape when released (Looper, 2002).

Carbon and nitrogen

The rate of biological (microbial) activity within the compost pile will be significantly influenced by the carbon to nitrogen ratio (C: N) (Collins, 2009). According to Kalbasi et al. (2005), a correct C:N ratio is essential for effective composting (Keener and Elwell 2000), as it provides enough energy and emits little

odor (Bagley, 2002; Franco, 2002). According to Collins (2009) and Franco (2002), appropriate C:N ratios should be between 15:1 to 35:1, While the C:N ratio of 25:1 seems to function best (Morse et al. 2001; Wilkinson, 2011). Insufficiently used nitrogen is released as ammonia from piles with a C:N ratio of less than 25:1 (Collins, 2009), which causes an unpleasant odor and air pollution (Morse et al. 2001). By adding a high-carbon co-compost material, such as sawdust, to the pile, the low C:N ratio issue can be solved (Morse et al. 2001). According to Seekins (2011), a C:N ratio more than 30:1 will slow down the composting process. Morse et al. (2001) found that all composting piles with very high C:N ratios have a tendency to heat more slowly than piles with a lower C:N ratio. By adding inorganic nitrogen to the pile, such as urea or ammonium nitrate, the high C:N ratio issue can be resolved (Collins, 2009).

Temperature

According to Mukhtar et al. (2004), temperature is also essential to the composting process. According to Keener and Elwell (2000), Mukhtar et al. (2004), and Seekins (2011), the action of the microorganisms inside the compost pile produces heat energy, which drives the rapid growth of thermophilic bacteria and encourages degradation (Harper et al. 2001; Langston et al. 2002; Ritz and Worley 2005; Ahmed et al. 2012). Additionally, exposure to high temperatures (135 to 160°F) for one to two days (Mukhtar *et al.* 2004; Kalbasi *et al.* 2005; Kumar *et al.* 2007) helps kill fly larvae and disease-causing microorganisms (e.g., bacteria, fungi and viruses), improving the safety of the finished product (Ritz and Worley 2005; Bonhotal *et al.* 2008; Collins, 2009; Seekins, 2011; Wilkinson *et al.* 2011; Bonhotal *et al.* 2014). Collins (2009) claims that unfavorable compost circumstances, such as a change in the C:N ratio, a reduction in oxygen supply, or an unfavorable moisture content, might lower the temperature inside the compost and cause the microbial community to return to a regime of lower temperature microorganisms. According to Glanville (1999), compost bins should be designed large enough to generate adequate internal heat and retain it for a longer period of time during cold weather.

Oxygen

Because carcass composting is an aerobic biodegradation process (Morse et al. 2001), oxygen is necessary for the microorganisms in the mixture (Ritz and Worley 2005; Seekins, 2011) to function at their peak and create an environmentally friendly product (Charnay, 2005; Collins, 2009; Turan, 2009; Wilkinson, 2011; Ahmed et al. 2012; Miller et al. 2016). Insufficient oxygen in the compost pile causes the process to turn anaerobic, which produces extremely odorous chemicals such ammonia, organic acids, and hydrogen sulphide (Henry, 2003). According to Glanville (1999), a compost pile must have a minimum of 5% oxygen concentration to maintain a process reasonably aerobic. This means that the compost pile must be mechanically stirred frequently. Mukhtar et al. (2004) claim that aeration significantly affects the final product's quality. The quality and kind of bulking material utilized have a significant impact on the oxygen supply to the compost pile. Mixtures containing too fine a bulking component (such as sawdust or litter) will restrict the oxygen supply to the microbes, slowing their growth. Reduced microbial growth results in composting temperatures that may not be high enough to kill pathogens, which lengthens the composting process (Ritz and Worley, 2005).

Porosity

According to Mukhtar et al. (2004) and Kalbasi et al. (2005), the porosity of the pile has an impact on the amount of oxygen available, the aeration process, temperature, microbial development, and the amount of time needed to finish

composting. According to Keener et al. (2001), the compost media should be sufficiently porous (between 35 and 45 percent) to ease the oxygen supply and optimize microbial growth, but not too porous to cause excessive cooling or drying (Harper et al. 2002; Looper, 2002; Wilkinson, 2011). Due to a deficiency in oxygen, piles with extremely low porosity or a predominance of very fine textures cannot heat up, whereas heaps with very high porosity can heat up quickly but cannot maintain high temperatures (Seekins, 2011). Keener et al. (2000) claimed that particle size controls the pile's porosity (Looper, 2002). A mixture should have a significant number of particles with a diameter of between 1/8 inch and one inch to achieve the best results (Kalbasi et al. 2005).

Bulking agent

Sawdust, shavings, wood chips and grill litter are all co-compost materials or bulking agents that have a variety of important uses (Wilkinson, 2011). They surround the carcasses, reducing their accessibility and attractiveness to mice, insects, and other wild animals (Mukhtar et al. 2004), and they offer additional carbon to microbes (Kalbasi et al. 2005). As an added benefit, adding co-compost material or bulking agents to the pile helps the pile maintain the correct ratio of carbon to nitrogen (C: N) for optimum composting (Mukhtar et al. 2004) and traps leachate and odors generated by decaying carcasses (Sander et al. 2002). By keeping the compost porous and maintaining the oxygen concentration within the pile, using somewhat coarse co-composting materials can help prevent odor and fly issues (Kalbasi et al. 2005; Ritz and Worley 2005).

To achieve a C:N ratio of 23:1 and a moisture content of about 55%, the compost recipe for composting poultry carcasses must contain 1 part by weight of carcasses, 2 parts by weight of used litter, 0.1 parts by weight of straw, and 0.25 parts by weight of water.

Composter construction and layout

The design and layout of composters have a big impact on how quickly materials decompose (Mukhtar et al. 2004). A dead bird composter should be carefully designed to prevent any operating issues down the road. Efficiency gains can be achieved through improved management and a well-designed composter. Based on the local climate, composter designs might differ from operation to operation, however all effective composters share a few characteristics (Collins, 2009). According to Mukhtar et al. (2004), a compost site should be situated in an area that is well-graded, well-drained, and elevated in order to achieve the best outcomes. This will prevent ground water and surface runoff from entering the facility (Ritz and Worley 2005; Seekins, 2011). The composter should also be placed and graded such that it is reachable all year round. According to Collins (2009), the size of a composter should be in line with the size of the poultry operation since, according to Ritz and Worley (2005), one cubic foot of primary compost space is needed for every pound of mortality. The primary composting capacity (number of primary bins) and the secondary composting capacity (number of secondary bins) will be equal. According to Ritz and Worley (2005), Collins (2009), and Seekins (2011), small bin composters are typically 6-8 feet wide by 5 feet tall, and 5-6 feet deep. Collins (2009) claims that the quantity of bins in a system relies on the size of each bin and the overall volume of bins needed (Morse *et al.* 2001).

The compost bins are often made of treated timber (Morse et al. 2001; Mukhtar et al. 2004) and placed on an impervious base or hard stand (Ritz and Worley 2005; Seekins, 2011; Wilkinson 2011). The bins should have a roof over them to protect the

contents from the elements (Morse et al. 2001), as well as to maintain the pile's proper moisture content (Ritz and Worley 2005; Collins 2009; Seekins 2011). On the other hand, bins without a roof are more susceptible to precipitation and weather changes (Mukhtar et al. 2004). An all-weather impermeable base helps reduce nutrient leaking into the soil, which reduces surface and groundwater contamination and stops pests and rodents from tunneling under the compost (Mukhtar *et al.* 2004; Bonhotal *et al.* 2008; Collins, 2009). Additionally, it facilitates facility cleanup (Ritz and Worley 2005). Similar to this, filling the bin will be simpler using a system of detachable drop-boards that slide into an upright channel at each end of the bin.

Composter operation and management

In the beginning, a primary bin should have a base layer of fresh litter that is 8 to 12 inches thick (Morse et al. 2001; Wilkinson, 2011). Fresh litter will start the process by supplying the necessary bacteria (Ritz and Worley, 2005), and it will also assist keep leachate from releasing its pungent odor (Wilkinson, 2011). The next step is to add a thin coating of bulking material, such as straw, coarse shavings, or peanut hulls. The bulking agent helps maintain ideal porosity for efficient composting as well as provides the carbon required for a microbial energy source. After the layer of bulking agent, to avoid heat loss and putrefaction, the bird corpses are positioned side by side in rows at least 6 inches away from the bin walls (Ritz and Worley 2005; Seekins, 2011). Each layer of the carcass can have a small amount of water added to it if necessary to get the moisture level up to the desired level; however, water usage should be moderate to prevent overly moist circumstances. In contrast, excessive moisture prevents the flow of oxygen, which anaerobically ends the process (Mukhtar et al. 2004). Then, a layer of 6 to 8 inches of litter must be spread over the carcasses (Ritz and Worley 2005; Wilkinson 2011). Problems with odor and flies will result from incomplete coverage. Dead birds, bulking material, and litter should be added in successive layers once the first layer is finished, up to a maximum height of 5 to 6 feet. Higher compost temperatures (over 170°F) brought on by excessive height can increase the likelihood of spontaneous combustion (Ritz and Worley 2005). To prevent exposed pieces or odors that draw vermin, flies, or rodents to the pile, the last layer of carcasses must be covered with a thick (8 to 10 inch) layer of litter (Morse et al. 2001; Mukhtar et al. 2004).

Within five to seven days after a bin is fully stocked, the temperature at the centre of the pile should increase to 135 to 160°F (Morse et al. 2001; CAST, 2008; Collins, 2009; Wilkinson, 2011). The decomposition process will be accelerated by the high temperature, which will also aid in the destruction of any harmful bacteria that may be present in the raw waste (Ritz and Worley 2005; Keener and Elwell 2006). A 36 inch probe-type compost thermometer can be used to measure the temperatures in the centre of the pile to ensure a thermophilic temperature range throughout the composting bin (Collins, 2009; Wilkinson, 2011). Make sure to probe the bin at several points to get the fullest image possible of its internal state (Mukhtar et al. 2004). A single temperature reading can be deceiving because it is common to detect hot and cold regions within the same bin.

According to Seekins (2011), the composting procedure typically involves two heating cycles and a curing stage. When the primary bin temperatures fall to 125 to 129°F (7 to 21 days later), the first heating cycle is finished (CAST, 2008). For a second heating cycle, the material is subsequently moved to the secondary treatment area (secondary bin) (Ritz and Worley 2005; Collins 2009; Wilkinson 2011). In order for another heat cycle to take place, the mixture needs to be aerated (Mukhtar et al. 2004; Ritz and Worley 2005; Seekins, 2011). Allow material to "cascade" from the

loader bucket as it is transferred to the second-stage area in order to ensure good turning and re-aeration (Collins, 2009). The harm to the environment will increase as a result of improper turning because it will cause odors and fly issues (Collins, 2009). When the secondary bin temperatures fall to or below 100°F, the second heating cycle is finished. After that, the material is moved to a storage area to finish the maturation process (Seekins, 2011; Wilkinson, 2011). When the compost's temperature drops to a level that is close to ambient or room temperature, the maturation period has ended. To ensure that the compost is fully stabilized, it should be stored in the storage yard for at least 30 days (Seekins, 2011).

Utilization of poultry waste

Land application

According to analysis, poultry waste, particularly litter, includes vital plant nutrients like potassium, nitrogen, and phosphorus (Martin and McCann 1998; Kelleher et al. 2002). Because of this, litter is utilized as an organic fertilizer in a number of countries (Bolan et al. 2010); nevertheless, due to issues with environmental degradation, its acceptance has been constrained (Stuven and Bock 2001). Unpleasant odors can be produced when ammonia and other volatile gases escape into the atmosphere (Tiquia, 2005). Furthermore, according to Boruadda et al. (2012), the dangerous greenhouse gases released by untreated litter are linked to ozone depletion, global warming, and climate change. Unprocessed litter applied in excess can cause eutrophication and degrade surface water resources (US EPA, 1996). To ensure the sustainability of the poultry business, it may be necessary to create a technique that offers a safer and more practical substitute for the land application of poultry waste (Kelleher et al. 2002; Szogi and Vanotti 2009).

Use in poultry feed

According to Fontenot et al. (1991), animal waste is more beneficial as feed than as fertilizer. Poultry waste, particularly litter, provides greater crude protein (Bhattachary and Fontenot 1966), accessible energy (Fontenot 1999), and nutrients that can be digested (Tadele 2015) than other animal wastes. In comparison to the TDN values discovered by Bhattachayra and Taylor (1975), Miron et al. (1990) revealed greater values for the organic matter digestibility in poultry litter. Only broiler litter, according to Muller (1975a), has a TDN value comparable to traditional feed additives. According to analysis, broiler litter has comparatively high protein content, with true protein making up around 45–67% of it, uric acid about 18–30%, and ammonia about 12–17%. Creatine (2-4%) and other nitrogen components make up a smaller portion. Additionally, according to Rankins et al. (2002), litter has a high concentration of various critical minerals that are crucial for animal nutrition. Feeding poultry manure has always been controversial and has led to a lot of research to support its safety.

Feeding to broilers

Numerous studies have been conducted to evaluate the feeding value of various types of chicken waste, including litter, manure, hatchery by-products, hen mortalities, rendered spent hens, and broiler offal. In this context, Elam et al. (1954) noticed a substantial improvement ($P<0.05$) in chick growth after feeding young chicks filtered suspension of autoclaved litter. Similar to this, hydrolyzed broiler litter has the potential to be included in chick feeds, according to Wehunt et al. (1960) However, research by Flegal and Zindel (1970) revealed that dry poultry manure concentrations above 5% led to a significant reduction ($P<0.05$) in chick growth. Later investigations by Fadika et al. (1975) fed developing Turkiyes dried layer manure at

doses of 5, 10, and 30% and discovered no significant change in weight increase or growth. The small decrease in feed efficiency coincided with the level of dietary manure rising. Similar findings were made by Lipstein and Bornstein (1971), who found that adding dry manure to the diet lowered the rate of growth and feed consumption in chicks. Additionally, it was suggested that broiler rations should include dried hatchery waste by Kempster (1945) and Wisman (1964). In their investigation, Ilian and Salman (1986) fed broiler chickens processed hatchery waste. When compared to birds fed 0% and 5%, broilers given 2.5% processed hatchery waste showed higher growth rates and feed efficiency ($P<0.05$). In a similar vein, Deshmukh and Patterson (1997) found that employing hatchery by-products was equivalent to or superior to a standard poultry diet for broilers.

Likewise, Rasool et al. (1999) discovered that adding hatchery waste meal to broiler diets at a level of 12% resulted in noticeably greater weight increase and feed efficiency. Similar to this, Kundu et al. (1986) found a significant ($P<0.05$) improvement in feed efficiency in diets that substituted hatchery waste meal for the dietary fish meal. Escalona and Pesti (1987), however, discovered that adding processed hatchery by-product meal to broiler diets at 5% level did not significantly alter feed efficiency ($P>0.05$). In subsequent studies, Agunbiade et al. (2007) found that diets in which hatchery waste meal completely replaced fish meal resulted in enhanced growth rate and feed efficiency ($P<0.05$), indicating that hatchery by-products can be used successfully in poultry feeding (Reddy, 1988; Dhaliwal et al. 1996). Furthermore, when complete hatchery waste meal was added to broiler diets, Abiola et al. (2012) discovered a non-significant difference in carcass characteristics and haematological markers ($P>0.05$). However, at 10% inclusion of whole hatchery waste meal in diet, feed consumption and growth rate increased ($P<0.05$). As the amount of whole hatchery waste meal in the diet increased, the cost of production reduced ($P<0.05$). Similar to this, numerous researchers have reported using hatchery waste in poultry diets as a way to cut feed costs (Babiker et al. 1991; Abiola, 1999; Abiola and Onunkwor 2004).

Processed chicks have also been used in poultry rations. Day old male chicks (cull) were autoclaved, dried, and then powdered before being added to poultry feed (Ravindra-Reddy and Rajasekhar-Reddy 1985). Similar to this, rendered fats and protein meals have been successfully introduced into animal feed (EPA, 2014), as demonstrated by Christmas et al. (1996), who fed broilers diets containing 0, 4, 8, or 12% rendered whole hen meal and observed no differences ($P>0.05$) in performance at any level. For a period of six weeks, Aparna and Patterson (1997) gave extruded cockerel chicks (5 and 10%) and a combination of cockerel chicks and shell waste (2.5 and 5%, respectively) to broiler chickens. Weight gain, feed conversion, and carcass characteristic parameters did not differ between the diets in terms of growth performance ($P>0.05$), but the group fed diets containing 5% cockerel chick shell waste had significantly higher ready-to-cook carcass and wing yield than the control group. Hossain et al. (2003) reported that live weight, feed conversion, profitability, and meat yield increased in broiler chickens with diets where broiler offal replaced the dietary fish meal, resulting in improved performance (Dafwang et al. 1986) at a reduced cost (Hamid, 1968). Similarly, Bulbul and Islam (1991) found that broiler chicken fed diets where broiler offal replaced fish meal had the lowest feed cost per kg weight ($P<0.05$).

Both Orga et al. (1964) and Fraga et al. (1989) noted some enhancements in broiler performance as a result of broiler offal incorporation in the diet. However, Pesti (1987) discovered that dietary broiler offal had little to no impact on broiler performance ($P>0.05$). Additionally, Kirkpinar et al. (2004) found that broiler

performance in terms of body weight, feed intake, and feed conversion efficiency were not significantly impacted (P>0.05) by the addition of chicken by-product meal to their diets. In terms of broiler performance, Bhargava and O'Neil (1975) also noted that poultry by-products and hydrolyzed feather meal were comparable to or superior to a wheat-soybean based control diet. In a similar vein, Mendonca and Jensen (1989) noted that 10% addition of poultry by-product meal in grill diet did not have a negative impact (P>0.05) on performance in terms of live weight gain, feed consumption, and feed conversion compared to control diet. Similar findings were made by Haque et al. (1991), who discovered that adding extruded poultry by-product meal (93 g/kg) to broiler diets had no negative effects (P>0.05) on body weight and feed utilization. Abiola (2001) revealed that values recorded for eviscerated weights, abdominal fat, and internal organs (liver, lungs, heart, and gizzard), across treatments, were not substantially different (P>0.05) from one another when cockerels were fed diets comprising hatchery waste meal.

Feeding to layers

Many researchers have documented the utilization of poultry waste as a feed resource. Flegal and Zindel (1969), Flegal (1971a), Flegal (1971b), and Flegal and Zindel (1972) fed laying hens diets including dried layer manure (10, 20, 30, and 40%). With the exception of layers given 10% manure, egg production, feed efficiency, and weight gain all declined as dietary manure level rose. However, as the amount of manure included in feed increased, the price of feed decreased. Similarly, Nesheim (1972) showed no significant difference (P>0.05) in the performance in terms of egg production and egg weight when feeding dry layer manure to laying hens at a level of 22.5%. The wheat-bran and dry layer manure diets' reduced calorie contents, however, were blamed for some variances in feed intake. Similarly, when dehydrated layer manure was fed to pullets at levels of 0, 12.5, or 25%, Flegal et al. (1972) observed no significant differences (P>0.05) in egg production and quality. In a similar vein, Quisenberry and Bradley (1968) discovered that layers fed diets including untreated litter and manure (10 and 20%, respectively) generally performed better than layers fed control diets. Wolford (1975), however, discovered that adding layer manure (10%) to the diets of caged Turkiye breeder hens did not significantly alter any performance metrics. Dry poultry waste was fed to laying hens at quantities up to 40% in 1971 by Flegal and Zindel, who reported no discernible differences in egg weight or eggshell thickness. For eggs from hens fed a diet containing 25% dried poultry waste, Biely et al. (1972) showed tendencies for lower percentages of large eggs and for lower Haugh unit scores.

Mahmud et al. (2015) fed laying hen diets comprising 4, 8, and 12% hatchery waste meal and found that when waste was fed at amounts as low as 4%, there were no appreciable variations (P>0.05) in egg quality metrics. Senkoylu et al. (2005) noted that adding feather meal or chicken by-product meal to layer feeds may cause Haugh unit values in eggs to decrease. Al-Harthi et al. (2009) discovered the opposite, demonstrating that the addition of hatchery waste meal to layer diets enhanced (P<0.05) shell quality. Abiola and Onunkwor (2004) discovered that completely substituting hatchery waste meal for fish meal in layer meals had no negative effects on the features of the eggs. Similar to this, Odunsi et al. (2013) found that substituting hatchery waste meal for fish meal in the diets of laying Japanese quail improved egg quality while costing less. Haematological variables weren't negatively impacted. Additionally, Ilian and Salman (1986) discovered that feeding processed hatchery waste to laying hens did not have a negative impact on their performance in terms of feed intake, body weight, egg production, feed conversion efficiency, or egg size.

Similar findings were made by Vande-Populiere et al. (1977), who discovered that the overall performance of laying hens on a diet supplemented with hatchery waste was on par with or superior to that of hens fed a control diet.
Chrappa et al. (1986) fed laying hens hatchery byproduct food at levels of 2 and 4%. In hens fed at a 2% level, egg production rose ($P<0.05$), and a 4% level boosted eggshell strength. Hatchery by-product meal was added to the layer diet by Zohari (1975) at 3.6, 7.25, 10.5, or 14.1%. While egg weight did not vary ($P>0.05$) at any level, egg production was significantly reduced ($P<0.05$) at the 7.25 and 14.5% levels. Alaba and Ekeocha (2012) noted that poultry hatchery waste meal can successfully replace fish meal in layer diets without compromising performance or egg quality. According to Wisman and Beane (1964), laying hen performance in terms of feed intake, body weight, feed efficiency, and daily egg output was unaffected by feeding 15% hatchery by-product meal. According to Damron et al. (2001), rendered whole hen meal could be used well in layer diets up to 10% level. To our knowledge, there is no literature currently available that looks at the utilization of composted dead birds in poultry feed. However, efforts are required to investigate such affordable options in order to effectively utilize the several million tonnes of this waste.

References

Abiola SS, Onunkwor EK. 2004. Replacement value of hatchery waste meal for fish meal in layer diets. Bioresour Technol. 95: 103-106.

Abiola SS, Radebe NE, Westhuizen CVD, Umesiobi DO. 2012. Whole hatchery waste meal as alternative protein and calcium sources in broiler diets. Arch Zootec. 61: 229-234.

Abiola SS. 1999. Yield of unhatched incubator eggs and its replacement value in soybean meal diets fed to cockerels. Trop J Anim Sci. 1: 74-78.

Abiola SS. 2001. Substitution of groundnut-cake for hatchery waste meal in finisher diets of cockerels. Sci Forum. 4: 48-53.

Agunbiade J, Salau KO, Adeyemi OA. 2007. Utilization of hatchery waste meal in cassava products based broiler finisher diets. In: Agiang EA, Agwunobi OO, Olawoyin OO. Proc. 32nd Annual Conference of Nigeria Society for Animal Production (NSAP) University of Calabar, Calabar, Nigeria. p. 275-276.

Ahmed ZAM, Sedik ZM, Alharery MD, Khalaf MA, Nasr SA. 2012. Microbial ecology of composting dead poultry and their waste. Global Vet. 9: 683-690.

Alaba O, Ekeocha AH. 2012. Replacement value of fishmeal by poultry hatchery waste meal in the diets of pullet growers and layers. Sci J Anim Sci. 1: 7-13.

Al-Harthi MA, El-Deek AA, Attia YA, Bovera F, Qota EM. 2009. Effect of different dietary levels of mangrove (Laguncularia racemosa) leaves and spice supplementation on productive performance, egg quality, lipid metabolism and metabolic profiles in laying hens. Br Poult Sci. 50: 700-708.

Aparna CD, Patterson PH. 1997. Preservation of hatchery waste by lactic acid fermentation. 2. Large scale fermentation and feeding trial to evaluate feeding value. Poult Sci. 76: 1220-1226.

Babiker SA, El-Sammani SE, Ismail EB. 1991. Incubator reject eggs as a protein supplement in the diets of broilers. Sudan J Anim Prod. 4: 83-93.

Bagley CB. 2002. Alternatives for dead animal disposal. Utah State University Wild West Veterinary Conference, Logan, Utah.

Bhargava KK, O'NEIL JB. 1975. Composition and utilization of poultry by-product and hydrolyzed feather meal in broiler diets. Poult Sci. 54: 1511-1518.

Bhattachary AN, Fontenot JP. 1966. Utilization of different levels of poultry litter nitrogen by sheep. J Anim Sci. 24: 1174-1178.

Bhattacharya AC, Taylor JC. 1975. Recycling animal wastes as a feedstuff: A review. J Anim Sci. 41: 1438-1457.

Biely J, Soong R, Seier L, Pope WH. 1972. Dehydrated poultry waste in poultry rations. Poult Sci. 51: 1502-1511.

Blake JP, Donald JO. 2002. Alternatives for the disposal of poultry carcasses. Alabama Agri Exp Station J. 12: 1130-1135.

Blake JP. 2004. Methods and technologies for handling mortality losses. World's Poult Sci J. 60:489–499.

Bolan NS, Szogi AA, Chuasavathi T, Seshadri B, Rothrock Jr MJ, Anneerselvam P. 2010. Uses and management of poultry litter. World's Poult Sci J. 66: 673-698.

Bonhotal J, Schwarz M, Brown N. 2008. Natural Rendering: Composting Poultry Mortality the Emergency Response to Disease Control. Available on Cornell Waste Management Institute's website: http://cwmi. css. cornell. edu/aifs. pdf. Accessed. 2:10-4.

Bonhotal J, Schwarz M, Rynk R. 2014. Composting animal mortalities.

Borugadda VB, Goud VV. 2012. Biodiesel production from renewable feedstocks: Status and opportunities. Renew Sust Energ Rev. 16: 4763-4784.

Bulbul SM, Islam MA. 1991. Feasibility of using unconventional feed in the poultry diet and formulation of economic ration with the inclusion of the unconventional resources. A report of a research project, sponsored by Bangladesh Agricultural Research Council (BARC). Research done in the department of Poultry Science, Bangladesh Agricultural University, Mymensingh.

Capucille DJ, Poore MH, Altier C, Rogers GM. 2002. Evaluation of Salmonella shedding in cattle fed recycled poultry bedding. Bov Pract. 36: 15-21.

CAST. 2008. Poultry carcass disposal options for routine and catastrophic mortality. Issue Paper 40. CAST, Ames, Iowa.

Charnay F. 2005. Composting of urban wastes in developing countries: Elaboration of a methodology for a production of compost. Doctorate thesis. Faculty of Sciences and Technology. University of Limoge, Paris, France. p. 229.

Chrappa V, Peter V, Boda K, Horvath I. 1986. Hydinarstvo- vedecki- prace-Vyskumneho- Ustavu- Chou-a-SI achtenia-Hydiny-v-Ivanke-Pri-Dunaji. 22:153-163.

Christmas RB, Damron BL, Ouart MD. 1996. The performance of commercial broilers when fed various levels of rendered whole-hen meals. Poult Sci. 75: 536-539.

Collins E. 2009. Composting dead poultry.

Conner DE, Blake JP, Donald JO, Kotrola JS. 1991. Microbiological safety and quality of poultry mortality composting. Poult Sci. 70: 29.

Coufal CD, Chavez C, Niemeyer PR, Carey JB. 2006. Measurement of broiler litter production rates and nutrients content using recycled litter. Poult Sci. 85: 398-403.

Crews JR, Blake JP, Donald JO. 1994. An economic evaluation of dead-bird disposal systems. Proc. National Poultry Waste Management Symposium, Athens, Georgia. National Poultry Waste Management Symposium Committee, Auburn University, Auburn, Alabama. p. 304-309.

Dafwang II, Cook ME, Pringle DJ, Sunde ML. 1986. Nutritional value of aerobically fermented poultry manure and offal (Fermway) for broiler chicks. Poult Sci. 65: 1965-1770.

Damron BL, Ouart MD, Christmas RB. 2001. Rendered whole-bird layer mortality as an ingredient in layer diets. J Appl Poult Res. 10: 371-375.

Deshmukh AC, Patterson PH. 1997. Preservation of hatchery waste by lactic acid fermentation. 2. Large-scale fermentation and feeding trial to evaluate feeding value. Poult Sci. 76: 1220-1226.

Dhaliwal AP, Shingari S, Sapra KL. 1996. Processing of the HW for feeding to poultry. Proc. World Poultry Congress. New Delhi, India.

Douglas MW, Parsons CM. 1999. Dietary formulation with rendered spent hen meal on a total amino acid versus a digestible amino acid basis. Poult Sci. 76: 1387-1391.

Elam JF, Jacobs RL, Couch JR. 1954. Unidentified factor found in autoclaved litter. Poult Sci. 33: 1053-1054. (Abstr.)

EPA (Environment Protection Agency). 2014. Food waste management scoping study.Available at: http://www.epa.gov/sites/production/files/2016-01/documents/msw task 11-2_foodwastemanagementscopingstudy_508_fnl_2.pdf.

Escalona PPR, Pesti GM. 1987. Nutritive value of poultry by-product meal. 3. Incorporation into practical diets (Research note). Poult Sci. 66: 1067-1070.

Fadika GO, Wolford JH, Flegal CJ. 1975. Performance and blood analyses of growing Turkiyes fed dehydrated poultry anaphage. Res Rep Mich State Univ Exp Stn.

Favoino E, Hogg D. 2008. The potential role of compost in reducing greenhouse gases. Waste Manag Res. 26: 61-69.

Flegal CJ, Sheppard CC, Dorin DA. 1972. The effect of continuous recycling and storage on nutrient quality of dehydrated poultry waste (DPW). Proc. Cornell Agric Waste Manage. Conf. p. 295-300.

Flegal CJ, Zindel HC. 1969. The utilizaton of dehydrated poultry waste by laying hens. Poult Sci. 48: 1807.

Flegal CJ, Zindel HC. 1970. The utilizaton of dehydrated poultry waste by laying hens. Poult Sci. 48: 1807.

Flegal CJ, Zindel HC. 1971. Dehydrated poultry waste (DPM) as a feed stuff in poultry rations. Proc. Int Sym on livestock wastes, ASAE, St. Joseph, MI. p. 305.

Flegal CJ, Zindel HC. 1972. The utilization of dehydrated poultry waste by laying hens. Poult Sci. 48: 1807.

Flegal CJ. 1971a. Dehydrated poultry waste (DPW) as a feedstuff in poultry rations. Livestock waste management and pollution abatement. Proc. Intern Symp on Livestock Wastes. p. 305-307.

Flegal CJ. 1971b. The effect of feeding dehydrated poultry waste on production, feed efficiency, body weight, egg weight, shell thickness and hatching score. Mich. Agric. Exp. Sta. Farm Sci. Res. Rpt. 117, Mich. St. Univ., East Lansing. p. 31-33.

Fogarty AM, Tuovinen OH. 1991. Microbiological degradation of pesticides in yard waste composting. Microbiol Rev. 55: 225-233.

Fontenot JP, De Baca RC, Glimp HA. 1991. Recycling Animal Wastes by Feeding to Enhance Environmental Quality1. Professional Anim Scientist. 7: 1-8.

Fontenot JP. 1999. Nutrient recycling: the North American Experience. Review. Asian-Aust J Anim Sci. 12: 642-650.

Fraga LM, Lon-Wo L, Palma A. 1989. Utilization of offal fat for broiler feeding. Revista Cuban de Ciencia Avicola (Cuba). 16: 111-116.

Franco DA. 2002. Animal disposal and the environmental, animal disease and public health related implications: An assessment of options. California Department of Food and Agriculture Symposium, Sacramento, California.

Glanville TD. 1999. Composting dead livestock: A new solution to an old problem. Iowa: University Extension, Iowa State University. Retrieved May 15, 2003.

Hamid MA. 1968. A comparative study of fishmeal, meat offals, vegetable protein and Teramycin (TM-S) on the growth rate of growing chicks. M. Sc. Thesis, Department of Poultry Science, Bangladesh Agricultural University, Mymensingh.

Haque AKMA, Lyons JJ, Vanderpopuliere JM. 1991. Extrusion processing of broiler starter diets containing ground whole hens, poultry by-product meal, or ground feathers. Poult Sci. 70: 234-240.

Harper AF, Estienne MJ, Collins ER. 2001. Composting as an environmentally safe means of dead pig disposal on Virginia swine farms. Suffolk, Virginia: Virginia Tech Tidewater Agricultural Research & Extension Center. Available at: http://vmirl.vmi.edu/ev/Paper%20Sessions/Early%20Bird/Absracts/Harper_ Abstract.htm

Henry ST. 2003. Dead animal disposal. Certification Program for Animal Manure Managers. Ghapter8. Glemson University Extension Services. Glemson, South Garolina. Available at: http: //-wv/w.clemson.edu/peedeerec/certifi/Gamm_p/Gh8/pch8_ 03a.pdf.

Hossain MH, Ahmmad MU, Howlider MAR. 2003. Replacement of fish meal by broiler offal in broiler diet. Int J Poult Sci. 2: 159-163.

Ilian MA, Salman AJ. 1986. Feeding processed hatchery wastes to poultry. Agric Wastes. 15: 179-186.

Imbeah M. 1998. Composting piggery waste: A review. Bioresour Technol. 63: 197-203.

Kalbasi A, Mukhtar S, Hawkins SE, Auvermann BW. 2005. Carcass composting for management of farm mortalities: A review. Compost Sci Util. 13: 180-193.

Kawata K, Nissato K, Shiota N, Hori T, Asada T, Oikawa K. 2006. Variation in pesticide concentrations during composting of food waste and fowl droppings. Bull. Environ. Contam. Toxicol. 77: 391-398.

Keener H, Elwell D, Monnin MJ. 2006. Mortality composting site selection and design options. In: Ohio livestock mortality composting manual, Ohio State University Extension. p. 9–12.

Keener H, Elwell D. 2006. Mortality composting principles and operation, In: Ohio livestock mortality composting manual, Ohio State University Extension. p. 1–7.

Keener HM, Elwell DL, Monnin MJ. 2000. Procedures and equations for sizing of structures and windrows for composting animal mortalities. Appl Eng Agric. 16: 681-692.

Keener HM, Elwell DL, Monnin MJ. 2001. Procedures and equations for sizing of structure sand windrows for composting animal mortalities. J Am Soc Agric Eng. 16: 681-692.

Keener HM, Elwell DL. 2000. Mortality composting principles and operation. In: Ohio livestock and poultry mortality composting Manual. The Ohio State University Extension, Ohio.

Kelleher BP, Leahy JJ, Henihan AM, O'Dwyer TF, Sutton D, Leahy MJ. 2002. Advances in poultry litter disposal technology – a review. Bioresour Technol. 83: 27-36.

Kempster HL. 1945. The use of dried incubator offal in chick rations. Poult Sci. 24: 396-398.

Kirkpinar F, Acikgoz Z, Bozkurt M, Ayhan V. 2004. Effects of inclusion of poultry by-product meal and enzyme-prebiotic supplementation in grower diets on performance and feed digestibility of broilers. Br Poult Sci. 45: 273-279.

Klemesrud MJ, Klopfenstein TJ, Lewis AJ. 1997. Limiting amino acids in meat and bone and poultry by-product meals. J Anim Sci. 75: 3294-3300.

Kube J. 2002. Carcass disposal by composting. Proc. Am Assoc Bov Pract. Madison Wisconsin. 35: 30-37.

Kumar VRS, Sivakumar K, Purushothaman MR, Natarajan A, Amanullah MM. 2007. Chemical changes during composting of dead birds with caged layer manure. J Appl Sci Res. 3: 1100-1104.

Kundu S, Biswas S, Ghosh TK. 1986. Feeding value of hatchery by product meal in broiler ration. Ind J Poult Sci. 21: 347-350.

Langston J, Carman D, Van Devender K, Boles Jr JC. 2002. Disposal of swine carcasses in Arkansas (MP397-5M-9-97N). Little Rock, Arkansas: Cooperative Extension Service, University of Arkansas. Available at: http://www.uaex.edu/Other_Areas/publications/HTML/MP397/composting_swine_carcasses.asp#Recipe

Lipstein B, Borstein S. 1971. Value of dried cattle manure as a feedstuff for broiler chicks. Isr J Agric Res. 21: 163.

Looper M. 2002. Whole animal composting of dairy cattle. Guide D-108. New Mexico State University, Las Cruses, NM.

Lu H, Castro AE, Pennick K, Liu K, Yang Q, Dunn P, Weinstock D, Henzler D. 2003. Survival of avian influenza virus H7N2 in SPF chickens and their environments. Avi Dis. 47: 1015-1021.

Mahmud A, Saima, Jabbar MA, Sahota AW, Hayat Z, Khan MZU. 2015. Effect of feeding hatchery waste meal processed by different techniques on egg quality and production performance of laying hens. Pak J Zool. 47: 1059-1066.

Malone B. 2006. Mass mortality composting programs. Proc. National Poultry Waste Management Symposium, Springdale, Arkansas. National Poultry Waste Management Symposium Committee, Auburn University, Auburn, Alabama. p. 29–34.

Malone G. 2005. Catastrophic mortality management. Proc. Pennsylvania poultry sales and service conference, Grantville, PA.

Marks J. 1997. New uses make ‘spent hens’ worth millions. News release: Extension and Agricultural Information, University of Missouri. Available at: http: //www.ext. Missouri.edu/agebb/news/jm 1141.htm.

Martin SA, McCann MA. 1998. Microbiological survey of Georgia poultry litter. J Appl Poult Res. 7: 90-98.

Mendonca Jr CX, Jensen LS. 1989. Effect of formulating diets with different assigned energy data for poultry by-product meal on the performance and abdominal fat content of finishing broilers. Poult Sci. 68: 1672-1677.

Michel Jr FC, Marsh TJ, Reddy CA. 2002. Bacterial community structure during yard trimmings composting. In: Microbiology of Composting, Insam H, Riddech N, Klammer S, Ed., Springer, Berlin, Germany. p. 25-42.

Miller LP, Flory GA, Peer RW, Bendfeldt ES, Hutchinson ML, King MA, Seekins B, Malone GW, Payne JB, Floren J, Malek E. 2016. Mortality composting protocol for Avian influenza infected flocks-FY2016 HPAI response.

Miron J, Solomon R, Yosef E, Ben-Chedalia D. 1990. Carbohydrate digestibility and nitrogen metabolism in sheep fed untreated or sulphur dioxide-treated wheat straw and poultry litter. J Agri Sci. 114: 115-121.

Morse DE, Friendshuh K, Hanks M, Iwan R, Schmidt D. 2001. Composting animal mortalities. Agricultural Development Division, Minnesota Department of Agriculture.

Mukhtar S, Kalbasi A, Ahmed A. 2004. Carcass disposal: A comprehensive review. National Agricultural Biosecurity Center Consortium, USDA APHIS Cooperative Agreement Project, Carcass disposal working group, Kansas State University. Kansas.

Muller Z. 1975a. Feed resources of West Malaysia with special reference to cattle rations on Majuternak cattle farms. German Agency for Tech. Cooperation, Ltd. (GTZ), Berlin.

Murphy DW. 1990. Disease transfer studies in a dead bird composter. Proc. National Poultry Waste Management Symposium, Raleigh, North Carolina. National Poultry Waste Management Symposium Committee, Auburn University, Auburn, Alabama. p. 25-30.

National Agricultural Biosecurity Center (NABC). 2004. Carcass disposal: a comprehensive review. National Agricultural Biosecurity Center Consortium, Carcass Disposal Working Group, Kansas State University, Manhattan, Kansas.

National Renderers Association (NRA). 2006. Essential rendering: All about the animal by-products industry. Meeker DL, Ed., National Renderers Association, Arlington, Virginia.

Nesheim MC. 1972. Evaluation of dehydrated poultry manure as a potential poultry feed ingredient. Proc. Waste Mgmt. Res., Proc. Cornell Agri. Waste Mgmt Conf. p. 307.

Nutsch A, Spire M. 2004. Carcass disposal: a comprehensive review. National Agricultural Biosecurity Center, Kansas State University, Manhattan, Kansas.

Ochetim S. 1993. The effects of partial replacement of soyabean meal with boiled feather meal on the performance of broiler chickens. Asian-Aust J Anim Sci. 6: 597-600.

Odunsi AA, Akinwumi AO, Falana OI. 2013. Replacement value of hatchery waste meal for fish meal in the diet of laying Japanese Quail (Coturnix coturnix japonica). Int Food Res J. 20: 3107-3110.

Olejnik B. 1995. Rendering appears to be solution to spent hen problems. Poult Times 42: 4-6.

Orga MS, Sidhu GS, Singh H. 1964. Systematic study for the evolvement of economic poultry ration. Analysis of common poultry feeds for proximate principles and preliminary feeding trails. Nutr Abst and Rev. (Series B). 34: 884.

Pesti GM. 1987. The nutritional value of poultry by-product meal. Department of Poultry Science, University of Georgia, Athens, USA, Nutrition Abstracts and Reviews (Series B) 58: 1851.

Quisenberry JH, Bradley JW. 1968. Nutrient recycling. Second Nat. Poult. Litter and Waste Mgmt. Sem., Texas St. College. p. 96-106.

Rankins DL, Poore MH, Capucille DJ, Rogers GM. 2002. Recycled poultry bedding as cattle feed. Vet Clin North Amer. Food Anim Prac. 18: 253-266.

Rasool S, Rehan M, Haq A, Alam MZ. 1999. Preparation and nutritional evaluation of Hatchery waste meal for broilers. Asian-Aust J Anim Sci. 12: 554-557.

Ravindra-Reddy V, Rajasekhar-Reddy V. 1985. Replacement of fish meal with male chick meal in broiler diets. Ind J Poult Sci. 20: 63-65.

Reddy VR. 1988. Utilization of poultry by-products by poultry. Poult Adv. 21: 39-43 (Nutr. Absts & Rev. 61:689).

Ritz CW, Worley JW. 2005. Poultry mortality composting management guide. Bulletin 1266. The University of Georgia, Cooperative Extension Service, Athens, GA.

Ryckeboer J, Mergaert J, Vaes K, Klammer S, DeClercq D, Coosemans J, Insam H, Swings J. 2003. A survey of bacteria and fungi during composting and self-heating processes. Ann Microbiol. 53: 349-410.

Sander JE, Warbington MC, Myers LM. 2002. Selected methods of animal carcass disposal (special report). J Am Vet Med Assoc. 220: 1003-1005.

Seekins, B. 2011. Best Management Practices for Animal Carcass Composting.

Senkoylu N, Samli HE, Akyurek H, Agma A, Yasar S. 2005. Performance and egg characteristics of laying hens fed diets incorporated with poultry by-product and feather meals. J Appl Poult Res. 14: 542-547.

Senne DA, Panigrahy B, Morgan RL. 1994. Effect of composting poultry carcasses on survival of exotic avian viruses: Highly pathogenic avian influenza (HPAI) virus and adenovirus of egg drop syndrome-76. Avi Dis. 38: 733-737.

Stuven R, Bock E. 2001. Nitrification and denitrification as a source for NO and NO2 production in high-strength wastewater. Water Res. 35: 1905-1914.

Szogi AA, Vanotti MB. 2009. Prospects for phosphorus recovery from poultry litter. Bioresour Technol. 100: 5461-5465.

Tadele Y. 2015. Utilization of farm animal organic waste as feeds for livestock and poultry. Adv Life Sci Technol. 32: 73-83.

Tiquia SM. 2005. Microbiological parameters as indicators of compost maturity. J Appl Microbiol. 99: 816-828.

Turan NG. 2009. Nitrogen availability in composted poultry litter using natural amendments. Waste Manag Res. 27: 19-24.

US Environmental Protection Agency. 2006. Disposal of domestic birds infected by Avian influenza – An overview of considerations and options, EPA530-R-06-009. Available at: http://www.epa.gov/epaoswer/homeland/flu.pdf. 30 p.

US EPA. 1996. National water quality inventory 1996 report to congress. Office of Water, U. S. Environmental Protection Agency, Washington, DC. EPA 841-R-97-008.

Vande-populiere JM, Kanungo HK, Walton HV, Cotterill OJ. 1977. Broiler and egg type chick hatchery byproduct meal evaluated as laying hen feedstuffs. Poult Sci. 56: 1140-1144.

Vinodkumar G, Saravanakumar VR, Ramakrishnan S, Elango A, Edwin SC. 2014. Windrow composting as an option for disposal and utilization of dead birds. Vet World. 7: 377-379.

Wehunt KE, Fuller HL, Edwards Jr HM. 1960. The nutritional value of hydrolyzed poultry manure for broiler chickens. Poult Sci. 39: 1057-1063.

Wilkinson KG, Tee E, Tomkins RB, Hepworth G, Premier R. 2011. Effect of heating and aging of poultry litter on the persistence of enteric bacteria. Poult Sci. 90: 10-18.

Wilkinson KG. 2011. On-farm composting of dead stock. In: Integrated Waste Management-Volume II. InTech.

Wisman EL, Beane WL. 1964. Utilization of hatchery by product meal by the laying hen. Poult Sci. 44: 1332-1333.

Wisman EL. 1964. Processed hatchery by-product as an ingredient in poultry rations. Poult Sci. 43: 871-876.

Wolford JH. 1975. Egg production and fertility of caged Turkiye breeder hens fed dehydrated poultry anaphage. Poult Res, Results, Michigan St. Univ.
Wood CW, Duqueza MC, Wood BH. 2010. Evaluation of nitrogen bioavailability predictors for Poultry Wastes. Open Agri J. 4: 17-22.
Xavier SAG, Stringhini JH, Brito AB. 2011. Feather and blood meal in pre-starter and starter diets for broilers. Rev Bras Zootec. 40: 1745-1752.
Zohari M. 1975. The use of hatchery by- product meal in poultry diet. J Vet Faculty Univ Tehran. 31: 43-54.

About The Authors

Dr. Muhammad Tahir Khan earned his PhD in poultry production from the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. He is a lecturer in the Department of Poultry Science, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interest includes nutritional manipulation of indigenous chicken and poultry waste management. He has published 27 research articles in national and international journals. Moreover, he is also a reviewer of some important international journals.
Email: mtahirkhan@cuvas.edu.pk , ORCID: 0000-0002-4734-3428

To Cite This Chapter:
Khan, M. T. (2023). **How composting can revolutionize poultry waste management: A comprehensive guide**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 326-341). ISRES Publishing

ASSISTED REPRODUCTIVE TECHNOLOGIES (ART) IN ANIMALS

Muhammad YOUNUS
Muhammad SAFDAR
Muhammad RIZWAN
Shafeeq Ur REHMAN
Aneeb Hassan KHAN

1. Introduction

A collection of medical techniques and procedures known as assisted reproductive technologies helps to facilitate conception and pregnancy. These techniques entail processing sperm, eggs, or embryos outside of the body before transferring them into the uterus or using a surrogate. When spontaneous conception is difficult or impossible, assisted reproductive technologies, or ART, refer to a broad range of medical interventions and procedures intended to play important role against infertility (allows the use of genetics of superior animals that may not be able to breed naturally), allows the use of genetics of parents (Dam and sire) thereby increases genetic gain etc.

1.1 Significance

The following domains are significantly affected by assisted reproductive technologies (ART).

- **Genetic Screening**: Genetic screening can be done with ART to lower the likelihood of hereditary diseases being passed down.
- **Research and Medical Advancements**: ART advances medical and scientific understanding of reproductive health.
- **Ethical and Legal Challenges**: It brings up moral and legal issues pertaining to embryonic status and reproductive rights.
- Should be about increased reproductive efficiency, genetic improvement, reduced risk of disease transmission, increased flexibility etc.

1.2 Historical Evolution

Reproductive biology has advanced significantly over the past 40 years thanks to our growing knowledge of cell and molecular biology. This is best demonstrated by the emergence of numerous the creation of assisted reproductive technologies (ART), characteristics of transgenic creatures, and most recently, mammals produced through Cloning somatic cells.

Lazzaro Spallanzani, an Italian priest and physiologist, established in 1779 that spermatozoa are necessary for fertilization by demonstrating in a lab experiment that a spermatozoon has a nucleus and cytoplasm (Johnston, 1963). The advancement of animal breeding and genetic improvement has been greatly aided by assisted reproductive technologies (ART).

1.2.1 Artificial Insemination (AI):

- 18th Century: Artificial insemination was first proposed for animals, with the majority of the initial trials concentrating on horses and cattle.
- 20th Century: Pigs, sheep, and poultry were among the many livestock species to which artificial intelligence (AI) technology was applied after techniques were refined.

1.2.2 Embryo Transfer (ET):

- 1890s: early trials of embryo transfer in mammals, including rabbits.

- 1950s: The creation of useful ET methods for cattle.
- 1970s: extensive use of ET across a range of livestock species.

1.2.3 In Vitro Fertilization (IVF):

- 1970: The first successful IVF in rabbits.
- 1978: In terms of human IVF, Louise Brown's birth represented a significant turning point as the first "test-tube baby" in history. Although unrelated to animals, this discovery had a big influence on the advancement of animal IVF methods.

1.2.4 Cloning: 1996: An important development in the field of animal cloning was the development of Dolly the sheep, the initial mammal cloned from a mature somatic cell. This accomplishment demonstrated somatic cell nuclear transfer's (SCNT) potential for cattle breeding.

1.2.5 Sperm and Oocyte Cryopreservation: The storage and transportation of genetic material for cattle breeding programs has been greatly enhanced by developments in cryopreservation methods for either sperm or oocytes.

1.2.6 In Vitro Embryo Production: The ability to produce embryos outside of an animal's body has been made possible by advancements in in vitro fertilization, in vitro maturation, as well as vitro culture techniques. This has opened up new possibilities for genetic improvement and more effective breeding programs.

1.2.7 Genome Editing: With the advent of CRISPR-Cas9 along with other genome-editing technologies, it is now possible to introduce beneficial traits and eliminate undesirable ones from animals through targeted genetic modifications.

1.2.8 Biotechnology and Genetic Markers: Genetic trait tracking in animal populations and the identification of better breeding candidates have been expedited by the integration of biotechnology, including genomics and genetic markers.

1.3 Importance in Animal Agriculture and Conservation

Rare and ecologically valuable species have undergone significant changes in many countries as a result of the advancement of agricultural technologies or the implementation of intensive production systems, which include breeding programs and forest management. Of the mammalian species with populations fewer than 1,000, 46 have become extinct in the last 200 years, and many more are endangered (Loskutoff et al., 1995). However, attempts have been made to conserve genomes &/or individual genes through the use of biotechnological &/or assisted reproductive technologies (ART) because of their significance in culture, history, and genetics. The preservation would enable, if necessary, the resuscitation of potentially extinct species in their original form. Genetic homogenization has been shown to have detrimental effects on both domestic and wild species of animals, including increased rates of juvenile mortality, subpar reproductive outcomes, and increased susceptibility to illness (Lasley et al., 1994).

The use of assisted reproductive technologies (ART) is essential to conservation and animal agriculture initiatives alike. These technologies cover a wide range of approaches and procedures intended to aid in animal reproduction, and depending on the situation, they can be used in different ways.

1.3.1 Animal Agriculture:

- **Genetic Improvement**: Farmers and breeders can enhance the genetic characteristics of livestock, including disease resistance, meat or milk production, and reproductive efficiency, by using ART. This can be accomplished by using methods that help guarantee that the most effective genetic material can be passed on to the following generation, such as embryo transfer and artificial insemination (AI).
- **Disease Management**: With the use of ART, animals with genetically resistant traits can be selectively bred, thereby limiting the spread of some diseases. Animals with desired disease-resistant traits can be multiplied through cloning and in vitro fertilization (IVF).
- **Accelerated Breeding Programs**: Generation intervals can be shortened and the rate of genetic advancement can be accelerated through ART-accelerated breeding. Increasing the productivity of livestock farming and satisfying the increasing demand for livestock products worldwide require this.

1.3.2 Conservation:

- **Endangered Species Conservation**: Keep rare and endangered species alive with the help of ART. Cloning, IVF, and artificial insemination are examples of techniques that can be used to reproduce offspring and maintain genetic diversity when natural reproduction is difficult or impossible because of factors including limited population size, genetic variation, or reproductive difficulties.
- **Cryopreservation**: It is possible to cryopreserve and store genetic material, including sperm, eggs, as well as embryos, for later use. This is crucial for conservation efforts because it can preserve the genetic diversity of threatened species for a long time.
- **Surrogate Mothers**: A closely associated species can act as a substitute female for embryos when a species is severely endangered or unable to reproduce naturally. This enables the preservation of distinctive genetic material.
- **Population Management**: Through meticulous breeding management and the reduction of inbreeding, ART can support the preservation of the genetic integrity of populations in breeding in captivity programs.

1.3.3 Disease Control and Research:

- **Disease Study**: Animal models for disease research and treatment can be produced through ART.
- **Disease Eradication**: ART can aid in the propagation of disease-free animals in cases of diseases affecting livestock, as well as supporting efforts to control and eradicate disease.

To sum up, ART is essential to animal husbandry as well as conservation. In agriculture, they facilitate population control, disease management, and genetic improvement; in the field of conservation, they are crucial for maintaining genetic diversity and averting the extinction of threatened species.

2. Basic ART Techniques

2.1 In Vitro Fertilization (IVF)

Assisted reproductive technology, or ART, is a fertility treatment that involves handling eggs or embryos outside of a female's body in order to increase the likelihood of successful pregnancies and healthy offspring. ART practices today include in vitro fertilization using or without the injection of sperm into the cytoplasm. It refers to a series of carefully planned, sequential steps known as an ART cycle, which includes ovarian stimulation, surgical egg extraction from the ovary (Transvaginal ovum retrival and ovum pick is now most commonly used in animals), sperm fertilization in a lab, and subsequent return of the embryo(s) to the female reproductive system. ART could include gestational carriers, donor sperm, and donor eggs. Among the procedures are ICSI and IVF. Situational indications exist for the transfer of either frozen or fresh embryos (Berntsen et al., 2019), where special physiological circumstances allow for the early healthy growth of the embryo. In this dynamic time frame of major waves of epigenetics during early development reprogramming, which is essential to the embryo's normal destiny, takes place.

A heifer can have multiple live offspring just a few months before her first estrous cycle thanks to assisted-reproduction technologies (ART). It is possible to carry out several ovulation embryo transfers (MOET) every six to eight weeks. However, ovum pickup can be carried out safely every two weeks, and in vitro production programs further increases the number of embryos developed per oocyte donor (Sirard et al., 2018).

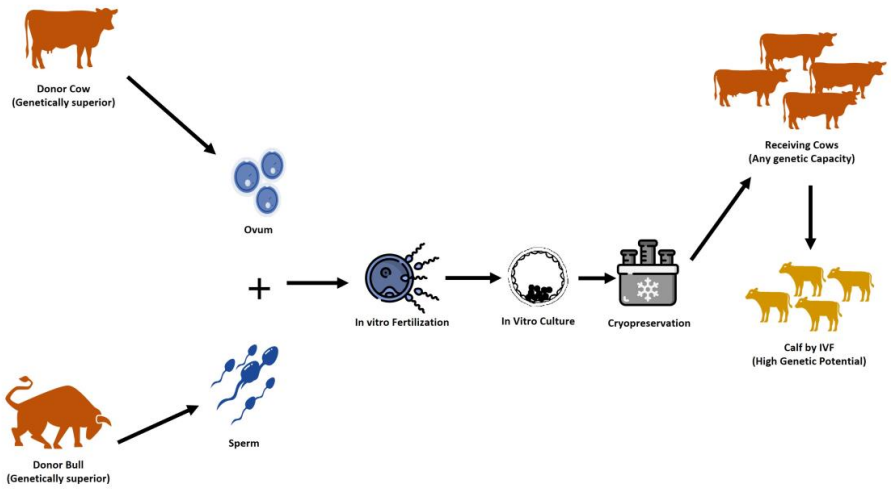


Figure.1 Step by step IVF process

2.2 Intra-Cytoplasmic Sperm Injection (ICSI)

Injecting a single sperm directly into an egg, a process known as intracytoplasmic sperm injection avoids the interactions between the sperm and the egg that occur during normal fertilization. Severe male infertility can be overcome with this method. Laparoscopy is used to perform the procedure. The creation of the ICSI method provided a fruitful remedy for infertility among males of a different etiology and has prompted a renewed interest in its possible application to the reproduction of farm

animals. ICSI is useful not only in clinical settings but also in the creation of transgenic animals and the investigation of fertilization mechanisms.

Since the very first report of ICSI success in hamsters (Uehara & Yanagimachi, 1976), live young have been born into rabbits, rats, sheep, humans, horses, cattle, and pigs through the transfer of embryos produced by ICSI. The direct injection of a single sperm is known as the ICSI micro-fertilization technique. Sperm head into the cytoplasm, or spermatozoa. Additionally, this method can be utilized to broaden the sperm vector system to produce transgenic animals. The principal indicator of severe male infertility for ICSI is caused by a variety of ejaculatory abnormalities. Whether they are testicular, epididymal, or spermatozoa.

In 1992, Palermo accidentally introduced a spermatozoon into the ooplasm of a human oocyte through a subzonal sperm injection procedure (SUZI), leading to the first successful intracytoplasmic sperm injection (ICSI) pregnancy. Initially, compared to SUZI, this method only slightly increased the rate of fertilization (Vanderzwalmen et al., 1996). Since the introduction of ICSI, the area of ART, or assisted reproductive technology, has not only grown significantly but scientists are also fascinated by the technique's success regarding the technique's functionality, the signs that applications, security, and the various factors that could evaluate its effectiveness. Up until 1992, as much as thirty percent of IVF treatments for male infertility resulted in fertilization failure, making the majority of cases of severe male infertility practically incurable. Nonetheless, the use of ICSI gives these patients a strong chance of having successful fertilization and developing healthy embryos that can be implanted.

Many of the upstream processes, such as gaining sperm motility, going through capacitation, and going through the acrosome reaction, are circumvented in ICSI in order to prepare the sperm for penetration of the egg. Attachment to the zona pellucida, fusion with the oocyte, and sperm incorporation. Throughout ICSI, a chosen cytoplasm is directly injected with the immobilized sperm of an oocyte following the oolemma's rupture. Luminous microscopy indicates that the fertilization period, which includes the extrusion of the pronuclei and the development of a second polar body is notably shorter following ICSI than following IVF (Nagy et al., 1994).

2.3 Embryo Transfer and Cryopreservation

In the field of assisted reproductive technology (ART), embryo transfer and cryopreservation are two crucial procedures that are mostly utilized in the context of IVF (in vitro fertilization) and fertility preservation. An important stage in the IVF procedure is called embryo transfer, during which fertilized embryos are inserted into a mother (recipient female/ Dam's) uterus in an attempt to conceive a child (to achieve pregnancy in herd or flock) pregnancy. The method of freezing and preserving biological materials—such as eggs, sperm, or embryos—for later use is known as cryopreservation.

Embryo transfer technology is a valuable tool that helps cattle grow more quickly and allows for the simultaneous use of the genetic contributions of both male and female animals. With the aid of ET, (Nicholas & Smith, 1983) reviewed that techniques such as MOET (multiple ovulation embryo transfer) or (embryo transfer) a quicker livestock development, quick elite animal growth, genetic gain, and quicker It is possible to develop herds and preserve uncommon genetic stocks.

The comparatively short fertile life span of mammalian oocytes and/or embryos has made continuous availability of viable, developmentally competent oocytes and/or

embryos essential to recent progress in IVP. Thus, keeping unfertilized oocytes would produce an easily accessible source, enabling the studies to be conducted at a convenient time and may, thus, be crucial in real life for the creation of a gamete bank where specific genetic combinations could be obtained.

Cloning

The process of producing an exact or nearly exact duplicate of an organism, cell, or sequence of DNA is known as cloning. Cloning is a potent technique that has the potential to reduce variation in genetics in experimental animals and multiply elite animals. It can be a tool for stem cell production as well as for conservation. Cells for medical use, such as in medicinal cloning. Using somatic cells for cloning provides chances to choose and breed animals with particular qualities (Das et al., 2003). "Dolly," a sheep, was the first animal created through somatic cloning (Wilmut et al., 1997). She was created from cells that were extracted from the udder of a 6-year-old Finn Dorset ewe and incubated in a lab for a few weeks. After that, individual cells were combined with genetically altered unfertilized eggs. Six days were spent cultivating 237 of these reconstructed eggs in temporary holders. Each egg now had the nucleus of a diploid from the adult animal. Nineteen eggs, which seemed to have progressed normally up to the blastocyst stage, were placed into Scottish Blackface ewes that served as stand-ins. After about 148 days, one gave birth to a live female lamb named Dolly. July was Dolly's birthday.

Through the use of cloning, it is possible to create thousands of identical copies of genetically modified and quantitative animals without using traditional breeding methods. To preserve the genetic diversity that is currently available, clone samples from a variety of animals may be used in remote locations where it is impractical to collect and store enough samples of semen or embryos. The local breeds have the potential to carry important genes that confer adaptation, particularly to heat or disease resistance. Cloning techniques can help prevent the extinction of these breeds, which is a pressing concern. Cloning could be used for xenotransplantation in the future to multiply humanized pigs whose organs could be transplanted into humans (Duszewska & Reklewski, 2007).

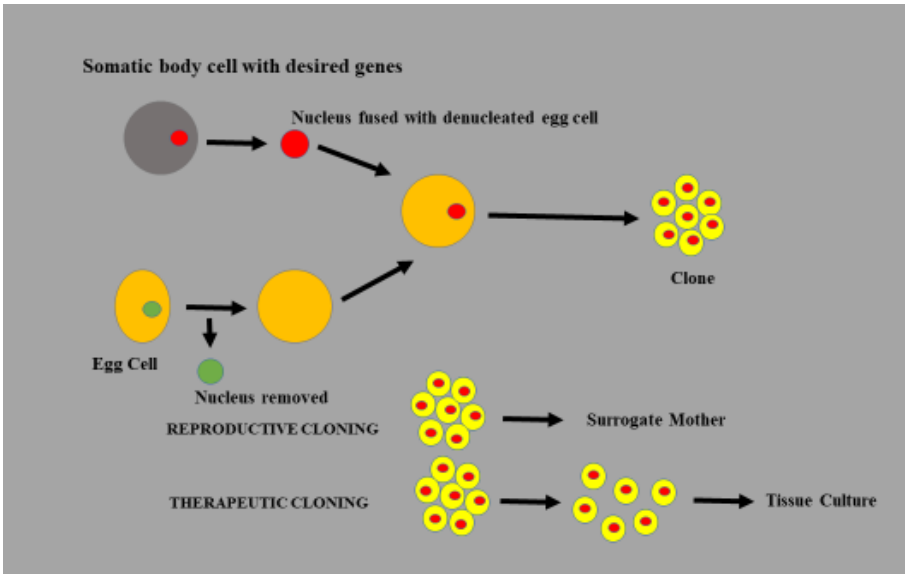


Figure.2. Steps of cloning process

3. Applications in Livestock Farming

Through the provision of more effective and regulated techniques for animal breeding and genetic advancement, Assisted Reproductive Technologies (ART) have completely transformed the livestock farming industry. These technologies have a number of benefits, including accelerating genetic advancement, improving breeding stock quality, and protecting priceless genetic resources. These are some important ways that ART is used in livestock farming.

3.1 Dairy and Beef Cattle: Enhancing Productivity and Genetic Diversity

A sustainable livestock farming system and the long-term prosperity of the industry depend on increasing the productivity & and genetic variety of dairy and beef cattle. Providers of milk, meat, and other products, including beef and dairy cattle are equally valuable. Gaining more genetic diversity and productivity can boost productivity, improve resistance to disease, and produce higher-quality goods. Here are some methods and things to think about in order to reach these objectives.

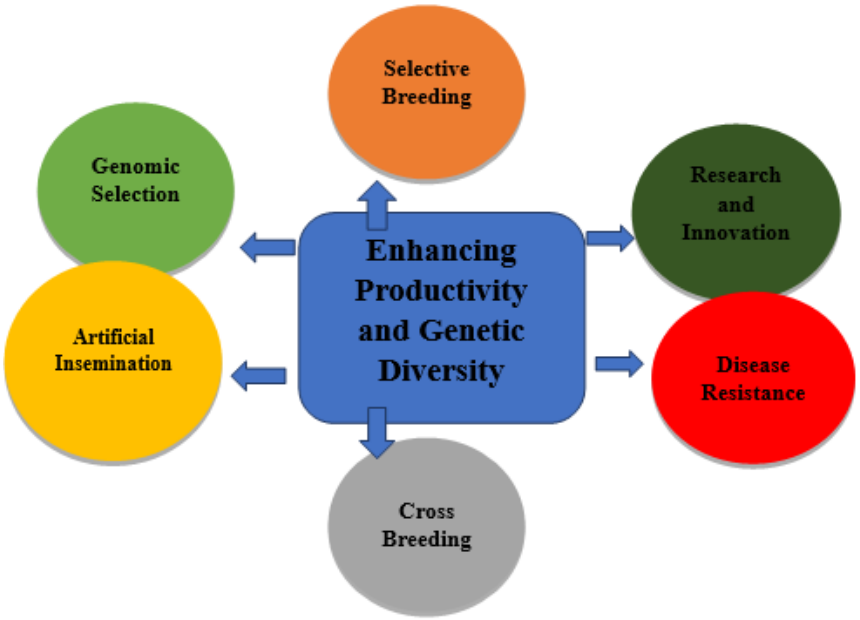


Figure.3. Enhancement of Productivity and Genetic Diversity in Dairy and Beef Cattle

Negative traits that jeopardize animal welfare, human health, and the sustainability of the environment have been found to be correlated with intense selection for highly heritable, productive, and economically viable traits. Breeders are now incorporating novel traits that prioritize economic efficiency, animal welfare, and environmental sustainability into their breeding programs or selection indices in order to address these issues (Miglior et al., 2017). The choice of index in dairy farming is determined by a number of characteristics, including feed effectiveness, emissions of methane, heat stress, hoof health, immune system reaction, milk composition, as well as reproductive traits.

1.1 Swine and Poultry: Optimizing Breeding Programs

For the production of premium eggs and meat, as well as to preserve the health and welfare of the animals, it is essential to optimize breeding programs for pigs and poultry. Improvements in rate of growth, efficiency of feed, resistance to diseases, or

meat and egg quality are among the features that these programs seek to enhance. Since the 1950s, the objectives of commercial chicken and pig breeding have been moving in that direction. Simultaneously, selection technology is growing in strength. Both of these developments allow animal breeding to contribute more and more to long-term food security.

According to (Lukefahr & Preston, 1999), it is unrealistic to think of intensive animal production systems as a way to guarantee food security for the rural poor. It is a different matter entirely to ensure food security for the (very large) global urban population. We think the breeding industry that supports technologically advanced animal production is crucial in this situation.

These are some essential factors and methods for improving swine and poultry breeding programs.

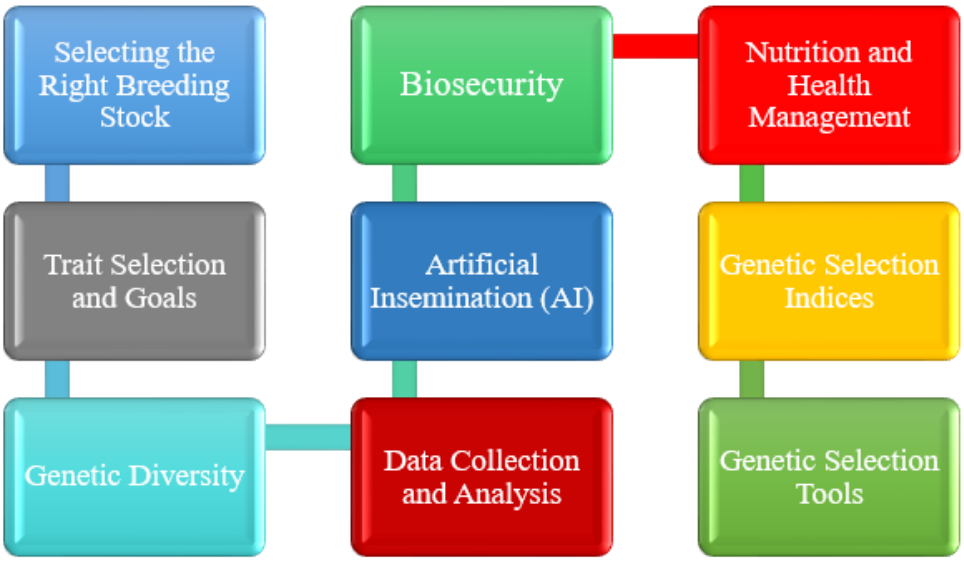


Figure.4. A flow chart of optimization for breeding programs

Optimizing breeding programs for pigs and poultry is a continuous process that needs commitment to genetic improvement, meticulous planning, and dedication. By putting these tactics into practice and keeping up with industry developments, you can improve the performance of animals, product quality, and overall farm profitability.

1.1 Small Ruminants: Challenges and Innovations

Small ruminants like sheep and goats are part of the livestock farming industry, which is essential to the world's agricultural economy because it produces valuable goods like milk and meat. Nonetheless, this industry faces a number of difficulties with regard to sustainability, productivity, and animal welfare. New ideas are always being developed to overcome these obstacles and enhance small-scale ruminant agriculture. The subsistence, economic, as well as social livelihoods of a sizable population of humans in developing nations depend heavily on small ruminants, such as sheep and goats, particularly native breeds. It is essential to evaluate production systems and genotypes economically. Even though it is frequently the intention, previous and current smaller ruminant genetic improvement programs hardly ever concentrate on the financial assessment of the genotypes and low-input, smallholder production systems. Dairy goats, for instance, have frequently been encouraged in communities without giving enough consideration to the medium- to long-term demand locally for

the milk or to its promotion or processing for consumers who live far away. To evaluate whether the anticipated rise in output from investing in genetic improvement programs will be profitable, the current marketing and production systems must be thoroughly characterized, with the inputs (expenses) as well as outputs (revenues) identified (Kosgey, 2004).

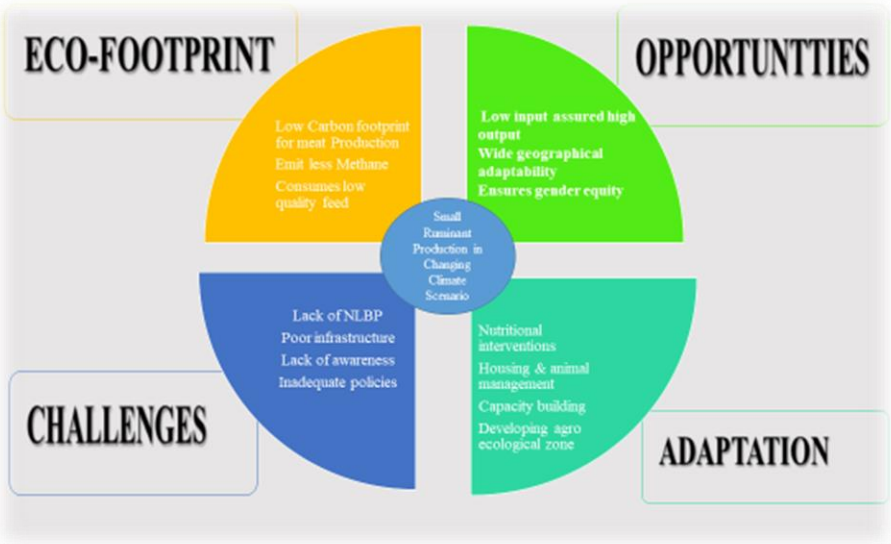


Figure 5. Possibilities and difficulties related to small-scale, environmentally intensive ruminant production methods

A vital part of global agriculture, small-scale ruminant farming confronts constant challenges as well as possibilities for innovation just like any other agricultural sector. For small ruminant farming to remain viable over the long term and satisfy the rising need for milk, meat, and other products worldwide, these issues must be resolved and sustainable practices must be implemented.

2. Wildlife Conservation and Endangered Species

The practice of conserving wild species of plants and animals, as well as their habitats, is known as wildlife conservation. It is essential to preserving ecological balance, biodiversity, and the general health of our world. The primary goals of wildlife conservation are to save species from extinction and to preserve their natural habitats.

2.1 ART Techniques for Endangered Mammals, Birds, Reptiles, and Amphibians

For the protection of threatened species of mammals, reptiles, birds, and amphibians, the use of ART can be extremely important. Threats to these species, such as habitat loss and low reproduction rates, can be mitigated with the use of these technologies. These are some ART methods that can be used with these various animal species.

I. **Mammals**:

- **In vitro fertilization (IVF)**: IVF allows for the collection of sperm and eggs from members of threatened or endangered mammal species, laboratory fertilization of the eggs, and subsequent implantation of the fertilized embryos into surrogate mothers of the same or closely related species.
- **Sperm and egg banking**: Future use and long-term storage are made possible by the cryopreservation of endangered mammal sperm and eggs.

- **Cloning**: By using preserved somatic cells and somatic cell nuclear transfer (SCNT), it is possible to clone endangered mammals and produce genetically identical individuals.

II. **Birds**:
- **Artificial insemination**: By gathering and fertilizing eggs with meticulously preserved sperm, this technique increases the genetic diversity of populations kept in captivity.
- **Embryo transfer**: It is possible to extract bird embryos from one individual as well as transfer them to another, maintaining genetic diversity and promoting.

III. **Reptiles**:
- **Incubation and hatchling care**: Enhancing survival rates and guaranteeing genetic diversity can be achieved through carefully monitoring hatchlings and carefully incubating reptile eggs.
- **Cryopreservation**: Reptiles can also benefit from sperm and egg banking, which preserves genetic material for later use.

IV. **Amphibians**:
- **Hormone-induced breeding**: Certain amphibians need particular environmental factors or hormonal therapies in order to reproduce. ART can help with these procedures.
- **Ex-situ conservation**: Amphibian populations can be kept under control in places like breeding facilities, which can help shield them from hazards like disease and habitat loss.

Mammals, reptiles, and amphibians that are endangered can be protected and helped to recover when these ART techniques are combined with other conservation efforts and habitat conservation. In captivity and occasionally during attempts to reintroduce these species into their native habitats, they are essential resources for preserving genetic diversity and boosting reproductive success.

4.2 Conservation Breeding: Maintaining Genetic Diversity

Captive breeding, or conservation breeding, is a tactic used in zoos, wildlife sanctuaries, or other specialized breeding facilities to preserve and enhance the genetic diversity of threatened or endangered species. This strategy is crucial for keeping species that are endangered from going extinct because of different threats like disease, habitat destruction, and poaching. It is crucial for a variety of reasons that variation in genetics in captive populations be preserved. First, genetic diversity among individuals within a population is essential for environmental change adaptation. Genetic variation is necessary for evolution. The concept of variation is especially crucial when reintroducing captive animals back into their natural habitat and adaptation is needed. Secondly, there is typically very little phenotypic variation in populations that have low genetic variation. Everybody is similar to each other (Wright, 1984).

One of the main objectives of captive breeding programs is to maintain genetic variety in captive populations. It is becoming more and clearer that reproductive technology can help captive breeding programs achieve this objective. The best way that reproductive technology can help captive breeding programs with this task is by creating methods that can both extend the reproductive life of current the founders as well as their close descendants and effectively increase the contribution of genetics of new wild creators to a population. In relation to reproductive technology and the preservation of genetic diversity, there are two main issues that need to be resolved:

First, what animals should be used to gather and preserve germplasm, and second, how should the germplasm be reintroduced into the population? This report focuses specifically on the first question—whose sperm should be collected—and briefly looks at some of the genetic variables that may be taken into account when making such decisions.

For multiple reasons, conservation breeding depends on preserving genetic diversity.

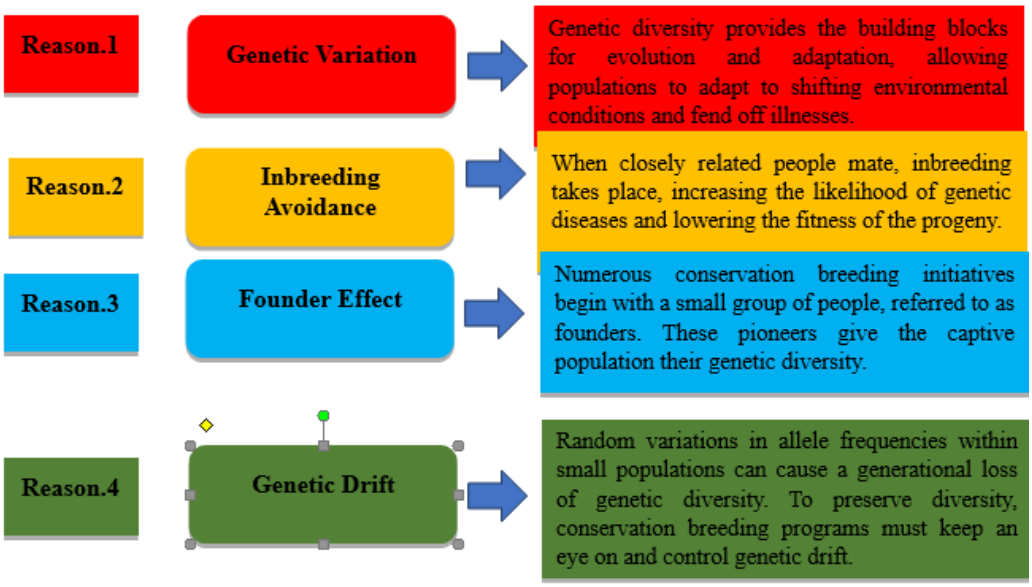


Figure 6. Major reasons to conserve Genetic Diversity

3. Challenges and Ethical Considerations

Recent years have seen tremendous advancements in ART, or assisted reproductive technology, for animals, providing chances to boost scientific research, conserve endangered species, and increase livestock production. They do, however, also present a number of difficulties and moral dilemmas. These are a few of the main concerns.

3.1 Challenges

- **Genetic diversity**: In small and isolated populations, inbreeding can be a serious problem, and if ART is not carefully controlled, it may unintentionally decrease genetic diversity. This may lead to a rise in the risk of genetic illnesses and a decline in population health as a whole.
- **Welfare of the animals**: Superovulation or multiple embryo transfer are two ART procedures that can cause physical and psychological stress in animals. When the animals involved in these procedures suffer injury or distress, ethical questions are raised.
- **Technical limitations**: Animal ART can be unreliable and have a low success rate. This may result in resource waste and possible injury to the animals undergoing these treatments.
- **Long-term health effects**: Uncertainty surrounds the long-term health effects of ART on animals. Veterinarians and researchers must evaluate any hazards to the health of the animals, including the results of hormone therapy and other interventions.

3.2 Ethical Considerations

- **Respect for animal autonomy**: Respecting the autonomy and welfare of animals is one of the ethical factors in ART for animals. These values mandate that animals receive considerate treatment and that their interests and welfare come first.
- **Informed consent**: There are ethical questions with these procedures because animals cannot give informed consent. Humans make decisions regarding the reproduction of these animals, and it is imperative that these choices are made with the animals' best interests in mind.
- **Welfare and suffering**: The potential suffering and distress that animals might go through during ART procedures is the focus of ethical concerns. One of the most important ethical considerations is minimizing pain and suffering for animals while also advancing their general welfare.
- **Environmental impacts**: Certain ART procedures, like cloning, may negatively affect genetic diversity and, consequently, the general well-being and adaptability of animal populations. This affects conservation initiatives.
- **Commercialization**: There are ethical questions raised by the commercialization of ART in animals, such as the cloning of valuable animals for financial gain. It may put money ahead of other moral obligations and the welfare of animals.
- **Transparency and regulation**: To guarantee that ART for animals adheres to moral principles and does not jeopardize the welfare of the participating animals, clear and regulated procedures are required.

There must be precise rules and regulations controlling the use of ART in animals in order to address these issues and ethical concerns. These rules ought to be founded in ethical precepts and scientific data, with an emphasis on minimizing harm, upholding animal autonomy, and advancing the welfare of the participating animals. In addition, to guarantee the ethical and responsible use of ART in animals, continued research and cooperation between researchers, ethicists, and legislators are crucial.

4. Future Prospects

There are numerous uses for assisted reproductive technologies (ART) in agriculture, conservation, and research involving animals. The future prospects in this field are promising and offer several exciting possibilities.

- **Improved Livestock Breeding**: In vitro fertilization (IVF), artificial insemination, and embryo transfer are examples of ART techniques that are already utilized to improve livestock breeding. Precision breeding, which uses genomics to select superior traits and slow the spread of genetic disorders, is expected to advance in the future, increasing the productivity of the meat and dairy industries.
- **Conservation Efforts**: In order to preserve rare and endangered species, ART is essential. Genetic diversity can be protected through the cryopreservation of embryos and gametes (eggs and sperm). By generating genetically diverse populations, the advancement of cloning as well as genome editing technologies such as CRISPR/Cas9 may also be utilized to save endangered species.
- **Biomedical Research**: Researching genetics, development, and reproduction in animals requires the use of ART. With the use of these technologies, researchers can examine the causes of infertility or reproductive disorders as well as simulate human reproduction in animals for scientific purposes. With fresh knowledge about the physiology and health of animals, this field will keep developing.

- **Disease Resistance**: Animals can be genetically engineered to become more resistant to disease, which has potential advantages for the environment and the economy. For instance, developing genetically altered animals resistant to particular illnesses can lessen the livestock farming industry's reliance on antibiotics and other drugs.
- **Environmental Sustainability**: ART can be used to enhance breeding plans that lessen animal agriculture's negative environmental effects. For instance, choosing animals that are more suited to a given environment or have lower methane emissions can support sustainability initiatives.
- **Transgenic Animal Production**: Transgenic animal production for industrial and medical uses can be facilitated by ART. It is possible to genetically modify animals so that their milk, blood, or other tissues contain useful proteins like antibodies or enzymes. Applications for this exist in biotechnology and pharmaceuticals.
- **Personalized Medicine**: By using ART to produce animals whose organs and tissues are specifically designed for human transplantation, the field of xenotransplantation may be able to address the organ shortage crisis in medicine.
- **Ethical and Regulatory Challenges**: As ART for animals progresses, moral and legal issues will come to the fore. The future of this field will depend on ensuring the welfare of animals used in ART procedures, addressing issues with cloning, genetic modification, and gene editing, and developing responsible guidelines.

5. Summary

To sum up, assisted reproductive technologies, or ART, have shown to be extremely useful resources for the management of reproduction and animal breeding. They provide a plethora of methods and procedures, including in vitro fertilization, artificial insemination, or transfer of embryos, which have greatly enhanced our capacity to protect endangered species, boost livestock productivity, and improve genetic diversity. Even though ART has significantly improved animal reproduction, more study and ethical thought must go into its future to guarantee its ethical and responsible use.

References

Berntsen, S., Söderström-Anttila, V., Wennerholm, U.-B., Laivuori, H., Loft, A., Oldereid, N. B., Romundstad, L. B., Bergh, C., & Pinborg, A. (2019). The health of children conceived by ART:'the chicken or the egg?' *Human Reproduction Update*, *25*(2), 137–158.

Chichi, D. V. (2021). In Vitro Fertilization, Fertility Frustrations, and the Lack of Regulation. *Hofstra Law Review*, *49*(2), 7.

Das, S. K., Majumdar, A. C., & Sharma, G. T. (2003). In vitro development of reconstructed goat oocytes after somatic cell nuclear transfer with fetal fibroblast cells. *Small Ruminant Research*, *48*(3), 217–225.

Duszewska, A. M., & Reklewski, Z. (2007). Uzyskiwanie zarodków zwierząt gospodarskich in vitro. *Medycyna Weterynaryjna*, *63*(12), 1522–1525.

Fishel, S. (2018). First in vitro fertilization baby—this is how it happened. *Fertility and Sterility*, *110*(1), 5–11.

Gajda, B., & Smorąg, Z. (2009). Oocyte and embryo cryopreservation–state of art and recent developments in domestic animals. *Journal of Animal and Feed Sciences*,

18(3), 371–387.
Johnston, D. R. (1963). The history of human infertility. *Fertility and Sterility*, *14*(3), 261–272.
Kosgey, I. S. (2004). *Breeding objectives and breeding strategies for small ruminants in the tropics*. Wageningen University and Research.
Lasley, B. L., Loskutoff, N. M., & Anderson, G. B. (1994). The limitation of conventional breeding programs and the need and promise of assisted reproduction in nondomestic species. *Theriogenology*, *41*(1), 119–132.
Loskutoff, N. M., Bartels, P., Meintjes, M., Godke, R. A., & Schiewe, M. C. (1995). Assisted reproductive technology in nondomestic ungulates: a model approach to preserving and managing genetic diversity. *Theriogenology*, *43*(1), 3–12.
Lukefahr, S. D., & Preston, T. R. (1999). *Human development through livestock projects: alternative global approaches for the next millennium*.
Miglior, F., Fleming, A., Malchiodi, F., Brito, L. F., Martin, P., & Baes, C. F. (2017). A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. *Journal of Dairy Science*, *100*(12), 10251–10271.
Nagy, Z. P., Liu, J., Joris, H., Devroey, P., & Steirteghem, A. Van. (1994). Time-course of oocyte activation, pronucleus formation and cleavage in human oocytes fertilized by intracytoplasmic sperm injection. *Human Reproduction*, *9*(9), 1743–1748.
Nicholas, F. W., & Smith, C. (1983). Increased rates of genetic change in dairy cattle by embryo transfer and splitting. *Animal Science*, *36*(3), 341–353.
Sirard, M. A., Grand, F. X., Labrecque, R., Vigneault, C., & Blondin, P. (2018). ASAS-SSR Triennial Reproduction Symposium: The use of natural cycle's follicular dynamic to improve oocyte quality in dairy cows and heifers. *Journal of Animal Science*, *96*(7), 2971–2976.
Uehara, T., & Yanagimachi, R. (1976). Microsurgical injection of spermatozoa into hamster eggs with subsequent transformation of sperm nuclei into male pronuclei. *Biology of Reproduction*, *15*(4), 467–470.
Vanderzwalmen, P., Bertin, G., Lejeune, B., Nijs, M., Vandamme, B., & Schoysman, R. (1996). Two essential steps for a successful intracytoplasmic sperm injection: injection of immobilized spermatozoa after rupture of the oolema. *Human Reproduction*, *11*(3), 540–547.
Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J., & Campbell, K. H. S. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature*, *385*(6619), 810–813.
Wright, S. (1984). *Evolution and the genetics of populations, volume 3: experimental results and evolutionary deductions* (Vol. 3). University of Chicago press.

About The Authors

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkiye. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 68 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.
E-mail: msafdar@cuvas.edu.pk , ORCID: 0000 0002 3720 2090

Muhammad Younus completed did his Graduation from Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan. He continues his post graduate study in Zoology at Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan. He is working on Biodiversity of species along with DNA barcoding of species as his main areas of interest.
Email: younus31302@gmail.com, ORCID: 0009-0005-5392-7031

Shafeeq ur Rehman graduated from the Cholistan University of Veterinary and Animal Sciences in Bahawalpur Pakistan. He continues his postgraduate study in Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur. He continues to work on FMD and PPR viruses as his area of interest. He is still working as a Research Assistant in FMDV and PPRV project in Cholistan University of Veterinary and Animal Sciences Bahawalpur Pakistan.
Email: shafeequrrehmanbhutta@gmail.com, ORCID: 0000-0003-3571-8226

Aneeb Hassan KHAN is an ambitious final year student pursuing a BS in Animal Sciences at Cholistan University of Veterinary and Animal Sciences, Bahawalpur. With a passion for Animal Nutrition, Breeding, Genetics, and Genomics, he has ventured into the captivating world of Molecular Modeling. Embarking on a journey into research, he aspires to merge his varied interests to contribute innovative insights to the scientific community.
Email: aneebkhan2020@gmail.com, ORCID: 0009-0007-1469-6415

Muhammad Rizwan is a graduate of Cholistan University of Veterinary and Animal Sciences, where he focuses on improving the yield of livestock to combat food shortages.
Email mrizwon@gmail.com, ORCID: 0009-0002-5782-0600

To Cite This Chapter:
Younus, M, et al. (2023). **Assisted reproductive technologies (ART) in animals**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 342-356). ISRES Publishing

HEMORRHAGIC SEPTICEMIA-MASTITIS VACCINE

QUDRATULLAH
Habib ur REHMAN

1. Economic impact of animal diseases in developed and developing countries

Animal diseases, in particular infectious diseases, have major adverse effects on livestock productivity as well as animal welfare worldwide. The costs of animal diseases are around 17% of turner over of the livestock sector in the developed countries while it is 35-50% in the developing countries (Bishop *et al*., 2010; Holden *et al*., 1996). Infectious diseases pose a significant challenge to all animal production systems, which sustain significant production losses from these diseases. Thus, animal diseases are a significant threat to food security worldwide. In addition, several infectious diseases do not respect the species barriers and about 70% of all infections are common to man and animals (zoonosis). Several options available to curtail the colossal economic losses associated with animal diseases include immunization, chemotherapy, improved management, nutritional and genetic improvement, diagnosis and removal of infected animals. Implementation of each of these options can add to solving the problems of productivity losses and zoonosis caused by these diseases (Bishop *et al*., 2010). Investment in animal health research has been shown to pay rich dividend by alleviating poverty in developing countries (Perry *et al*., 2002). According to Garcia *et al.* (2003), a comparison of average milk yield across different countries would reveal that one New Zealand cow produces as much milk as 3 dairy animals in Pakistan, whereas one American cow produces milk that is nearly equal to milk produced by 7 Pakistani cows. This dramatic difference in the milk yield of Pakistani dairy animals *vis-à-vis* dairy cows of advanced countries can be attributed to a verity of factors including genetics, nutrition, management and diseases etc.

1.2 Mastitis

According to Bishop *et al* (2010), in terms of economic losses, mastitis is the single most important disease in cows in developed counties and is also of major importance in developing countries. Avenues of economic losses include milk loss, premature culling, and cost incurred on replacements, occasional mortality, treatment costs and the cost of discarded milk. Treatment of clinical cases not only involves the costs of antibiotics and other therapeutic agents, but may also create opportunities of antibiotic and drug residues in the milk of treated animals. Furthermore, in livestock production systems where farmers are paid according to milk quality, elevated milk somatic cell count (SCC) as a result of clinical and sub clinical mastitis may affect the profitability of dairying. Selection against subclinical mastitis using SCC is now widely practiced in countries where dairying is well developed. Thus, selection for resistance against clinical mastitis is one of the important components of dairy cattle breeding programmes in Scandinavian countries.

Khan and Chaudhry (2001) analyzed the data on 2704 lactations of 993 Nili-Ravi buffaloes to investigate the frequency and behavior of short and complete lactations. Lactation milk yield up to 44 weeks (308 days) was included and lactations with less than 56 days were excluded from the analysis. Of 2107 lactations of less than 14 weeks durations, the causes of drying could be determined from the farm record for 534 lactations. Mastitis accounted for 31% of these lactations for drying during the course of shorter than normal lactations. A meta-analysis of investigations conducted for one year period (1994-1995) on economically important diseases of livestock in Punjab, Pakistan ranked mastitis at the 5th slot. This disease was

responsible for 8% of the total pecuniary losses inflicted by all livestock diseases in this province (Khan *et al*., 1994-95).

1.3 General aspects of hemorrhagic septicemia (HS)

Hemorrhagic septicemia (HS) is an acute highly fatal, septicemic disease occurring primarily in water buffaloes (*Bubalus bubalis)* and cattle but occasionally in other domestic and wild mammals (De Alwis, 1992; Adlakha and Sharma, 1992). Water buffalo is far more susceptible to this disease than cattle (De Alwis, 1999; Khan *et al*., 2006). The etiologic agent of HS is a Gram negative bacterium, *Pasteurella multocida* (Tabatabaei *et al*., 2007; Carter and De Alwis, 1989). *Pasteurella multocida* has at least five serotypes designated as A, B, C, D and E. Serotypes B: 2 and E: 2 are two common serotypes of *P. multocida* associated with HS in animals in Asia and Africa, respectively (Benkirane and De Alwis, 2002). In serotype classification, the letter is used to denote the capsular antigen that was determined originally by Carter (1984) with the use of indirect hemagglutination test. The numeral 2 denotes the somatic or O antigen that was determined originally by Namioka and Bruner (1963) by using agar gel diffusion precipitin test developed by Heddelston and associates.

Hemorrhagic septicemia (HS) is one of the most important diseases of bovines in South Asian and Middle Eastern countries. Epidemiological studies conducted in India over a period of approximately 13 years (1974-1986) indicated that HS was placed mortality wise first and morbidity wise second as compared to 4 other epizootic diseases namely, foot and mouth disease (FMD), rinderpest, anthrax and black quarter (Dutta *et al*., 1990).

1.3.1 Carrier state

Carriers animals are the source of the organism for other animals. Cattle and buffaloes are considered to be the principal carriers of *P. multocida*, but other animals such as pigs, sheep, goats and horses can also act as carriers. The incidence of HS has been shown to be directly proportional to the number of carriers in the animal population. Thus in India in low incidence areas, no carriers were detected, whereas in the moderate and high incidence areas, the carrier rates were 1.9 and 5-6%, respectively (Adlakha and Sharma, 1992). Regular vaccination has been shown to eliminate the carrier status. (De Alwis, 1984).

1.3.2 Transmission

Pasteurella multocida is spread by inhalation, ingestion of contaminated feed, water, direct contact, and fomites etc. The causative agent of HS is mostly shed into the oropharynx, lymphatic tissue associated with the upper respiratory tract, and periodically in nasal secretions during stress (poor food supply, close herding, and wet conditions). HS pathogen does not remain alive for log time, but can survive for a few hours or days in damp soil. The favorable condition for transmission is rainy season and humid environment (Anonymous**,** 2012).

1.3.3 Association of HS with other diseases

Some other infectious diseases may predispose to occurrence of hemorrhagic septicemia. Foot and mouth disease causes immunosuppression (Golde *et al.,* 2014; Maddur *et al.,* 2010) and thus can precipitate an attack of HS or some other bacterial infections. Elshemey and Abd-Elrahman(2013) reported that 56.32% (n=2600) of 4616 cow and buffalo fattening calves infected with SAT_2 serotype of FMD virus showed signs of hemorrhagic septicemia (*P. multocida* B_2 serotype) with a case fatality rate of 13.92%. All of 5630 calves had been vaccinated with an inactivated

FMD vaccine containing antigens of serotype A and O. Kumar *et al*. (2007) reported outbreaks of concurrent pasteurellosis (*P. multocida* serotype B: 2) and classical swine fever that occurred in Indian Punjab. Overall mortality, morbidity, and case fatality rate were 77.5, 88.2, and 87.8%, respectively, in pigs ≤3 months of age, and 8.2, 20.5, and 40%, respectively in older pigs. More marked pneumonic lesions were recorded in cases from which *P. multocida* was isolated.

1.3.4 Pathogenesis

Hemorrhagic septicemia is associated with *P. multocida* serotype B: 2. It is a Gram negative bacterium that secretes lipopolysaccharide (LPS), also named as endotoxin, in blood stream of the host on its lysis. All manifestations of the disease are due to these endotoxines which lead to endotoxemia associated sepsis (De Alwis, 1992; Horadagoda *et al*., 2001). Endotoxines induce many complex deleterious biological reactions in the host. They stimulate a cascade of endogenous mediators such as the coagulation and arachidonic acid systems. Endotoxin also activate polymorphonuclear cells, monocytes and macrophages. When monocytes and macrophages sense the presence of entotoxin, they release special peptides called cytokines.

Horadagoda *et al* (2002) studied the clinical changes alongwith acute phase responses (including tumour necrosis factor; TNF α) in 6 buffalo calves following iv administration of *P. multocida* B:2 endotoxin @ of 1µg /kg in 10 ml of phosphate - buffered saline. There was a rapid onset of clinical signs (dullness, sternal recubency, fever, excessive salivation and respiratory difficulty) following administartion of endotoxin. These clinical signs lasted for about 12 hours. Serum TNF α levels increased rapidly within one hour post inoculation of endotoxin, reaching their peak levels (8-150 ng/ml) at 1-2 hours post inoculation and then declined rapidly to baseline values at 3-5 hours post inoculation. Other acute phase responses included leukopenia, decreased serum levels of iron and zinc. In addition, there was a delayed but prolonged increase in haptoglogin from 12 hours post inoclution that reached its peak from 60 hours post inoculation. Control buffalo calves (n=3) showed neither clinical signs nor the acute phase serum changes mentioned above. The results of this study confirmed the notion that endotoxin has a cardinal role in the pathogenesis of *P. multocida* B:2 associated pasteurellosis of buffalos, commonly knowns as hemorrhagic septicemia.

1.3.5 Rainy seasonal occurrence

Although, occurrence of HS has been reported throughout the year (Dutta *et al*., 1990), its incidence much higher in hot and humid weather (Sivakumar *et al*., 2012). Chowdhury *et al.* (2014) recently reported an outbreak of HS in a buffalo herd in the month of December. High humidity coupled with high environmental temperature and stressful conditions prompts growth of the *P. multocida* and shortens the incubation period of the pathogen by nearly 30 hours, resulting in HS outbreaks with high case fatality rate (De Alwis, 1995).

1.3.6 Clinical signs and post mortem findings

Hussain *et al.* (2014) recorded an outbreak of pneumonic pasteurellosis (*P. multocida)* in cattle and buffaloes in district Sahiwal of Pakistani Punjab. Clinical signs observed included fever, anorexia, brisket edema, profuse salivation, mucopurulent nasal discharge, protruded tongue with open mouth breathing, submandibular edema and dyspnea with respiratory grunts. Necropsy of the dead animals reveled severe pneumonia with consolidation of lungs, froth in trachea, clotted blood in heart, intense pleural adhesions, accumulation of serosanguinous fluid

in pericardial and peritoneal cavities. Acute HS with severe respiratory distress has also been reported in wild ruminents (Dhoot and Upadhey, 2001).

1.3.7 Treatment

Owing to generally a peracute nature of the disease, treatment of HS is not effective, and there is need to find effective therapeutic agents for the treatment of HS. This disease. According to Shahzad *et al.* (2013), treatment was effective only when instituted at the early stage of the disease. These workers also reported that ciprofloxacin, ceftiofur hydrochloride + tylosin were the most effective antibiotics for treatment. Nonetheless, the workers did not mention the cure rates of these antibiotics. Egyptian workers (Elshemey and Abd-Elrahman, 2013), treated an outbreak of HS that occurred in the wake of SAT_2 FMD infection in cow and buffalo fattening calves. Table 2.1 gives the cure rate of 9 different treatment protocols used to manage this HS outbreak.

Table 1. Cure rate of 9 different treatment protocols used to manage an outbreak of HS (*Pasteurella multocida* serotype B_2) that occurred in the wake of SAT2 FMD infection in cow and buffalo fattening calves in Alexandria Egypt Adapted from Elshemey and Abd-Elrahman (2013)

Treatment protocol number	Treatment protocol	No. of animals Treated	Cure rate from H.S	
			No. of animals that recovered from HS	Percent cure rate
1	Ceftiofur sodium	470	423	90
2	Lincspectin and gentamycin.	350	280	80
3	Cefotaxime sodium.	905	815	90.05
4	Tylosine and gentamycin	150	105	70
5	Tultrathromycin	170	85	50
6	Sulpha + Trimethoprim	70	14	20
7	Oxytetracycline LA	200	40	20
8	Amoxycillin	75	0	0
9	Florfenicol	210	0	0

Hussain *et al.* (2014) treated sick animals suffering from pneumonic pasteurellosis with antipyretic, steroids and antibiotic drugs (nature of drugs used, their dosages and duration of treatment not stated by the authors). However, the treated cows and buffaloes did not show improvement in signs and death ensued in a very short period (2-4 hours) in per acute cases.

Ashraf *et al.* (2009) compared florfenicol, florfenicol + flunixin meglumine, amoxicillin and amoxicillin + flunixin meglumine for the treatment of HS in buffalo calves (a total of 40 buffalo calves aged 6-18 months). The recovery rate (survival) in calves treated with florfenicol + flunixin meglumine was 90%, whereas in calves

treated with amoxicillin + flunixin meglumine was 60%. Calves treated with florfenicol alone had a recovery rate of 80%, whereas 50% of the calves treated with amoxicillin alone survived. Chowdhury *et al.* (2014) treated HS affected buffaloes with (a) Inj. Ceftiofur sodium @ 1gm I/M for 5 days, (b) Inj. Meloxicam @ 20 ml I/M for 5 days, (c) Inj. Prednisolone @10 ml I/M for 5 days (in tapering doses). The buffaloes with early stage of disease responded to the treatment.

Raza *et al.* (2000) investigated a total of 50 animals (39 buffaloes and 11 cattle) suffering from hemorrhagic septicemia (HS), selected from the field and divided into 3 groups (A, B and C). Group A (20 animals) was treated with protocol A i.e. norfloxacin (Norfloxillin; Tarobina) + diclofenac sodium +Novacoc forte (Richter Pharma). Group B (20 animals) was treated with protocol B which was essentially the same as protocol A except that Novacac forte was omitted. Group C (10 animals, control) was treated with one of conventional therapies of HS, i.e gentamicin + dipyron. The disease severity index of experimental animals under each treatment protocol was recorded at 0, 12, 24, 48, and 72 hours from the start of treatment. All treatments were repeated at 12, 24, and 48 hours after start of treatment as the sitaution warranted. The survival percent was 85, 80 and 30 amongst animals treated respectively with protocol A, B and C. Three animals in group A and 2 animals in group B died after recovering completely. Excluding these animals from the recovered ones, net survival (percentage) was 70, 70, and 30 in groups A, B and C, respectrively. In terms of reduction of severity of disease, there was significance difference ($P \leq 0.05$) between protocol C and A, between protocol C and B at 12 and 48 hours after treatment but there was non-significant difference at hours 24. Both treatment protocols A and B exploiting the use of a quinolone (norfloxacine) plus a non–steroidal anti-inflammatory drug (diclofenac sodium) with or without a toxin neutralizer and circulatory–stimulant (Novacoc forte) were more effective than the conventional treatment i.e gentamicin + dipyron for the treatment of HS.

1.3.8 Morbidity, mortality and case fatality rates

Hemorrhagic septicemia or pasteurellosis is probably the most serious disease of buffaloes and there are outbreaks which cause heavy mortality and morbidity (Dhillion *et al.,* 1971; Saini *et al.,* 1991; Joshi*et et al.,* 1987; Dhand *et al.,* 2002a). The death rate in buffaloes is three times more than in cattle (Bain *et al*., 1982). Field observations of 9 districts of Punjab, Pakistan showed 9% mortality, 11% incidence, and 87% case fatality rates of hemorrhagic septicemia in buffalo, whereas these values were 2.5, 4, and 62% in cattle (Sheikh *et al.,* 1996).

Taking cues from the previously reported observation that fever increases the survival rate in reptiles, Kluger and Vaughn (1978) investigated the relationship between fever and survival in rabbits infected with *Pasteurella multocida.* A statistically significant correlation was recorded between the fever magnitude and survival. As fever increased up to 2.25 ºC, the survival rate increased. Survivl rate decreased as fever increased.

1.4 Prevalence and economic significance of HS in Pakistan

Hemorrhagic septicemia is a common and important infectious disease of buffaloes and cattle in Pakistan (Khan *et al*., 1991; Ashraf *et al.*, 2011). With a mortality rate near 70% (May *et al*., 2001), this disease was responsible for causing pecuniary losses worth Rs 2.17 billion *per* annum in 1996 in the Punjab province alone

(Anonymous, 1996). Owing to an increase in animal population (cattle n=41.2 millions; buffalo n= 35.6 millions) in 2014-2015, and escalation of prices of animals, the current monitory losses may be at least 2 magnitude higher than these figures. Using an active surveillance system, Khan *et al*. (1991) reported an estimated economic loss of Rs 689900 due to HS in 10 randomly selected villages of Tehsil Lahore. During 2000-2005, field workers of FAO project entitled "Support for Emergency Prevention and Control of Main Trans-boundary Diseases in Pakistan" conducted a Participatory Disease Surveillance. An active surveillance of trans-boundary animal diseases and other important diseases throughout the country (10% villages were randomly selected throughout Punjab, Sindh, Khyber Pakhtunkhwa, Azad Jammu and Kashmir and Northern Areas) was conducted by 17 teams comprising of 5 active field veterinarians each. The highest prevalence (49%) of HS was recorded in district Khanewal, Punjab. On the other hand, the lowest prevalence (0.78%) was recorded in Jamshaid Saddar town of Karachi. The highest prevalence (75.64%) was recorded in Faisalabad district, while lowest (1.53%) in Bajaur agency (Farooq *et al.*, 2007). Based on information gleaned from veterinary officers in 9 districts of Pakistani Punjab, Sheik *et al.* (1996) documented 9% mortality, 11% incidence, and 78% case fatality rates of hemorrhagic septicemia in buffalo, whereas these values were 2.5, 4, and 62% in cattle. Disease incidence was higher in 0 to 24 month old animals and groups of less than 10 animals. Farooq *et al.* (2011) determined the sero-surveillance, morbidity, mortality and case fatality rate of HS in cattle and buffaloes in Dera Ghazi Khan district, Punjab, Pakistan. The average geometric mean titers (GMT) recorded against HS in diseased buffaloes and cattle were 5.7 and 6.1, respectively. The morbidity, mortality and case fatality rates respectively were 57.58, 52.30 and 90.83% in young buffalo calves. The corresponding values for adult buffaloes were 3.17, 1.92 and 60.65%, respectively. In case of young cattle calves, morbidity, mortality and case fatality rates were 8.63, 5.27 and 61.11%, respectively; the corresponding values in adult cattle being 4.83, 2.18 and 45.23%, respectively. This study demonstrated that the mortality, morbidity and case fatality rates due to HS were higher in young calves than in adults both in buffaloes and cattle. It also reinforced the notion that buffaloes are more susceptible to HS than cattle. Recently, Khan *et al*. (2011) reported an outbreak of HS in buffalo calves (aged 6-11 months; n=54) kept at the Livestock Experimental Station, University of Agriculture, Faisalabad, Pakistan. This outbreak occurred in August and September, 2009. Morbidity and mortality rates in this outbreak were 100% and 31.48%, respectively. Mortality peaked on 8th day despite institution of a theoretically effective treatment (Amoxicillin long acting, ceftiofur sodium, prednisolone acetate + dexamethasone sodium phosphate and meloxicame). It is pertinent to note that this outbreak occurred despite vaccination in June with a reputed oil based HS vaccine inoculated as per the manufacturer's recommendation.

Khan *et al.* (2006) reported the findings of a comparative sero-surveillance and clinical epidemiological study conducted on HS in cattle and buffaloes in district Malakand, NWFP (current name of the province is Khyber Pakhtoon Khwa). The average geometric mean titer (GMT) recorded against HS in buffaloes ranged from 4.12-46.98, whereas in cattle the corresponding values ranged from 4.45-46.40. In young buffalo calves (the age not mentioned by the authors), mortality rate, morbidity rate and incidence rate, were 21.19, 95.25 and 22.25%, respectively. In adult buffaloes, the corresponding values were morbidity, mortality and case fatality rates were 5.49, 1.65 and 30%, respectively. In case of young cattle calves, mortality, morbidity, and case fatality rates were lower than those in young buffalo calves, being

1.77, 3.94 and 45%, respectively. The values for morbidity, mortality and case fatality rates in adult cattle were 2.51, 0.39 and 15.79%, respectively.

Ahmad and Naz (2000) reported HS incidence of 6.8% in buffalo calves maintained at the Livestock Experiment Station, Bahadurnagar, Okara, Pakistan. Riaz *et al*. (1992) conducted an active surveillance from September 1989 to August 1990 in district Gujrat, Pakistan. A total of 1025 farmers were interviewed. HS incidence of 1.31% in cattle and 7.24% in buffaloes was recorded.

With the establishment of Directorate for Animal Disease Surveillance and Reporting System in Punjab under the administrative control of Director General (Ext), Livestock and Dairy Development, Punjab, the disease incidence reports communicated by field veterinarians through District Livestock Officers/ District Disease Reporting Officers across Punjab during the month of July to September, 2009 were incorporated to prepare the first quarter report on the distribution of notifiable animal diseases in the province (Anonymous, 2009). FMD PPR, CCPP, ET, HS, BQ and Ecthyma are the diseases among all the contagious diseases that were reported during the reporting period. A total of 208 outbreaks of 23 contagious diseases were reported for different diseases including HS (82), FMD (38), ET (19), PPR (12), BQ (9), CCPP (9) as main prevalent diseases in the reported area. HS, FMD, ET, PPR, BQ and CCPP respectively affected 247, 148, 74, 50, 9 and 159 heads of animals while 95, 22, 16, 13, 5 and 12 died, respectively. Prevalence and incidence of HS, FMD, ET, PPR, BQ and CCPP were found to be higher than other contagious diseases. HS and BQ were reported from Faisalabad, Kasur, Rahim Yar khan and Chakwal, DG Khan, Rahim Yar Khan, respectively. FMD was found to be present in district Bahawalpur, Dera Ghazi Khan and Faisalabad. FMD is a disease that affected highest number of animal heads in Punjab with a low mortality rate. PPR and ET were reported from district Bahawalpur, DG Khan, Faisalabad, Lahore and Bahawalpur, Chakwal, DG Khan, and Faisalabad, respectively. It was concluded that HS, FMD, ET, PPR, BQ and CCPP were the diseases of main concern in the reported areas of Punjab during the months of July to September, 2009.

Khan (2013) reported that no case of HS was brought to the notice of District Diagnostic Laboratory Rahim Yar Khan during the period from May – July, 2012.

1.5 Hemorrhagic septicemia vaccines

Use of vaccines for the control of pasteurellosis in animals is since long in vogue. Shilston (1923) while reviewing the information on hemorrhagic septicemia (pasteurellosis) (that was published in the book edited by Wooldridge, 1923) cited several authors and investigators who used pasteurellosis vaccines for the control of this group of infectious diseases associated with *P. multocida.* For want of availability of these archaic reports, the work of some of these investigators has been reproduced almost verbatim from Shilston (1923) in the ensuing paragraph Oreste and Armmani (1887) attenuated the *Bacillus. Bubalisepticus* (*P. multocida*) by passage through pigeons and employed the blood of these birds after they had died from the inoculation of the organism, for the immunization of the buffaloes. Three injection of 0.1 ml of the blood were given. By growing the organism at temperature of 30^0C to 32^0C, reduced the virulence of *P. multocida* so that the cultures could be employed to give protection to the buffaloes and sheep. Lignieres (1902) prepared a polyvalent vaccine with strains of the hemorrhagic septicemia bacillus from cattle, sheep, horses, pigs, fowls, and dogs. Cultures of the organisms of uniform virulence were kept at a temperature of 42^0 to 43^0 C. for five days to produce the first vaccine and for two days for the second vaccine. The vaccines were injected subcutaneously in doses of from 0.15 to 1 ml according to the size of the animals, at interval of 12-15 days and were

assessed protective against the disease in all species of animals for a period of about one year. Mohler and Eichhorn (1912) employed vaccines prepared by Lignieres method of protecting the buffalo herd in the Yellowstone Park against hemorrhagic septicemia which was causing a heavy mortality. The organisms were injected subcutaneously with 1 ml of each vaccine at an interval of ten days, and no further cases of disease occurred during the succeeding 12 months. These investigation also tested the immunization power of vaccines prepared in the same way as those of Lignieres but to which 5% carbolic acid had been added, thus making them dead vaccines. They found that the carbolized vaccine gave protection against an inoculation of the virulent organism, but, as judged by complement fixation test of serum of the treated animals, this protection was of shorter duration than after injections of uncarbolized vaccines. Holmes tested the immunity conferred by dead vaccines prepared in various ways from cultures of *P. multocida*. Broth cultures of a virulent strain of the organism heated to 65^0 C. for half an hour and carbolized was found to protect young cattle and buffaloes, when injected subcutaneously in dose of 5-10 ml for a period of 6 weeks ageist an inoculation of the virulent organism. The vaccine was considered harmless, only causing in some cases slight swelling which disappeared in a few days, protection was not established until four days after the injection of vaccine. After 150,000 doses of vaccine were employed annually in India as a prophylactic measure in areas where the disease is enzootic at periodic, inoculations, being carried out at the beginning of the winter rains and monsoon season. Muktesar Laboratory in India adopted serum-live culture inoculation method for the control of HS. This method is briefly as follows: Buffaloes and hill cattle are first injected subcutaneously with a protective dose of immune serum and 4 hours later receive 0.1 ml of 48 hours old broth culture of virulent hemorrhagic septicemia bacilli; the initial protection may also be conferred by an injection of dead vaccine followed in ten days by 1ml of virulent culture. A temperature reaction and local edema usually follows the inoculation of the culture; when this has subsided, after 8-10 days an injection of 1ml broth culture was given. Further injections of 5, 25, 100 and 500 ml of culture were given at intervals of ten days and the animals were given at intervals of 10 days. The animals are then bled for serum; the injections and bleedings were repeated, the dose of culture being gradually increased up to 100 ml. An injection of the serum confers an immediate immunity lasting for a period of 3-4 weeks. It has been very effective when employed during outbreaks of hemorrhagic septicemia, the mortality being at once arrested; the serum also possesses some curative power.

Aggressins were one of the earliest immunizing agents used against HS, blackleg, anthrax and certain other human and animal diseases. These are the substances (including but not limited to capsular material, bacterial protein, secretions, excretions, enzymes and toxins) secreted and excreted by certain organisms under favorable conditions of growth, which have the property of inhibiting phagocytosis by a specific action on the leucocytes and reticulo-endothelial system. Owing to their deleterious effect on the tissues of the host and leucocytes and reticulo-endothelial system, they may facilitate the rapid development of normally sublethal infections of disease- producing organisms, resulting in death. Injection of aggressins into the body causes the production of specific anti-aggressins (Scott, 1931). Gochenour, (1924) cited by Scott, (1931) developed an aggressin for the immunization of animals against hemorrhagic septicemia. The hemorrhagic septicemia aggressin is probably the least efficient, due to the peracute nature of the disease. Losses from shipping fever were reportedly nearly twice as great among cattle vaccinated with HS aggressins, or other vaccines including bacterins at the stock yards

or on arrival at the farm as among the cattle not treated with these immunizing agents (Scott, 1931).

Currently, vaccination against HS is the single most important control intervention in countries plagued by this disease. A variety of vaccines containing either whole local isolates of *P. multocida* or their components have been developed and tested under control and filed conditions (Verma and Jaiswal, 1998; Shivachandra *et al*., 2011). A few of these vaccines have found their wide spread field use. One of the hallmarks of these vaccines is a considerable variation in duration of immunity conferred (Shivachandra *et al*., 2011). Plain broth formalin killed bacterins, alum precipitated and aluminium hydroxide gel adjuvanted vaccines have been used until recently. Their immunity lasts for 4-6 months and their protective efficacy is about 60% (FAO, 1998; Verma and Jaiswal, 1998; Tasneem *et al*., 2009; Shivachandra *et al*., 2011). Qureshi and Saxena, (2014) recently reported that cattle vaccinated with conventional alum precipitated HS bacterin did not develop and sustain adequate levels of antibody for long duration. Owing to their shorter duration of immunity, these vaccines are now being supplanted by oil adjuvant vaccines that give both a higher degree of protection and a longer duration of immunity up to a year (Verma and Jaiswal, 1998; Tasneem *et al*., 2009). Oil adjuvant HS vaccines have the drawback of high viscosity and consequently difficulty in syringibility (De Alwis, 1984). In order to address these difficulties, a double emulsion and multiple emulsion vaccines endowed with thin viscosity and long stability have been developed that gave immunity similar to oil adjuvant vaccines (Muneer and Afzal, 1989; Muneer *et al*.,1994; Verma and Jaiswal, 1997; Verma and Jaiswal, 1998). A score of studies have investigated live streptomycin-dependent *P. multocida* (Wei and Carter, 1978; De Alwis *et al*., 1980; Lu and Pakes, 1981; Verma and Jaiswal, 1998; Dagleish *et al*., 2007; Ataei *et al*., 2009) and *P. multocida* + *P. haemolytica* vaccine (Catt *et al*., 1985). De Alwis *et al*. (1980) investigated a live mutant strain of *P. multocida* that was administered as a single dose containing 10^{10} to 10^{11} viable organism. This vaccine immunized 75 % of cattle and 100% of buffalo. The number of live microorganism used in this vaccine is seemingly so high that it precludes the practical utility of this vaccine in the immunological control of HS. In a subsequent study, De Alwis, (1981) reported that natural *P. multocida* infection of buffalo calves give considerably higher immunity than that conferred by vaccine which can be interpreted to mean that a live vaccine, using a suitable mutant, may prove superior to the existing ones(Adlakha and Sharma, 1992).A live HS vaccine containing *P. multocida* serotype B: 3, 4 was administered by intranasal aerosol in Myanmar (Burma) to more than 15 million cattle and buffaloes between 1989 and 1999 (Myint *et al*., 2005). Field observations of veterinary officers in 9 districts of Pakistan, Punjab have pointed out the occurrence of field outbreaks of HS despite vaccination with alum- precipitated *P. multocida* bacterin prepared in public sector (Sheik *et al*., 1996). In order to obtain maximum protection from HS vaccine, it is recommended that the vaccines be prepared from *P. multocida* strains circulating in the regions of intended use. FAO recommends a live avirulent HS vaccine prepared from a *P. multocida* serotype B: 3 of fallow deer origin for use in Southeast Asia (Carter, 2005). Despite vaccination and improved management, occurrence of disease outbreaks is a regular feature each year, especially in endemic areas of the country (Afzal and Muneer, 1990). Pakistani workers (Shahzad *et al*., 2013) reported an outbreak of HS in buffalo calves that had been vaccinated with alum precipitated HS vaccine two months earlier. Arif *et al.* (2013) recently documented that subunit or whole bacterin based *P. multocida* vaccines were less immunogenic than an anti-idiotypic vaccine prepared against *P. multocida* B: 2.

Hemorrhagic septicemia is predominantly a disease of young cattle and buffaloes. How soon after birth, the calves should be vaccinated against this disease is a very pertinent question in the immunization program of dairy animals. Japanese workers (Otomaru *et al.,* 2015) in a bid to answer this question investigated the dynamics and duration of antibody titers against *P. multocida* in Japanese black calves. To this end, 20 unvaccinated calves from two Japanese black breeding farms in Japan were investigated. Serum samples were collected from these calves at 1, 4, 8, 12, 16 and 20 weeks after birth. Similarly, serum samples were also obtained from their dams once at 1 week after calving. Serum antibody titers against *P. multocida* in calves at 1 week of age correlated very well with those in their dams at 1 week after calving. Maternally derived antibody titers against *P. multocida* in experimental calves fell to their lowest values at 4 weeks of age. One of the findings relevant to immunization against *P. multocida* was that calves started producing antibodies against this organism by themselves between 4 and 8 weeks of age. In the light of the findings of this Japanese study, vaccination against *P. multocida* can probably be started after the calves have attained an age of 4 weeks.

Nadeem *et al*. (2010) conducted a study to determine the effect of prophylactic application of levamisole in hemorrhagic septicemia (HS) vaccinated buffalo calves. A total of 60, 5 month old buffalo-calves were randomly selected from the Livestock Production Research Institute Bhadurnagar (LPRI) Okara, Pakistan and divided into 3 groups (G1, G2 and G3) of 20 calves each. Animals in G1 group were vaccinated with 3ml (I/M) injection of a commercially available oil based HS bacterin (NIAB-HS™) while animals in group G2 received levamisole @ 0.5mg/kg body weight two days prior to vaccination with this bacterin. Group G3 served as non-vaccinated and non-medicated control. IHA based geometric mean titers (GMT) against *P. multocida* and Lymphocytes Proliferation Assay (LPA) were used as evaluation criteria. Geometric mean titers (GMT) were significantly higher (P>0.05) in calves of group G2 (21.5) as compared to GMT values (14.79) recorded in calves of group G1. Similarly, the values (0.317±0.041) of LPA in calves of group G2 were significantly higher than the corresponding values (0.043±0.002) recorded in calves of group G1. The values of GMT and LPA in non–vaccinated, non–medicated calves (group G3) were 2.5 and 0.043±0.002, respectively. It was concluded that prophylactic application of levamisole helps in mounting a better humoral and cellular immune response to *P. multocida* vaccine.

1.6 Importance of transferrin binding protein A as an immunogen

IROMPs (Iron regulated outer membrane proteins), in particular TbpA (transferrin binding protein A) has been proposed as a vaccine candidate immunogen for *P. haemolytica*, *Neisseria meningitides*, and *Haemophilus influenza*. The rationale for targeting TbpA as an immunogen is three folds (Singh *et al*., 2011). Firstly, this is one of the important IROMPs involved in acquisition of iron from the host and is essential for overcoming the iron restriction imposed by the host iron binding i.e. transferrin. Secondly, it is present at the cell surface of the bacterial cell and expressed by bacteria when growing inside host body in response to iron depleted conditions. Its expression is enhanced by incorporation of iron chelators like 2, 2-dipyridyl in the culture media (Srivastava, 1998). Transferrin binding protein is one of the important virulence factors of *P. multocida*. Thus, Veken *et al*. (1996) reported a positive association of TbpA with HS causing strains of *P. multocida* in bovine whereas the strains of non HS serotypes did not express this protein. Singh *et al.* (2011) evaluated the potential of TbpA as a DNA vaccine against HS in a mouse model. The TbpA gene of *P. multocida* serotype B:2 was cloned in a mammalian expression vector alone

and along with murine IL2 gene as immunological adjuvant to produce monocistronic and bicistronic DNA vaccine constructs, respectively. The immune response to DNA vaccines was determined on the basis of serum antibody titers and lymphocyte proliferation assay. Mice vaccinated with DNA vaccines showed significantly higher antibody titers and cell mediated immune response than the non-vaccinated mice. The bicistronic DNA vaccine elicited a superior immune response and protection following challenge as compared to monocistronic construct. These investigators concluded that DNA vaccine offers the promise of an effective immunological approach for the control of HS.

1.7 Montanide® adjuvanted HS vaccines

In a bid to develop a stable, easily injectable HS vaccine with a long duration of immunity, Pakistani workers (Muneer and Afzal, 1989) prepared and evaluated two oil-adjuvant vaccines *viz.* water-in-oil emulsion (final product incorporating Marcol 52, Montanide® 103 and antigen in ratios of 6:1:3) and double emulsion (Marcol 52, Arlacel A and Tween 80 plus antigen) of *P. multocida* Robert's type 1. These preparations were evaluated on only 10 buffalo calves aged ≥ 4 months which showed sustained antibody response throughout the entire duration of experiment (=240 days). The pitfall of small number of test subjects (buffalo calves) in this study were addressed by Muneer *et al*. (1994) by evaluating three oil adjuvant vaccines of *P. multocida* 6: B. in terms of level and duration of antibody levels in buffalo calves (n=45). The three vaccines evaluated were (a) water-in-oil emulsion (= single emulsion; OAV1) containing Marcol 52, Montanide® 888 and antigen incorporated at a ratio of 6:1:3, (b) double emulsion (OAV2) formulated to contain Marcol 52 (63%), Arlacel A (7%) and Tween 80 (1.5%) + antigen (28.5%), (c) vitamin E adjuvant vaccine (OAV3) incorporating Vit. E (62%), Montanide® 888 (8%) and antigen (30%). ELISA-based *P. multocida* antibody titers elicited by these vaccines remained substantially higher than those recorded by using traditional alum precipitated bacterin following sensitizing and booster vaccination spaced 2 months apart. All three vaccines induced immune response in buffalo calves that persisted beyond 270 days post-vaccination. On experimental challenge at 6 and 12 months after booster vaccination, the calves vaccinated with OAV1, OAV2, and OAV3 withstood experimental challenge with virulent *P. multocida* (6×10^8 cfu) given subcutaneously. Also, there was no untoward reaction like pyrexia or swelling at the inoculation site. Contrarily, calves dose with alum precipitated bacterin (the traditional HS vaccine) showed signs of HS on post-vaccination challenge at 6 and 12 months, *albeit* they also recovered. Non-vaccinated animals (= control groups) died after showing overt signs of HS on challenge at both 6 months and 12 months post vaccination. Sotoodehnia *et al*. (2005) developed a Montanide® oil ISA-70 adjuvanted HS vaccine that was injected at the dose rate of 3 ml (2 mg dry weight/ml) into 5 calves by intramuscular route. The sera collected were tested by passive mouse protection test (PMPT). The results of PMPT showed 100% protection up to 150 days and 66 to 83% up to 200 days post-vaccination. In active mouse protection test (AMPT), 4 log of protection was recorded. The investigators concluded that immunity induced by this Montanide® HS vaccine was protective for calves against HS. Multiphasic emulsions (e.g Montanide® ISA 201) reportedly induce long term immunity and protected cattle against hemorrhagic septicemia for 1 year after only one vaccination (Reddy *et al*., 1995).

1.8 Prevalence and economic significance of mastitis in Pakistan

Mastitis is one of the most prevalent and economically important diseases of dairy animals in Pakistan. Table 2.2 depicts animal, clinical, subclinical, quarter, and blind quarter based prevalence of mastitis in some selected studies in Pakistan.

Table 1. Reported prevalence of mastitis in cows and buffaloes in some selected studies conducted in Pakistan

Prevalence rate (%)	No. of animals investigated	Species (cows/buffaloes)	Locale of study	Reference
33.33[4]	200	Buffalo	Hyderabad	Soomro *et al.* (1997)
77.98[1],58.75[3]	300	Buffalo	Attock	Bachaya *et al.* (2005)
27[4],4[2],10[5] 36[4],5.5[4], 8[5]	50 50	Buffalo crossbred cows	Faisalabad	Khan and Muhammad (2005)
29[1],7[2],32[3] 41[1],12[2],24.75[3]	100 100	local cows crossbred cows	D. I. Khan	Akhtar *et al.* (2012)
36.38[4],24.60[2] 33.67[4],18.21[2]	382 291	Buffalo Cow	Tehsil Burewala	Hameed *et al.* (2012)

1= Animal based; 2= Clinical signs based; 3=Quarter based; 4=Subclinical based; 5=Blind quarters based.

Hussain *et al.* (2005) reported the findings of a farmers' participatory surveillance survey of livestock diseases in Islamabad capital territory. They used the technique of proportional piling to estimate the relative prevalence of livestock diseases in the area. In the execution of the study, 100 beans (or pebbles in some cases) were dispensed to the farmers and they were asked to make piles according the relative incidence of 5 most prevalent diseases. The results indicated that HS, FMD and mastitis were the 3 most common disease conditions observed by the participating farmers. Gender based stratification of the disease incidence data revealed that 15.8 and 16.3% of the male and female livestock keepers, respectively in Islamabad capital territory had noticed occurrence of mastitis in their cows and buffaloes.

A recently reported study conducted under Pakistan Strategy Support Program of USAID by Ashfaq *et al.* (2014) estimated and evaluated on the basis of prevalence of main livestock diseases that effect on diary animal productivity and farm incomes in five tehsils of district Faisalabad. Three villages were selected from each tehsil and ten livestock farmers from each village picked randomly to collect information. Farmers categories into three on the basis of adult cows and buffaloes owned were as follows: small (1-3 animals), medium (4-6 animals), and large farmers (more than 6 animals). The study focused on the negative impact on milk production and farm incomes due to mastitis, parturient hemoglobinuria, FMD, and tick infestation. The morbidity or mortality rate, incidence rate, and case fatality rates of each disease were determined. The economic losses related with these diseases were appraised. In addition, economic returns associated with controlling these 4 diseases were calculated in the form of benefit-cost ratios. Overall, 9.01% of total economic losses

associated with these 4 diseases were caused by mastitis. Small, medium and large farmers, respectively sustained 14.09, 8.34 and 9.59% of the total economic losses caused by 4 diseases selected for survey information. Riaz *et al*. (1992) conducted an active surveillance from September 1989 to August 1990 in district Gujrat, Pakistan. A total of 1025 farmers were interviewed. Mastitis incidence of 4.04% in cattle and 5.48% in buffaloes was recorded. A meta-analysis of investigations conducted over a one year period (1994-1995) on economically important diseases of livestock in Punjab (Pakistan) ranked mastitis at the 5th slot, accounting for 8% of the total pecuniary losses inflicted by all livestock diseases in this providence (Khan *et al*., 1994-1995).

1.9. Role of *Staphylococcus aureus* and *Streptococcus agalactiae* in mastitis

In countries lacking application of mastitis control programs, contagious organisms (*S. aureus* and *Str. agalactiae*) are the two most predominant mastitis pathogens (Pankaj *et al*., 2013). Collectively, these two pathogens are responsible for nearly 75% cases of this disease in cows and buffaloes (Allore, 1993). Pakistani worker (Shakoor, 2006) while reviewing the reported prevalence of pathogens associated with mastitis in Pakistani studies (n=16, conducted between 1966-2002) concluded that *S. aureus* was the most prevalent mastitis pathogen in buffaloes and cows followed by *Str. agalactiae* , *E. coli*, and mixed infections.

1.10 Mastitis vaccines

High prevalence rate, poor response to antibiotics, development of antibiotic resistance and consumers concern about residues of antibiotics/antibacterials in milk and meat of the treated animals have spurred interest in the control of mastitis through vaccination (Tollersrud, 2001). The idea of use of mastitis vaccine for control and treatment is not new. According to Wooldridge, (1923), vaccines of various kinds e.g. stock vaccines containing a mixture of streptococci, staphylococci, and *Bacterium pyogenes*, were in wide use in 1920s to prevent or treat bovine mastitis. This author also stated that in herds in which there has been a series of clinical cases, favorable results have been reported from the use of so called stall-specific vaccines (autogenous vaccines).The production of autogenous mastitis vaccines entails isolation of the organism (s) responsible for the outbreak from several cows, its mass cultivation followed by treatment of the whole of the milking herd with this vaccine made therewith. In order to prevent outbreaks, it was recommended that doses of dead vaccine should be given during the dry period. In the subsequent period, investigations by French (Richou and Thienlin., 1955), and English workers Derbyshire *et al.* (1961), demonstrated that use of staphylococcal mastitis vaccines had a definite increase in immunity against certain strains of staphylococci as reflected by a decrease in the spread of this infection coupled by a clear reduction of the number of acute cases of mastitis. An equal effect against all strains of staphylococci was, however, not noticed. Notwithstanding this limited success, as emphasized by Derbyshire *et al.* 1961) further research into mastitis vaccinology is clearly warranted. (Heidrich and Renk, 1967). During the past 3 decades, a variety of mastitis vaccines were investigated and a few of these are now commercially available (Ahmad, 2009; Pereira *et al.,* 2011; Rashid, 2011). Monovalent and polyvalent mastitis vaccines developed and evaluated at the Mastitis Research Lab. Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan were found effective in mastitis control and treatment (Shakoor, 2006; Athar, 2007; Yousaf, 2009; Ahmad, 2009; Rashid, 2011). Recently, Leitner *et al*. (2011) reported the efficacy and safety of a recombinant Montanide® ISA 206 *S. aureus* mastitis vaccine in dairy cows.

This vaccine was based on Target of RNAIII Activating Protein (TRAP), which is a membrane associated 167AA protein constitutively expressed in all strains of *S. aureus* and coagulase negative staphylococci. This recombinant TRAP (rTRAP) *S. aureus* mastitis vaccine was safe, immunogenic and elicited a humoral immune response that remained high for at least 160 days post booster shot.

1.10.1 Montanide® (Seppic, France) adjuvanted mastitis vaccines

Montanide® ISA are fairly a new generation of ready-to-use adjuvants which allow the manufacture of different type of emulsions: water in oil (W/O), oil in water (O/W), or water in oil in water (W/O/W). They can be based on mineral oil, non-mineral oil or their mixture. Adjuvants designed for veterinary and human vaccines were reviewed by Aucouturier *et al.* (2001). According to these authors, W/O/W (e.g. Montanide® ISA 201 VG, a multiphasic emulsions) can induce long and short term immune response. Low viscosity (thus an easy syringibility) is another desirable attribute of this class of Montanide®. After vaccination, the antigen in the external water phase is immediately available to the immune system like aqueous formulations while antigen entrapped in the internal aqueous phase is slowly released like W/O emulsions. Water in oil in water (W/O/W) emulsions elicit superior immune response as compared with aluminium hydroxide in cattle. They are well tolerated and induce a strong stimulation of cellular immune response. According to Aucouturier *et al.* (2001) screening studies in mice with viral, bacterial or parasitic antigens indicated that W/O/W emulsions induce higher IgG2a antibody levels than other type of emulsions. IgG2 is the most desirable antibody for protection against *S. aureus* mastitis (Watson, 1975) because neutrophils have receptors for this antibody. Tollersrud *et al*. (2002) reported that *S. aureus* capsular polysaccharide type expressed on the surface of formaldehyde inactivated whole cells emulsified in the Montanide® gave a strong and long lasting immune response. Tollersrud, (2001) reported that Montanide® ISA-70 enhances a stronger immune response to α, β-toxins than carbopol vaccine tested in sheep. Yousaf *et al.* (2009) evaluated the effect of a Montanide® ISA 206 adjuvanted *S. aureus* bacterin-toxiod on the prevalence and incidence of mastitis in cows. A total of 60 California mastitis test and surf field mastitis test negative cows in their first and second month of lactation were selected and assigned randomly to 3 groups (C1, C2, and C3). Montanide® ISA-206 adjuvanted *S. aureus* bacterin –toxoid (in 5 ml doses) was injected I.M twice with 4 weeks spacing to animals of group C1 and C2. Levamisole (2.5mg/kg body weight) was administered *per os* to cows of group C2 after first and second shots of vaccine. Cows in group C3 served as control (non -vaccinated and non- medicated with levamisole). Prevalence and incidence rates of mastitis were determined. The results showed that maximum number of quarters were found positive in group C3 with maximum cumulative prevalence (27.5%) while minimum cumulative prevalence (11.25%) was noted in group C2 followed by group C1 (13.75 %). There was no significant variation in cumulative incidence between group C1 and C2. Montanide® ISA-25 and ISA-206 (Seppic, France) as adjuvants in cattle and pigs are much better than conventional aqueous formulations to retain potency and to enhance the antibody response (Barnett *et al*., 1996). No toxicity (even after booster dose) by the use of these vaccines can be seen (Castrucci *et al*., 1993). It is reported that Montanide® adjuvanted vaccine is less viscous, less irritant and easily injected than conventional Freund's adjuvanted vaccine (Cook *et al*., 1990).The Montanide® adjuvanted vaccine elicited superior and rapid immune response at any time period which was maintained for longer time (Patil *et al*., 2002). Ahmad and Muhammad, (2008) demonstrated that a locally

prepared *Staphylococcus aureus* and *Streptococcus agalactiae* aluminium hydroxide adjuvanted mastitis vaccine was immunogenic when tested in rabbits.

1.11 Need for a combined HS–Mastitis vaccine ('combo' or 'cocktail' HS – mastitis vaccine)

Combined vaccines ('Combo vaccines') are vaccines containing combination of two or more vaccinal antigens from different pathogens blended in one vaccine vial/bottle. These vaccines usually target more than one disease. The use of combination vaccines is a pragmatic strategy to overcome the constraints and problems associated with multiple injections of individual vaccines. Combined vaccines containing bivalent, trivalent as well as polyvalent microorganisms (bacterial and /or viral) including *P. multocida* have been developed to reduce the number of vaccine doses and reduce the cost of production as well as to expand the spectrum of protective immunity against multiple infections (Verma and Jaiswal,1998; De Alwis,1999). Combined vaccines targeting HS, black quarter, FMD, infectious bovine rhinotracheitis, adeno virus type-3 parainfluenza and some other respiratory pathogens have been developed and tested.(Srivastava *et al*., 1975; Osman *et al*., 1990).

The use of combined vaccines lessens the cost for extra health care visits, facilitates the addition of new vaccines and diseases into immunization programs (CDC, 2011). Geering and Lubroth, (2002) opined that during visits by FMD vaccination teams to livestock farming communities, it may be desirable to combine FMD vaccination with vaccination against other diseases (e.g. HS, contagious bovine pleuropneumonia, sheep and goat pox). Combined vaccination not only conserves resources but is also helpful in achieving a higher level of farmer cooperation in the FMD control program. According to Hussain et al. (2005), HS and mastitis are among the 5 most important diseases that affect the local animals in Pakistan. An immunization program based on the use of combined HS- mastitis vaccine may potentially be associated with the following advantages:

- Less frequent handling of the candidate vaccinate cows and buffalos
- Inclusion of mastitis in the vaccination program. By and large a 'do nothing' policy is in place in Pakistan as far as mastitis control is concerned.
- Less cost of vaccination

Efficacy of a bivalent vaccine *viz.* FMD and HS was compared to individual FMD and HS vaccines in rabbits by Altaf *et al.* (2012). The investigators concluded that this combined vaccine induced higher antibody titers against both FMD and HS than the individual FMD (prepared from O serotype) and HS vaccine (alum precipitated). Srinivasan *et al.* (2001), compared the serological response of cattle to a combined FMD, rabies, HS, black quarter vaccine to individual component vaccines containing FMD, rabies, *P. multocida,* and *Clostridium chauvoei* antigens. A statistically non- significant variation in the serological response was elicited by individual component vaccines and combined vaccine containing antigens of all four disease pathogens. As far as could be ascertained from the available literature, a combined HS-mastitis vaccine has not been evaluated thus far. According to Chakrabarti, (2002), a combined H.S-black quarter vaccine is available in India. It is a combination of inactivated antigen of *P. multocida* type-I and *Clostridium chauvoei*. This is stabilized and adjuvanted with BAIF aluminium hydroxide gel aluminox. The manufacturer claims that this vaccine protects animals against both hemorrhagic septicemia and black quarter.

In Pakistan, because of extremely small herd size (more than 80% animals kept in groups of 3-4 animals/family; Jost, 1980; Tuefel, 1998), widely rampant

poverty and illiteracy, lack of any milk quality premium program adoption of the standard mastitis control practices i.e. post milking antiseptic teat dipping and dry period antibiotic therapy as advocated by the National Mastitis Council Inc, USA (Nickerson, 1994) is conceivably difficult. Even on well organized private dairy farms and those in public sector, mastitis control is not in place. Against this backdrop, vaccination against the common mastitis pathogens holds the promise of an adjunct/alternative mastitis control strategy (Koiranen, 1977).

1.12 Mastitis in heifers and justification of use of combined HS-mastitis vaccine

Future of a dairy herd hinges to a considerable extent on the milk producing potential of replacement animals i.e. heifers. Investment in general management and health of the heifers is reflected in good herd replacements. Managmental and health deficiencies during the period intervening between calf birth and its first calving can drastically decrease the milk yield of heifer as she enters the lactating herd. On many farms, calves suffer from the vice of suckling each other's navel and developing udder .The occurrence of subclinical intramammary infections (IMI) in non-lactating heifers was once considered rare but in recent years, studies have indicated that the prevalence of IMI in heifers can be as high as 97% in some herds (Trinidad *et al*., 1990b). These infections are important because infections acquired during the prepartum period may damage developing secretary tissue, increase the SCC (Trinidad *et al*., 1990a; Trinidad *et al*., 1990b; Nickerson *et al*., 1995; Oliver *et al*., 2003) and reduce lifetime milk production (Oliver *et al*., 2003, De Vliegher, *et al*., 2004). Therefore, the control of IMIs in heifers is receiving increased attention. Staphylococci (primarily coagulase-negative Staphylococci but also *Staphylococcus aureus*) are the most frequent isolates recovered from heifers but environmental pathogens such as Streptococci are also frequently recovered (De Vliegher, 2004). Use of a combined HS–mastitis vaccine incorporating antigens of *P. multocida*, *S. aureus* and *Str. agalactiae* in heifers may not only potentially reduce the incidence of HS but also reduce the incidence and prevalence of *S. aureus* and *Str. agalactiae* associated mastitis.

Suboptimal immune response to bovine vaccines is an important problem and several interventions can be used to overcome this problem. Arthington and Havenga, (2011) demonstrated that an injectable trace minerals supplement (MultiMi®, Fort Collins, Colorado, USA) containing 15, 40, 10 and 5 mg/ml of CU, Zn, Mn (all as disodium EDTA salts),and Se (as Na selenite) when administered concurrently with a bovine viral vaccine (Titanium® 5; AgriLabs, St. Joseph, Missouri, USA) containing bovine herpes virus (BHV1),bovine viral diarrhea virus (BVDV1), BVDV-2, bovine parainfluenza virus type 3,and bovine respiratory syncytial virus did not impair humoral immune responses in beef calves. In addition, concurrent administration of injectable trace minerals supplement and BHV-1 vaccine enhanced the production of neutralizing antibodies to BHV-1 in previously naïve beef calves. No such investigation seems to have been conducted on hemorrhagic septicemia vaccine.

References

Adlakha SC and SN Sharma, 1992. Infectious diseases. In: Tulloh NM and JHG. Holmes (editors) Buffalo Production. Elsevier, Amsterdam, Netherland.

Afzal M and R Muneer, 1990. Development of a combined vaccine for haemorrhagic septicemia and foot and mouth disease. Pak Vet J, 10: 67-69.

Ahmad R and NA Naz, 2000. Incidence and therapy of pasteurellosis in buffalo

calves. Pak Vet J, 20: 101-102.

Ahmad T and G Muhmmad, 2008. Evaluation of ***Staphylococcus aureus*** and ***Streptococcus agalactiae*** aluminium hydroxide adjuvanted mastitis vaccine in rabbits. Pak J Agri Sci, 45: 353-361.

Ahmad T, 2009. Therapeutic efficacy of an aluminium hydroxide adjuvanted Staphylococcus aureus and Streptococcus agalactiae bacterin-toxoid alone and in combination with antibiotics for the treatment of clinical and sub-clinical bubaline mastitis. Ph.D Thesis, Deptt Clinical Medicine and Surgery, Univ Agri Faisalabad, Pakistan.

Akhtar A, Khairani-Bejo, A Umer, J Tanweer and Habibullah, 2012. Prevalence of mastitis and identification of causative pathogens in local and crossbred cows in Dera Ismail Khan. Pak J Sci, 64: 265-268.

Allore HG, 1993. A review of the incidence of mastitis in buffaloes and cattle. Pak Vet J, 13: 1–7.

Anonymous, 2012. Haemorrhagic Septicaemia. In: OIE Manual of Standards for Diagnostic Tests and Vaccines, 3rd Ed. Office International De Epizootics, Paris, pp: 739-751.

Arthington JD and LJ Havenga, 2011. Effect of injectable trace minerals on the humoral immune response to multivalent vaccine administration in beef calves. J Anim Sci 90: 1966-1971.

Ashfaq M, G Muhammad, Shamsheer-ul-Haq, A Razzaq, 2014. Effects of livestock diseases on dairy production and In-comes in district Faisalabad, Punjab, Pakistan. Pakistan Strategy Support Program-International Food Policy Research Institute Working Paper No.023.

Ashraf A, H Tariq, S Shah, Nadeem, I Manzoor, S Ali, A Ijaz, S Gailani and S Mehboob, 2011. Characterization of *Pasteurella multocida* strains isolated from cattle and buffaloes in Karachi, Pakistan. African J Microbiol Res, 5: 4673-4677.

Ashraf S, A Omer, M Ijaz, UN Chaudry and MM Ali, 2009. Efficacy of florfenicol against hemorrhagic septicemia in buffalo calves. Pak J Zool Supp Ser, 9: 119-122.

Aucouturier J, L Dupuis, and V Ganne, 2001. Adjuvants designed for veterinary and human vaccines. Vaccine, 19: 2666–2672.

Bain RVS, MCL De Alwis, GR Carter and BK Gupta, 1982. Haemorrhagic septicemia. FAO Animal and health paper No.33. FA0, Rome, Italy.

Barnett PV, L Pullen, L Williams and TR Doel, 1996. International bank for foot-and- mouth disease vaccine: Assessment of montanide ISA 25 and ISA 206, two commercially available oil adjuvants. Vaccine, 14: 1187-1198.

Benkirane A and MCL De Alwis, 2002. Hemorrhagic septicaemia, its significance, prevention and control in Asia. Vet Med Czech, 47: 234-240.

Bishop S, M de Jong, D Gray, 2010. Opportunities for incorporating genetic elements into the management of farm animal disease: policy issues. Commission on Genetic Resources for Food and Agriculture. Background study paper No.18. Accessed from www.fao.org/ag/magazine/bsp 18-e.pdf.

Carter GR, 1984. Serotyping *Pasteurelia multocida*. Methods in Microbiol, 16: 247-258.

Carter GR, 2005. Hemorrhagic septicemia. In: Kahn, C. M (ed.). The Merck Veterinary Manual. 9th Ed. Merck & Co., Inc. Whitehouse Station, New Jersey, USA. pp: 607-609.

Carter GR, and MCL De Alwis, 1989. Haemorrhagic septicaemia. In: Pasteurella and Pasteurellosis, Adlam C and JM Rutter (eds.) Academic Press, London. pp: 131-160.

Castrucci G, M Ferrari, V Angelillo, F Rigonat and L Capodicasa, 1993. Field evaluation of the efficacy of romovac 50, a new inactivated, adjuvanted bovine rotavirus vaccine. Comp Immunol Microbiol Infect Dis, 16: 235-239.

CDC, 2011. Morbidity and mortality weekly report (MMWR), general recommendations on immunization, and recommendations of the advisory committee on immunization practices (ACIP), 60: 1-64.

Chakrabarti A, 2002. Hemorrhagic septicemia.A text book of preventive veterinary medicine. pp: 255-262.

Chowdhury M, J Mitra, S Sarkar, T Samanta and BB Roy, 2014. PCR and electron microscopy based diagnosis of an outbreak of haemorrhagic septicemia in buffalo and its control in a farm of West Bengal, India. Explor Anim Med Res, 4: 86-94.

Cook DR, HT Hill and DR Kinker, 1990. Efficacy of a killed gpX deleted pseudorabies virus vaccine. Can J Vet Res, 54: 438-445.

De Alwis MCI, 1981. Mortality among cattle and buffaloes in Sir Lanka due to haemorrhagic septicaemia. Trop Anim Hlth Prod, 13:195-202.

De Alwis MCL, 1984. Haemorrhagic septicaemia in cattle and buffaloes. Rev Sci Tech Off Int Epiz, 3: 707-730.

De Alwis MCL, 1992. Haemorrhagic septicaemia. A general review. Br Vet J, 148:99-112.

De Alwis MCL, 1995. Haemorrhagic septicemia in cattle and buffaloes.In:W.Donachie et al. (ed.), Haemophilus, Actinobacillus and Pasteurella. Plenum Press, New York, pp.9-24.

De Alwis MCL, 1999. Haemorrhagic septicaemia. Australian Center for international Agriculture Research (ACIAR) monograph no.57, Canberra, Austalia, pp: 1-34.

De Alwis MCL, GR Carter, and MM Chengappa, 1980. Production and characterization of streptomycin dependent mutants of Pasteurella multocida from bovine haemorrhagic septicaemia. Can J Comp Med, 44: 418-422.

De Vliegher S, 2004. Udder Health in Dairy Heifers - some epidemiological and microbiological aspects. Ph.D. Thesis, Dept. of Repro, Obstetrics and Herd Health, Fac Vet Med Ghent, University, Belgium.

Derbyshire JB, I Davidson and CD Wilson, 1961. Symposium on staphylococcal mastitis. Vet Rec, 73: 1011-1033.

Dhand NK, RM Gopal, J Singh, DR Sharma, KS Sandhu and PK Sindhu, 2002. Disease outbreaks in the field and emerging disease an overview. Proceedings of the animal husbandry officers workshop department of veterinary and animal husbandry extension, agricultural university (ed KB Sing, H.K Verma) pp: 1-8.

Dhillion SS, MS Kwatra and PN Dhingra, 1971. In intestinal form of pasteurellosis following rinderpest vaccination in Punjab. Bulletin of international epizootics 75:335-341.

Dhoot VM and SV Upadhye, 2001. Pasteurellosis in a chital deer (Axis Axis) in captivity. Zoos Print J, 16: 428-429.

Dutta J, BS Rathore, SG Mulick, R Singh and GC Sharma, 1990. Epidemiological studies on occurrence of hemorrhagic septicemia in Inida. Ind Vet J, 67: 893-899.

Elshemey, TM and AH Abd-Elrahman, 2013. Hemorrrhagic septicemia outbreak as a

consequence of SAT_2 FMD infection in buffalo and cattle in Alexandria province,

Garcia O, K Mahmood and T Hemme, 2003. A review of milk production in Pakistan with particular emphasis on small-scale producers. PPLPI working paper no.3. International Farm Comparison Network (IFCN) of FAO. P: 5.

Geering WA, and J Lubroth. 2002. Preparation of Foot-and- Mouth Disease Contingency Plans .FAO Animal Health Manual. No.16. FAO, Rome Italy P: 51.

Golde WT, T deLosSantos, L Robinson, MJ Grubman, N Sevilla, A Summerfield and B Charleston. 2011. Evidence of activation and suppression during the early immune response to foot-and-mouth disease virus. Transbound Emerg Dis, 4:283-290.

Hameed S, M Arshad, M Ashraf, M Avais and MA Shahid, 2012. Cross-sectional epidemiological studies on mastitis in cattle and buffaloes of tehsil Burewala, Pakistan. J Anim Plant Sci, 22: 371-37.

Heidrich HJ and W Renk, 1967. Diseases of the Mammary Glands of Domestic Animals. Translated from German by LW Van, Den Heever, WB Saunders Co. Philadelphia USA.P. 205.

Holden S, S Ashley, and P Bazeley, 1996. Improving the delivery of animal health services in developing countries-A literature review. Accessed from: http://www.theidlgroup.com/documents/improving the delivery of Animal Health Services in Developing Countries. Pdf.

Horadagoda NU, JC Hodgson, GM Moon, TG Wijewardana and PD Eckersall, 2001. Role of endotoxin in the pathogenesis of haemorrhagic septicemia in the buffalo, Microbial Pathogenesis, 30: 171-178.

Horadagoda NU, JC Hodgson, GM Moon, TG Wijewardana and PD Eckersall, 2002. Development of clinical syndrome resembling haemorrhagic septicemia in the buffalo following intravenous inoculation of *Pasteurella multocida* B: 2 endotoxin and the role of tumour necrosis factor-alpha. Res. Vet Sci, 72: 194-200.

Hussain M, MA Malik, Z Fatima, and MR Yousaf, 2005. Participatory surveillance of livestock diseases in Islamabad capital territory. Intl J Agric Biol, 7: 567-570.

Hussain R, F Mahmood, A Khan, M Z Khan, and A B Siddique, 2014. Pathological and molecular based study of pneumonic pasteurellosis in cattle and buffalo (*Bubalus bubalis*). Pak J Agri Sci, 51: 235-240.

Joshi VB, KK Baxi and DS Sambyal, 1987. Prevalence of *pasteurella multocida* in domestic animals and birds of Punjab, Journal of research, Punjab agricultural university 24: 484-486.

Khan A, KS Saleemi, MZ Khan, ST Gul, M Irfan, S Qamar, 2011. Hemorrhagic septicemia in buffalo (*Bubalus bubalis*) calves under sub-tropical conditions in Pakistan. Pak J Zool, 43: 295-302.

Khan A, U Saddique, R Ahmad, H Khan, Y Mohammad and M Zubair, 2006. Sero-serveillance of hemorrhagic septicemia in cattle and buffaloes in district Malakand, NWFP. J Agri Biol Sci, 1:11-14.

Khan FM. 2013. Second Quarterly Notifiable Disease Forecast Report of District Rahim Yar Khan. 58-60/ADIO/RYK/02.05.21.1-8.

Khan MA, MS Khan, HR Chaudhry, J Khan and S Saleem, 1994-1995. Meta analysis of epidemiological and economical losses and ranking order of buffalo and cattle disease in Punjab. Project report on Epidemiological of Major Livestock Diseases in Pakistan. pp: 73-90.

Koiranen L, 1977. Significance of vaccination for prevention of mastitis. Suomen Elainlaakarilehti. 83: 77-78.

Lignieres and spitz, 1902. Bul Soc Cent Med Vet, 20: 487.

Maddur MS, S Kishore, AK Chockalingam, S Gopalakrishna, N Singh, VVS Suryanarayana, and MR Gajendragad, 2010. The relationship between cellular immune response to foot-and-mouth disease virus Asia 1 and viral persistence in Indian cattle (*Bos indicus*). Res in Vet, 89: 36-40.

Muneer R and M Afzal, 1989. Preliminary studies on improved oil-adjuvant vaccine for haemorrhagic septicaemia in buffalo calves. Rev Sci Tech Off Int Epiz, 8: 999-1004.

Muneer R, M Hussain and AB Zahur, 2005. Efficacy of oil based haemorrhagic septicaemia vaccine: A field trial. lntl. J Agri Biol, 7: 571-573.

Muneer R, S Akhtar and M Afzal, 1994. Evaluation of three oil-adjuvant vaccines against Pasteurella multocida in buffalo calves. Rev Sci Tech Off Int Epiz, 13: 837-843.

Myint A, TO Jones, and HH Nyunt, 2005. Safety, efficacy and cross-protectivity of a live intranasal aerosol haemorrhagic septicaemia vaccine. Vet Rec, 256: 41 – 45.

Nadeem M, I Hussain, T Ahmad, AS Qureshi and A Yousaf, 2010. Effect of prophylactic application of levamisole in buffalo-calves immunized with haemorragic septicemia vaccine. In: Crudeli GA, EM Patino and JL Konard (Guest Editors).Proc. 9th World Buffaloes Congress , Buenos Aires, Argentina, pp: 451-454.

Namioka S, and DW Bruner. 1963. Serological studies on *Pasteurella multocida*, IV. Type distribution of the organisms on the basis of their capsule and O groups. Cornell Vet, 53:41-53.

Nickerson SC, 1994. Progress in the development of mastitis vaccine. Proc. National Matitis Council, Inc, Arlington, USA, PP: 133-134.

Nickerson SC, WE Owens and RL Boddie, 1995. Mastitis in dairy heifers: initial studies on prevalence and control. J Dairy Sci, 78: 1607-1618.

Oliver SP, MJ Lewis, BE Gillespie, H H Dowlen, EC Janicke and RK Robers. 2003. Milk production, milk quality and economics benefit associated with prepartum antibiotic treatment of heifers. J Diary Sci, 86:1187-1193.

Pankaj A Sharma, R Chhabra and N Sindhu, 2013. Sub-clinical mastitis in Murrah buffaloes with special reference to prevalence, etiology and antibiogram. Buffalo Bulletin. 32: 107- 115.

Pereira UP, DG Oliveira, LR Mesquita, GM Costa, and LJ Pereira, 2011. Efficacy of Staphylococcus aureus vaccines for bovine mastitis: a systematic review. Vet Microbiol, 148:117-124.

Perry BD, McDermott, JJ, Randolp TF, KR Sones, and Thornton, PK. 2002.Investing in animal health research to alleviate poverty. International Livestock Research Institute (ILRI), Nairobi, Kenya.

Rashid I, 2011. A short-term field evaluation of a mastitis vaccine prepared from a biofilm producing local isolate of Staphylococcus aureus, M Phil Thesis, Deptt Clinical Medicine and Surgery Univ Agri Faisalabad, Pakistan.

Raza MA, G Muhammad, M Athar and M. Zargham Khan, 2000. Comparative efficacy of three protocols for the treatment of haemorrhagic septicemia in buffaloes and cattle. Pak Vet J, 20: 35-39.

Reddy GS, KA Rao and VA Srinivasin, 1995. Immunity conferred by oil-adjuvant haemorrhagic septicaemia vaccine. Indian J Anim Sci, 66: 703-704.

Riaz M, MS Khan, MA Khan, AA Rabbani and M Arshad. 1992. Investigation on

epidemiology and economic losses of major livestock diseases in district Gujrat. Pak Vet J 12: 86-88.

Richou R and G Thienlin, 1955. Sur la prevention et le traitment des mammities. Recueil Med Vet, 131: 73-85.

Saini SS, DR Sharma, BS Gill, MS Kwatra, J Singh, JK Sharma, SS Dhillion and Ramneek (1991). Reemergence of hemorrhagic septicemia in Punjab. Ind J ani sci 61: 1178-1180.

Scott JP. 1931. Aggressins: An outline of the development of the theory and notes on the use of these products. J Bacteriol, 22: 323-337.

Shahzad W, R Munir, M Asif, MS Sagar, M Altaf, W Aslam, G Akbar and F Mehmood, 2013. Prevalence, molecular diagnosis treatment of field isolates of toxogenic *Pasteuralla multocida* in a hemorrhagic septicemia outbreak in Nili-Ravi Buffalo calves at Livestock Experiment Station, Bahadurnugar, Okara, Pakistan.

Sheikh MA, M Anzam and AR Shakoori, 1996. Observations on haemorrhagic septicemia in Pakistan Livestock. J of Vete Med, Series B.43: 293-304.

Shilston AW, 1923. Haemorrhagic septicemia (pasteurellosis). In: Wooldridge, GH. (editor) Encyclopedia of Veterinary Medicine, Surgery and Obstetrics. Vol 1 (Veterinary Medicine). Henry Frowde and Hodder and Stoughton, Lodon. PP: 103-112.

Singh S, VP Singh, PS Cheema M Sandey, R Ranjan, SK Gupta, and B Sharma,2011. Immune response to dna vaccine expressing transferrin binding protein a gene of Pasteurella multocida.Braz J Miceobiol.42 (2): 750-760.

Sivakumar T, A Thennarasu, and JSI Rajkumar.2012. Effect of season on the incidence of infectious diseases of bovine in Tamilnadu. Elixir Meteorol 47: 8874-8875.

Soomro SA, KB Mirbahar, MA Memon, and MI Memon, 1997. Prevalence of clinical and sub-clinical mastitis in buffalo at Hyderabad, Sindh. Pak J Agri Agril Engg Vet Sci, 13: 28-30.

Srivastava NC, PC Harbola and SS Khera, 1975. Preliminary observations on combined vaccination against haemorrhagic septicaemia and black quarter. Indian Vete J, 53: 168–172.

Srivastava SK. 1998.Immunogenicity of Pasteurella *multocida* grown in iron restricted medium. J Appl Anim Res, 13: 137-144.

Tabatabaei M, GRM Jula, AR Jabbari and M Esmailzadeh. 2007. Vaccine efficacy in cattle against hemorrhagic septicemia with live attenuated aroA mutant of Pasteurella multocida B: 2 strain. J Cell Anim Biol, 1: 62-65.

Tasneem k, B Zamir, S Ali, ZJ Gill and A Raza, 2009. Haemorrhagic septicaemia: A review. Pak J Sci, 61: 10-13.

Tollersrud T, L Zernichow, SR. Andersen, K Kenny and A Lund, 2001. *Staphylococcus aureus* capsular polysaccharide type 5 conjugate and whole cell vaccines stimulate antibody responses in cattle. Vaccine, 19: 3896-3903.

Trinidad P, SC Nickerson and TK Alley, 1990a. Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. J Dairy Sci.:73:107-14.

Trinidad PSC. Nickerson and TK Alley, 1990b. Efficacy of intramammary treatment in unbred and primigravid dairy heifers. J Am Vet Med Assoc 197:107-114.

Verma R and TN, Jaiswal, 1997. Protection, humoral and cell- mediated immune responses in calves immunized with multiple emulsion haemorrhagic septicaemia vaccine. Vaccine, 15: 1254-1260.

Verma R and TN, Jaiswal, 1998. Haemorrhagic septicaemia vaccines. Vaccine. 16: 1184-1192.

Watson DL, 1975. Cytophylic attachment of ovine IgG2 to autologous polymorphonuclear leucocytes. Aust J Exp Biol Med Sci, 53:527-529.

Wooldridge GH, 1923. Encyclopedia of Veterinary Medicine, Surgery and Obstetrics Vol.II, Veterinary Surgery and Obstetrics, 2nd Ed. Henry Frowde & Hoddrer and Stoughton, London.P:1559.

Yousaf A, G Muhammad, Sajjad ur Rahman, M Siddique and MZ Masood, 2009. Effect of montanide adjuvanted Staphylococcus aureus bacterin-toxiod on prevalence and incidence of mastitis in cows. Pak J Agri Sci, 4: 119-123.

To Cite This Chapter :

Qudratullah & Habib Ur Rehman (2023). **Hemorrhagic Septicemia-Mastitis Vaccine**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 357-378). ISRES Publishing

IMPORTANCE OF MICROENCAPSULATION IN VALUE ADDED DAIRY PRODUCTS

Koushik DAS
Kunal M. GAWAI
Dhinal H. PATEL
Subrota HATI
Manishkumar P. PARMAR

1. Introduction

The microencapsulation technology is the packaging of different ingredients inside tiny closed capsules that release ingredients over time at calculated rates in response to specified processing and ecological conditions (*e.g.*, shear force, temperature, enzymatic action, pH, fermentation, *etc*.). Ingredients used for regulating colour, texture, flavour and preservation are frequently added to foods during industrial food manufacturing (Champagne and Fustier, 2007).

The prepared food industry, bread industry, and ingredient suppliers themselves are the sectors that have most likely employed capsulated ingredients. Encapsulating enzyme for accelerated cheese ripening was the most researched application of encapsulated components in the dairy industry's prior usage, which was quite limited. However, introduction of functional foods has been proven to be a gateway in extending the use of encapsulated chemicals (Augustin, 2003).

Reasons for using microencapsulation in food industry are:

a) To lessen the core's reactivity with respect to the environment.
b) To slowdown the core material's transfer rate to the outer environment.
c) To regulate the core material's release beneficial to produce ideal latency before perfect stimulation.
d) To hide the primary flavour.
e) To achieve uniform distribution in the coating medium while diluting core component when used in minute quantities.
f) The core material should be handled more easily to prevent lumping, place the main raw materials more evenly throughout a mixture by providing most suitable exterior surface matching with the materials in the mixture as a whole, transform a liquid into a solid, encourage simple mixing of the main ingredient (Shahidi and Han, 1993).

1.1 Microencapsulation methods

Table 1. Different methods of encapsulation (Encapsulation Methods and Properties of Capsules, LLS Health CDMO, 2019)

Process	**Approximate Size range**	**Morphology**	
		Matrix	**Core-shell**
Solvent Cast/grind	1 µm – 5 mm	✓	
Spray chilling	1 µm - 100 µm	✓	✓
Spray drying	1 µm - 100 µm	✓	
Vibrating-nozzle (narrow size distribution)	10 µm – 5 mm	✓	✓
Emulsification/solvent evaporation	100 nm – 5 mm	✓	

Melt extrusion	300 µm - 5000 µm	✓	✓
Spray-coating/pan coating	100 µm – 5 mm		✓
Polymer phase separation from solution	100 nm – 5 mm		✓
Coacervation	1 µm – 5 mm		✓
Interfacial polymerization	100 nm – 5 µm		✓
Suspension polymerization	100 nm – 5 mm	✓	
Extrusion/spheronization (extrusion/micropelletization)	1 mm – 5 mm	✓	

1.1.1 Selection criteria for proper method

The following factors must be addressed while constructing and selecting an appropriate encapsulating procedure -

- ✓ The active ingredient's physicochemical properties and the functionality in the finished product.
- ✓ The necessary processing conditions for the survival of the encapsulated ingredient in final product.
- ✓ Prior to usage, the storage conditions of encapsulated component.
- ✓ The storage conditions of the final product.
- ✓ The physical qualities of the encapsulated substance (particle size, density, stability).
- ✓ The mechanism(s) and trigger(s) for releasing the active component from microcapsules.
- ✓ If there are any cost constraints (Mishra, 2015)

1.2 Release Rates

One microcapsule can typically achieve zero, half, or first-order release rates based on the core material -

- ➢ When the core is a pure substance that is capable of releasing through the wall of a microcapsule, "zero" order occurs.
- ➢ Matrix particles frequently experience half-order release.
- ➢ When the substance at the core is a solution, first-order release occurs (Karel and Langer, 1988).

1.3 Mechanisms for release of microcapsules

The coating structure gives protection to core material from environmental conditions and controls the release of core materials (Dziezak, 1988).

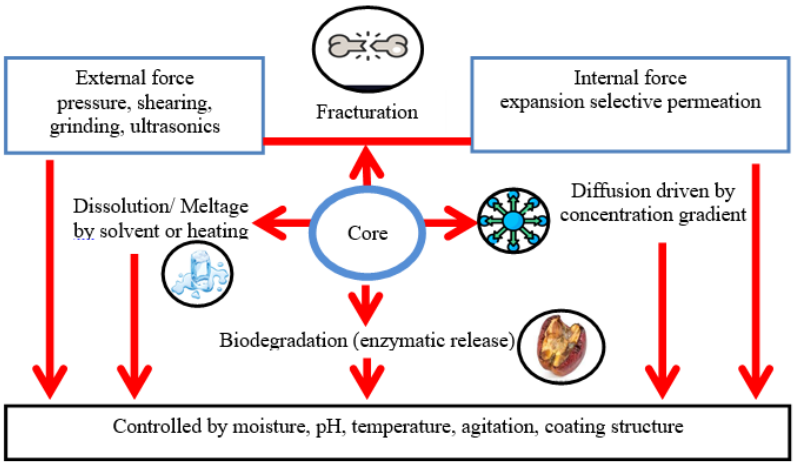


Figure 1. Release mechanisms of microcapsules

2. BENEFITS

a) Enhanced stability results in a longer shelf life and greater efficiency - The core can be shielded from exposure to hazardous environmental conditions by becoming entrapped within a wall material.
b) Controlled release - different conditions, including dissolution, temperature, pressure, pH, and enzymes, can cause the release mechanism to occur, depending on the microencapsulation process and wall material chosen.
c) Ease of handling and precision - by lowering stickiness, hygroscopicity, or changing melting point, microencapsulation improves the flow ability and handling of active substances. It can also enable the conversion of liquid material into readily manipulated, free-flowing dry particles.
d) Taste, colour, and odour masking.
e) Avoiding interactions between the various elements in a formula (Arenas-Jal *et al*., 2020).

3. POTENTIAL INGREDIENTS FOR ENCAPSULATION

Organic acids, flavours, sugars, probiotics, prebiotics, vitamins, minerals, colours, ω-3 oils, phytochemicals (Shahidi and Han, 1993).

4. CHALLENGES

Probiotics and many chemicals must frequently undergo a special processing step in order to be encapsulated, which complicates the production process and raises the cost of the item. Encapsulated components are normally only used when there is an issue with the functional food's processing. Functional foods with probiotics pose some challenges (Champagne *et al*., 2005):

a) Proper strain selection
b) Sufficient microbial population
c) Toxicity issues
d) Stability of microbe in processing steps
e) Storage stability of the probiotic strain
f) Probiotic population in the product
g) The impact on sensory characteristics

The probiotic strain itself may be an element of scalability of product as an authentication of probiotic growth. As a result, strains are now chosen based more on their capacity to have an impact on health than on their technological attributes.

The following circumstances have possibilities to be deleterious to probiotic viability during the processing of dairy products:

• Starter cultures produce inhibitory compounds (fermented milks)
• High temperature processing
• Low temperature processing
• Presence of oxygen
• Various elements (flavours, salt)
• Drying (Dried milk products).

In addition to those encountered during production, the harmful effects of acid (cheese), oxygen (yogurt, blends), humidity (powdered blends) during storage are also present.

5. ENCAPSULATION IN DIFFERENT PRODUCTS

Microencapsulation of various bioactive compounds can be done in an extensive area of dairy products. These dairy products are divided into fermented and non-fermented categories to facilitate understanding. When the two categories are examined, it is clear that examples related to fermented foods outnumber those of non-fermented foods.

5.1 Fermented milk products

Consumer interest in products that support health and wellbeing is rising nowadays. Since ancient times, fermented milk products have been known to have positive effects. Due to the metabolites produced during the fermentation process, like– bacteriocins, lactic acid, fermented dairy products regulate the gut microflora and inhibit proliferation of pathogens. According to research, consuming dairy products that have undergone fermentation helps in reducing the risk of type 2 diabetes, heart disease, and stroke (García-Burgos *et al.,* 2020).

The final product quality as well as the physico-chemical features of the bioactive chemicals influence the choice of encapsulation methods and delivery mechanism. Bioactive substances that are encapsulated and supplied must not unfavourably change the final product's appearance, texture, or mouthfeel. Delivery methods for bioactive substances must be simple to scale up, affordable, and made using ingredients that are safe for consumption (McClements, 2018).

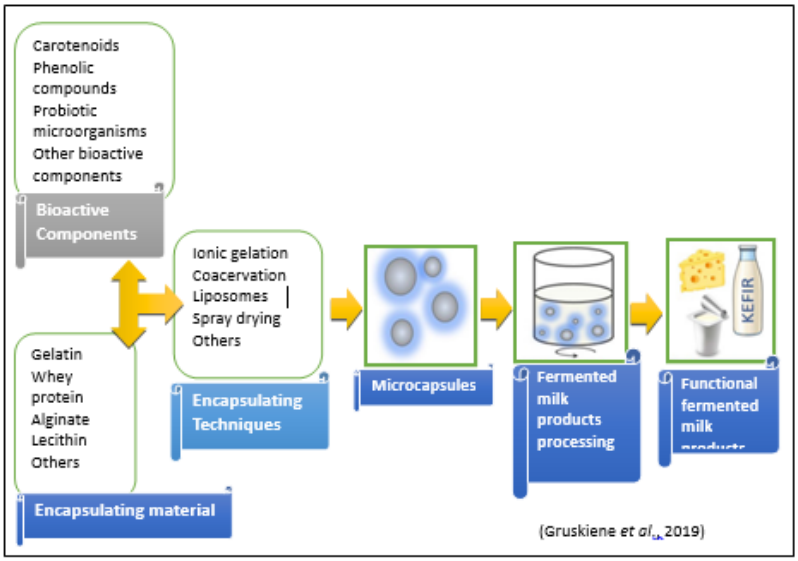


Figure 2. Delivery of bioactive components to fermented milk products through microencapsulation ((Gruskiene et al., 2019)

5.1.1 Yoghurt fortification with microencapsulated bioactive ingredients

5.1.1.1 Carotenoids

All photosynthetic organisms, as well as several bacteria and fungi, biosynthesize a class of isoprenoid chemicals known as carotenoids. They are vibrant red, orange, and yellow natural colours. Carotenoids are mostly consumed by humans as dietary components of fruits and vegetables. Consuming carotenoids is linked to improved immune system performance, cumulatively help in decreasing risk of cancer, cardiovascular disease, macular degeneration (age-related) and type 2 diabetes. Additionally, a few of them have provitamin A action (Rodriguez-Concepcion *et al.,* 2018)

5.1.1.2 Phenolic Compounds (Phenolics) Containing Components

Another significant class of compounds that are added to yogurt in encapsulated form are phenolics. These secondary metabolic products of plants and their extracts often provide phenolics for consumption. Phenolics have positive benefits mainly showing antioxidant, antimicrobial and anti-inflammatory activities and anticarcinogenic effects (Shahidi and Ambigaipalan, 2015). Applications and uses of phenolic compounds are frequently constrained, nevertheless, by their poor bioavailability, unpleasant taste, and instability in food. The microencapsulation method makes it easier to introduce phenolics into different food matrices.

Table 2. Encapsulation of carotenoids in bioactive form for yoghurt's fortification

Bioactive Component	Encapsulation technique	Encapsulation material	Observations	References
Tomato peel extract	Electro-spinning	Zein, gelatin	Similar properties in terms of pH, syneresis, acidity, and viscosity to the control yogurt sample. The ability of yogurt to scavenge free radicals has enhanced by 40–60%.	İnanç Horuz and Belibağli, 2019
Red Pepper waste extract	Freeze drying, spray drying	Whey proteins	When compared to the control sample, the sensory and overall acceptance scores for the fortified yogurt were higher.	Šeregelj *et al.,* 2019
β- Carotene	Spray-dried emulsion followed by fluidized bed coating	Maltodextrin or sodium caseinate for emulsification and hydroxypropyl	Powders that were coated were utilized to color the yogurt. Yogurt's color barely changed	Coronel-Aguilera, and San Martín-González, 2015

		cellulose for coating.	after 4 weeks of storage.	
β- Carotene	Spray drying; coacervation for beads	Maltodextrin for spray drying; Chitosan and alginate for beads	In yogurt, spray-dried beta-carotene released more during digestion and was incorporated into micelles than beta-carotene encapsulated in chitosan-alginate beads.	Donhowe *et al.*, 2014

Table 3. Encapsulation of phenolic compounds in yogurt

Bioactive Component	**Encapsulation technique**	**Encapsulation material**	**Observations**	**References**
Grape seed extract	Spray drying	Whey proteins and arabic gum	At a final concentration of 1%, encapsulated grape seed extract produced equivalent sensory characteristics, viscosity, acidity, water-holding capacity, and color to the control. Antioxidant activity increased by four times.	Yadav *et al.*, 2018.
Orange peel extract	Coacervation	Whey proteins and arabic gum	Yogurt's physicochemical and organoleptic qualities were not adversely affected.	El-Messery *et al.*, 2019.
Sour cherry extract	Spray-dried coated liposomes	Lecithin for liposomes preparation and chitosan for their coating.	Up to 14 days of storage, the addition of LP did not affect the color characteristics of yogurt. Regarding total phenolic content and antioxidant capability, encapsulation	Akgün *et al.*, 2020.

			gave the extract stability.	
Hibiscus calyx extract	Double emulsion and following ionic gelation.	Rapeseed oil and pectin	By using atomization and dripping-extrusion techniques, microparticles were produced. Yogurt was mixed with the microparticles at a 20% (w/w) ratio. Compared to dripping methods, the aesthetic acceptance of yogurt enriched with atomized microparticles was higher, but the preservation of beneficial components was lower.	De Moura *et al.*, 2019.

5.1.1.3 Other Bioactive Components

a) Research on adding vitamin D3 to yogurt was published in certain articles. This fat-soluble vitamin is vulnerable to oxidation, low pH, and UV rays (Markman and Livney, 2012).
According to Leskauskaite *et al.* (2016), vitamin D3 was administered as an oil-in-water emulsion stabilized by whey protein alone or in combination with carboxymethylcellulose. It was also enclosed in casein micelles (Sharifan *et al*., 2020). During yogurt's shelf life, encapsulated vitamin D3 remained remarkably stable. Moreover, during the bioavailability study, encapsulated vitamin D3 showed feasible behaviour (Sharifan *et al.,* 2020). The researchers came to the conclusion that adding vitamin D3 to yogurt improves its nutritional value while also being healthy and helping people avoid vitamin deficiencies.

b) As there is high iron bioavailability in iron-fortified dairy products, yogurt is considered as an appropriate delivery system for iron (Kim *et al.,* 2003). Iron is encapsulated to eliminate its impact on sensory properties, stability of product, and lipid oxidation (Mehansho, 2006). Iron is used as a core material for encapsulation and it’s uses are primarily in the form of ferrous sulfate, ferric ammonium sulfate, ferrous bisglycinate and ferrous lactate. Typically, iron is packaged in conjunction with vitamin C, which aids in the metabolism of nonheme iron and promotes nonheme iron absorption. Yogurt fortified with ferrous bisglycinate and ferrous lactate failed to retain sensory qualities, however yogurt fortified with encapsulated ferrous sulfate was identical to the control sample (GilliardNkhata *et al.,* 2015).

c) Yogurt is thought to be a particularly appealing food system for adding PUFA because it can be stored at lower temperatures, which maintains fatty acids stable. The most significant ones are EPA and DHA that are mainly found in fish oil. To enhance PUFA intake on a regular basis and mask fish oil's unpleasant flavour, various encapsulating techniques were explored for delivery into yogurt.
Unsaturated fatty acids are guarded from degradation by the encapsulation. After three weeks of storage, the yogurt with free fish oil had a lower peroxide value and contained more EPA and DHA, according to the authors. Overall, yogurt with added fish oil capsules has acceptable sensory properties (Bakry *et al.,* 2019; Tamjidi *et al.,* 2012).

5.1.1.4 Probiotics

As encapsulated probiotics had a greater survival rate than nonencapsulated bacteria, it was determined that the encapsulation of *Lactobacillus paracasei* by the gelation procedure using gellan gum and sodium caseinate was an effective technique for delivering bacteria. Pectin and casein were combined as the wall components in a sophisticated coacervation procedure to create the *Lactobacillus acidophilus* strain's microcapsules (Shoji *et al.,* 2013) and in combination of coacervation and ionic gelation where WPC and pectin were used as coating material, respectively (Ribeiro *et al.,* 2014).
In both examples, the addition of *Lactobacillus acidophilus* to yogurt showed reduction in post-acidification values and high viability during storage conditions. The combination method's microcapsules had a detrimental impact on the yogurt's texture. While comparing to the one without encapsulation, it was less acceptable.
To create protein-based microcapsules of *Lactobacillus rhamnosus* GG for yogurt fortification, soy protein isolate crosslinked by transglutaminase was used (Li *et al.,* 2016).
Probiotics encapsulated with whey protein showed improved bioavailability in the colon, according to research by Aljouni *et al*. (2020).

5.1.2 Fortification of Microencapsulated Bioactive Ingredients in Cheese

5.1.2.1 Phenolic Compounds

Cheese is an ideal food for developing functional food. Given the industrial scale of production, plant extracts rather than pure components are used to fortify cheese in order to reduce production costs. When cheese is stored, adding free phenolics to it can affect the cheese's texture, color, and flavor. Additionally, phenolics interact with elements of milk and reduce their antioxidant effects (Haratifar and Corredig, 2014).

a) Sodium alginate was used as a coating material and atomization/coagulation approach was used to encapsulate rosemary, fennel, and chamomile extracts. The produced microcapsules were added to cottage cheese, but neither their composition nor color was altered. While comparing to the free form of extracts, antioxidant activity was at par under storage more effectively (Caleja *et al.,* 2016; Ribeiro *et al.,* 2016).
b) The microcapsules were made by sonicating and then spray drying in order to encapsulate olive derived phenolics and delivered them into white soft cheese. Maltodextrin and proteins from skim milk were used as building components. The process guaranteed the cheese's constant antioxidant activity for the entire 30 days of storage (Farrag *et al.,* 2020).

5.1.2.2 Probiotics

Table 4. Probiotic encapsulation in cheese

Cheese Type	Microorganism	Encapsulation technique	Encapsulation material	Observations	References
Cheddar cheese	*Bifidobacterium bifidum*	Emulsification/ internal gelation	κ-carrageenan or sodium alginate	Over a 35-day period, the vitality of the bacteria that were encapsulated decreased more slowly. Under simulated gastrointestinal circumstances, the encapsulant sodium alginate performed better than κ-carrageenan.	Afzaal *et al.*, 2020
Iranian UF white cheese	*Lactobacillus plantarum*	Complex coacervation followed by spray drying or freeze drying	Whey protein isolate and gum arabic	Phytosterol was coencapsulated with bacteria. Its coencapsulation enhanced the survival of bacteria in cheese for 91 days of storage 4˚C in contrast to encapsulated bacteria alone or free cells.	Sharifi *et al.*, 2021
Soft goat cheese	*Lactobacillus plantarum*	Spray drying	Skim milk	The encapsulated bacteria were found to have a high level of 8.82 log CFU/g after 8 weeks of cheese storage, while the number of loose cells dropped to 6.9 log CFU/g. Encapsulated bacteria had no effect on the cheese's sensory quality, chemical	Radulović *et al.*, 2017

				makeup, or pH level.	

5.1.2.3 Other compounds

a) Carotenoids function as coloring agents or health-promoting substances in cheese. Recently research conducted on adding encapsulated tomato extract as lycopene source to Appezeller cheese and Queso Blanco cheese was conducted. Maltodextrin was used as a coating material while the emulsion was spray-dried to create microcapsules. Cheese's sensory qualities were enhanced by the tomato flavor and increased yellowness (Kwak *et al.,* 2016; Jeong *et al.,* 2017).

b) Very few studies have focused on providing cheese with omega-3 fatty acids from vegetable as well as animal sources. There is a lot of ALA in chia seed oil. A calcium caseinate-stabilized chia oil emulsion was used to fortify sheep's milk cheese. The method of manufacturing cheese and the quality of the microorganisms during the ripening period were unaffected by the enrichment (Muñoz-Tébar *et al.,* 2019).

c) There was a higher rate of proteolysis when encapsulated enzymes were used in cheese production as compared to free enzyme. Enzyme capsules were mixed with milk during cheese making. Cheeses with additional gum capsules had more moisture than cheeses without them. Throughout the ripening process, in comparison to the control sample, other cheese samples treated with enzyme microcapsules displayed much higher percentage of proteolysis. Cheeses prepared with κ-carrageenan (wall material) capsules containing protease, the degree of proteolysis increased. The release of protease from capsules was consistent with differences in the sensory and textural qualities of treated and control cheeses (Kailasapathy and Lam, 2005).

5.1.3 Kefir fortification

Kefir made functional with encapsulated structured lipids was developed by Yüksel-Bilsel and Sahin-Yesilcubuk (2019). Arabic gum and gelatin were used as coating material in the coacervation process to create the microcapsules. As a crosslinking agent, transglutaminase was used and it is fortified in kefir and it was kept for 10 days at 4ºC and a satisfactory level of pH, acidity, and color was sustsained. Additionally, a study on *in vitro* digestion found that kefir had no inhibitory effect on oil release.

Kefir is probiotic in nature, but it can also be supplemented with them in order to get the recommended daily intake of bacteria. Encapsulation typically boosts the probiotics' viability throughout cold storage and in the stomach's acidic environment. Incorporation of *Bifidobacterium animalis* ssp. *lactis* BB12 harbored alginate beads (González-Sánchez *et al.,* 2010) and *Lactobacillus paracasei* KS-199 in alginate fibers (by electro-spinning) (Yilmaz *et al.,* 2020) serve as examples of this.

5.1.4 Fermented milk

✓ A donkey milk based fermented beverage was formulated with sunflower oil by emulsion method. Target was to create a beverage to scale up the low energy intake, texture enhancement, and provide healthier qualities. Two strains of S. *thermophilus* were added for the fermentation that was able to produce EPS and folic acid. Folic acid content in the final product was >10 times than that of donkey milk (0.16 ±0.03 g/100 mL), and remained steady for 20 days. This formulation is an excellent alternative for those who have an allergy to cow milk protein (Tidona *et al*., 2015).

- ✓ As microencapsulation by emulsion method is a useful method to include lipophilic bioactive chemicals in beverage systems, CLA was incorporated in chocolate milk drink formulation. CLA shows numerous health benefits as diabetes control, LDL cholesterol reduction, anti-carcinogenic effect, etc. (Lopes *et al.,* 2011).
- ✓ In a study, six functional cereal based fermented milk beverages were formulated with *Lactobacillus plantarum* and *S. thermophilus* capsules seperately that were enriched with folate and riboflavin. Products were wheat-fermented milk (WFM), barley-fermented milk (BFM), and corn-fermented milk (CFM). The findings showed that WFM with *S. thermophilus* and CFM with *Lc. plantarum* had the highest levels of folate, while BFM with *S. thermophilus* and *Lc. plantarum* had the highest levels of riboflavin. Thus, using encapsulating probiotics improves their gastrointestinal survival and raises the availability of bioactive compounds in beverage systems (Sharaf *et al.,* 2015).
- ✓ A study was conducted to determine whether high pressure homogenization may successfully generate functional fermented milks by microencapsulating two probiotic cultures - *Lactobacillus paracasei* A13 and *Lactobacillus salivarius* subsp. *salivarius* CET 4063. Exo-polysaccharides, volatile molecule synthesis, and factors affecting the mouthfeel of fermented milk were assessed throughout storage. Because of the encapsulation, the hyperacidity phenomenon was lessened. The improvement of the starter's viability and the product's sensory qualities depended heavily on the products' decreased acidity as a result of microencapsulation. Opposite to *Lb. salivarius* CECT 4063, *Lb. paracasei* A13 exhibited a higher resistance to the gastric conditions. But in contrast a reduction in EPS production was observed due to microencapsulation. According to the probiotic strain and microencapsulation method used, the volatile profiles revealed unique characteristics (Patrignani *et al.,* 2017).

5.2 Non-fermented products

Due to rise in health consciousness people are more prone to fermented products rather than non-fermented products. Though people consume nonfermented dairy products according to their requirements (desert, food supplements etc.). Microencapsulation can be a useful technique to enhance their experience.

5.2.1 Incorporation of probiotic microcapsules in Ice cream

Table 5. Microencapsulation of different components in Ice cream

Substance to be encapsulated	**Encapsulation technique**	**Encapsulation Material**	**Observation**	**Reference**
Lactobacillus casei & *Bifidobacterium lactis*	Modified Emulsion	Calcium alginate	During the same length of storage at the same temperature, probiotic survival increased by 30%. There was no detectable difference between two	Homayouni *et al.,* 2008

			samples in terms of color, body texture, or flavour of the product.	
Phenolics from pomegranate peel	Spray drying	Maltodextrin	Free and microencapsulated ice cream were sensorily similar.	Çam *et al*., 2014
Flaxseed oil	Patent (Application number: 2030/DEL/2014)		ω-3 enriched ice cream (butter scotch and strawberry) was oxidatively stable and organoleptically superior. According to the formulation serving size of 100 g can provide 45% of the RDA for ALA.	Gowda *et al*., 2018
Pistachio peel extract	Spray Drying	Maltodextrin	Pistachio peel extract increased the melting resistance, first dripping time, antioxidant activity, and total phenolic content of ice cream mixtures whereas overrun decreased.	Ghandehari Yazdi *et al*., 2020

5.2.2 Incorporation of probiotic microcapsules in Milk

Table 6 . Microencapsulation of different components in Milk

Substance to be encapsulated	**Encapsulation technique**	**Encapsulation Material**	**Observation**	**Reference**
Korean mistletoe extract	Emulsion	Medium chain triacyl-glycerol and polyglycerol monostearate	The TBA test revealed that encapsulated extract had lower chemical oxidation than control sample.	Kim *et al*., 2008

Vitamin C and iron	Spray drying	Poly glycerol mono stearate	Milk sample with unencapsulated L-ascorbic acid and encapsulated iron had the lowest TBA value up to 5 days of storage whereas other treatments showed higher TBA values.	Lee *et al.*, 2004
Bifidobacterium breve	Emulsion	Inner layer: hard oil made from coconut Outer layer: starch and gelatin	Under gastric and small intestine settings, the number of microencapsulated bacteria remained remarkably steady.	Jung *et al.*, 2007
Chito oligosaccharide	Spray chilling	polyglycerol monostearate	Astringency, bitterness, color and other sensory properties of milk were unaffected by the incorporation of microencapsulated chitooligosaccharide.	Choi *et al.*, 2006

5.2.3 Incorporation of probiotic microcapsules in Dried milk

Table 7. Microencapsulation of different components in Dried milk

Substance to be encapsulated	**Encapsulation technique**	**Encapsulation Material**	**Observation**	**Reference**
Turmeric oleoresin	Spray drying	Gum acacia, maltodextrin, and dairy whitener	The spray dried powder has a desired curcumin concentration and >95% encapsulation efficiency. Results for Antioxidant activity and bioavaibility in rat model as promising.	Ipar *et al.*, 2022
Lactobacillus rhamnosus	Extrusion	Sodium alginate	Product supplemented with encapsulated probiotic strain showed a greater reduction in *Bacillus cereus* count rather than the control sample	Abdel-Rahman *et al.*, 2019

Ghee flavor extract	Spray drying	WPC-80 and guar gum	Ghee used in creating various low-fat and ghee-flavor enhanced goods in the pharmaceutical and food industries might benefit from the encapsulation of ghee flavorants.	Duhan *et al.*, 2021

5.3 Using dairy ingredients for encapsulation

Early food industry applications of microencapsulation included masking unwanted flavours, regulating the desirable flavour release, and converting liquid into powdered forms for ease of use and extended shelf life. The food sector is increasingly using encapsulation technology to safeguard and regulate the distribution of food ingredients and bioactive components.

Table 8. Various bioactive compounds and their potential benefits

Core type	Example	Potential effect
Oil	Milk fat, ω-3 oils	Enhanced targeted release and storage stability
Flavour	Mint, orange	Flavour preservation and controlled release in mouth
Food additive	Leavening agents	Controlled release during baking
Minerals	Salts of iron	Target delivery on intake, avoid unfavorable interactions (such catalyzing fat oxidation)
Phytochemicals	Carotenoids, carotene, flavanoids, polyphenols, lycopene, lutein tocopherols, phytosterols,	Environmental protection and interaction with food matrix, targeted delivery when ingested
Probiotics	Lactobacilli, bifidobacteria	Enhanced survivability in storage and acidic conditions in stomach

(Corredig, 2009)

5.3.1 Components used as encapsulating ingredients

Due to their properties, dairy components are highly sought-after as encapsulating materials and are promptly available as food-grade constituents. Milk proteins,

lactose, milk fat and its fractions, and milk fat globule membrane (MFGM) can all be used as materials for encapsulation.
For designing functional micro-encapsulated components – core stability, solubility, physico-chemical characteristics of encapsulating ingredients and method of encapsulation should be properly looked into.

Table 9. Different components used as encapsulating ingredients

Encapsulant material	**Examples**	**Characters**
Protein	Pea proteins, gelatin, hydrolysed proteins casein, whey proteins, soy proteins, egg	Gelling, emulsifying, viscosity building
Glucose syrup and sugar	Mono-, di- and oligosaccharides, glucose syrups	Low viscosity at high solids and the capacity to produce amorphous solids after dehydration
Polysaccharide	Carboxymethylcellulose, pectins, alginates, chitosan starch, maltodextrins, gums,	Film formation, stabilization of emulsions, ability to create amorphous solids after dehydration and gelling
Fat and waxes	Animal fats (e.g. milkfat), waxes (e.g. beeswax) vegetable fats (e.g. canola oil)	Lipophilic core solubilization, embedding matrix, water barrier characteristics, and film formation
Surfactant	Glycolipids, tweens, spans mono- and di-glycerides, phospholipids (e.g. lecithin)	Emulsifying

(Corredig, 2009)

Different structured capsules can be created to encapsulate components depending on the target application. These can be categorized as either matrix structures or the traditional core-shell structures, where the active core is confined. Capsules with multiples layers and with several cores are variations of this.
conventional oil-in-water (O/W) emulsions, solid lipid particles (O/W), multilayer-stabilized emulsions, multiple emulsions (W/O/W), nano-emulsions produced by high-pressure homogenization, and liposomes are examples of capsules having core-shell architectures. As examples of enclosed systems with matrix structures-lipospheres, hydrogels, and biopolymeric micro- and nanoparticles with functional cores can be found (Kamyshny and Magdassi, 2015).

Encapsulating properties of dairy ingredients

Encapsulants are needed for being able to gel, interface stabilization, solubilize actives, and produce amorphous matrices, among other crucial qualities. A variety of dairy ingredients naturally contain these qualities.

Table 10. Encapsulating property of dairy ingredients in different systems

System	Coating material	Properties
Emulsions	Milk proteins – caseins, whey proteins, hydrolyzed milk proteins, milk protein isolates	Emulsion stabilization, film formation
	Milk fat and its fractions	Carrier of active cores
Dried emulsions	Milk proteins – hydrolyzed milk proteins, caseins, whey proteins,	Stabilizing emulsion prior to drying, emulsion matrix of dried particle
	Lactose	Amorphous matrix formation on drying
	Milk fat	Carrier of active core, secondary coating material, moisture barrier
Hydrogel	Milk proteins – casein, whey proteins	Gel phase formation, good system for embedding cores
Lipospheres	Milk fat	Active core entrapment
Coacervates	Milk proteins – casein, whey proteins	Interaction with biopolymers that have opposite charges to create a distinct phase
Liposomes	Milk phospholipids, milk fat globule membrane	Bilayer vesicles stabilization

(Corredig, 2009)

5.3.1.1 Milk fat

Milk fat is primarily made up of triglycerides (98%) and has been found to have more than 400 fatty acids and 200 triglycerides, demonstrating complexity of structure. In order to distinguish the functional and physical characteristics of milk fat, such as crystallization behaviour and melting point, the fractionation procedure gives milk fat varied triglyceride compositions.

For encapsulation, fractions of milk fat with a known melting point can be employed, allowing for more precise control over the release of key components.

Emulsion-based systems can also utilise milk fat. Tocopherol was enclosed in a caseinate O/W emulsion made from a milk fat fraction high in stearin to guard the antioxidant against deterioration while in storage (Relkin *et al*., 2008).
In a W/O/W emulsion utilizing milk fat, Magee *et al*. (1981) established a technique for producing microencapsulated enzyme-substrate flavoring systems. The mixture of the enzyme and the substrate was shielded from ionic and pH conditions by encapsulation. Encapsulating the enzyme and its substrate enhanced the quantity of flavouring ingredients and decreased enzyme loss during cheese production.

5.3.1.2 Milk fat globule membrane (MFGM)

The MFGM is a comprised of proteins, glycolipids and phospholipids. It serves as a natural barrier against milk fat lipolysis and is particularly nutrient-dense. MFGM material behaves differently when used to stabilize emulsions compared to milk.
A research work carried out by Corredig and Dalgleish (1998) claim that O/W emulsions stabilized by MFGM have lower susceptibility to interfacial displacement because of low molecular weight surfactants in comparison with milk protein-stabilized emulsions and remain unaffected by the addition of extra milk proteins (caseins, whey).
Liposomes made from MFGM phospholipids had lower membrane penetrability, thicker membrane, higher phase transition temperature, better environmental stability (*e.g.*, heat, pH, of divalent cations) than liposomes made from soy phospholipids (Thompson *et al*., 2006).

5.3.1.3 Milk proteins

Casein and whey proteins make up the majority of the proteins in milk. Numerous enclosed systems have been constructed using both of these proteins and these differ from one another.
For instance, there are differences in the isoelectric point of caseins (4.6), the structure of whey proteins (globular), heat sensitivity (caseins are more heat stable than whey proteins), emulsifying properties (whey proteins construct denser interfaces than casein), and gelling properties (casein gels can form in the presence of Ca^{2+}, whey protein denaturation often occurs before gelation). In encapsulated systems, proteins are chosen based on their functional properties (Augustin and Udabage, 2007).

5.3.1.3.1 Caseins

Probiotics such *Bifidobacterium lactis*, *Lactobacillus casei*, *Lactobacillus paracasei*, and *Lactobacillus rhamnosus* have been encapsulated by combining casein with emulsification. Using transglutaminase to cross-link the protein is one way to accomplish this. Such cross-linking causes the casein to gel, trapping bacterial cells as a result (Heidebach *et al*., 2009).
It was shown that caseins interact with polysaccharides to form complexes that resemble coacervates. Casein coacervation results from lowering the pH below its isoelectric point. According to reports, the coacervation process is appropriate for encapsulating volatile chemicals (Koupantsis *et al*., 2014).
After being emulsified, flaxseed oil, quillaja saponin, and sodium alginate and caseinate were added. The calcium chloride solution was then injected with droplets using an encapsulating device to promote gelation. It was proven that sodium caseinate prevented lipid oxidation successfully for 50 days of storage at 55 °C (Chen *et al*., 2017).

5.3.1.3.2 Whey proteins

The main whey protein found in cow's milk is called β-lactoglobulin (β -LG), and it naturally transports hydrophobic molecules on its molecular nanoscale. Cholesterol, several aroma compounds (such as aldehydes and ketones), fatty acids (such as conjugated linoleic acid, palmitate and oleate), retinoic acid and vitamin D are examples of chemicals that react and bind within its hydrophobic calyx (Kontopidis *et al*., 2004). Numerous hydrophobic bioactives, including vitamin D and cholesterol, have been shown to strengthen a protein's ability to withstand heat denaturation (Puyol *et al*., 1994). This might make it possible to make β -LG more stable during the production of food and give us more control over how well it can distribute particular bioactives in food systems.

5.3.1.3.3 Milk protein hydrozylates

Several studies have demonstrated that proteolysis modifies milk proteins' activity, specifically their gelation and surface-active properties. The type of milk protein and the hydrolysis circumstances determine how proteolysis affects the physical functionality of proteins.
Casein's emulsifying activity could be increased by a small amount of hydrolysis (2–10%), as demonstrated by Chobert *et al*. in 1988. According to Euston *et al*. (2001), WPC's ability to emulsify was increased by hydrolysis (10-27%). Additionally, it has been demonstrated that protease-treated whey proteins cause gel formation to occur more quickly following heat treatment than the intact proteins do. By varying the hydrolysis conditions, gel characteristics can also be changed (Otte *et al*., 1996). These characteristics make milk protein hydroxylates efficient encapsulants and may encourage the creation of novel delivery systems.

6. CHARACTERIZATION OF MICROCAPSULES

Chemical, physical, physicochemical, and even organoleptic approaches could be used to describe microcapsules. These techniques are among the cutting-edge physical or physicochemical analyses that can determine or directly see the structure of the manufactured microcapsules. Microcapsules can be characterized using a variety of methods, including electron microscopy (EM), radio tracers, fluorescence quenching, ultrasonic absorption, electron spin resonance (ESR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy.

6.1 Scanning Electron Microscopy (SEM)

Whether the coating can provide required quality depends on the flow characteristics of the capsule's inner and outside microstructure as well as position of core material inside the microcapsule. All of these parameters can be studied by SEM as described by Rosenberg *et al* (1985). Core material distribution in microcapsules, the mechanics of capsule production, and the effects of moisture uptake on microcapsule's properties might all be investigated using the SEM approach.

6.2 Electron Spin Resonance (ESR) Spectroscopy

ESR is a technique for detecting how electrons behave (dynamically) inside of a suitable molecule and for investigating various processes by determining the electron environment. Quantities, types, nature, environments, and behaviours of unpaired electrons can all be learned via ESR observations. The only method for non-

destructively and selectively measuring free radicals in any sample phase is through the use of ESR devices (gas, liquid or solid) (Kim and Baianu, 1991).

6.3 Nuclear magnetic resonance (NMR) Spectroscopy

For inducing transitions between nuclear spin energy levels, NMR spectroscopy that is a subfield of absorption spectroscopy is used by utilizing radio frequency electromagnetic radiation. The NMR signal, sometimes referred to as the "free induction decay" signal, is created when the nuclear spins return to equilibrium state thus inducing voltage in a detecting coil.

In complex systems, like food, NMR techniques offer quick, sensitive and nondestructive ways to gather extensive data on intermolecular interactions and molecular dynamics. It is now well known that the NMR approach is speedier and more suited for characterizing microcapsules (Shahidi and Han, 1993).

6.4 Isoelectric point

Neutral pH is the pH range where molecules have no electrical charge. A molecule's value can affect how soluble it is at a certain pH. The micro electrophoresis device measures the electrophoretic mobility of the microsphere and makes it simple to determine the isoelectric point. The mobility is related to the amount of charge on the surface, the way that the microcapsules behave when exposed to ions, or both. (“Characterization of microcapsules”. n.d.)

6.5 Encapsulation efficiency

Encapsulation efficiency (EE %) can be calculated using below formula:

$EE\ \% = (W_t/W_i) \times 100\%$

Where W_t = total (final) amount of the incorporated material obtained in microcapsules

W_i = the total quantity of incorporated material added initially during the preparation

W_i and W_t can be determined by chromatographic or spectroscopic method. The integrated molecule will become soluble and be quantifiable if the material employed to construct the capsule shell is a polymer, which can be dissolved in a solvent. If the inserted molecule is not soluble in that solvent, the capsules can be placed in a solution containing the desired molecule to extract it (Arab *et al*., 2019).

6.6 Angle of contact

By measuring the angle of contact, researchers can establish whether microspheres are hydrophilic or hydrophobic and so better understand their wetting properties. The presence of the adsorbed component affects this solid-specific thermodynamic property. Angle of contact is defined where the solid, air, and water merge. By placing a droplet in a circular cell that is mounted above the objective of an inverted microscope, the advancing and receding angle of contact may be recorded. (“Characterization of microcapsules”. n.d.)

6.7 Microcapsule yield

The yield of the microcapsules is calculated by dividing the actual weight of the microcapsules over the theoretical weight of compound to be encapsulated and coating material, multiplied by 100 (Thu and Krasaekoopt, 2016).

$EY\ (\%) = M^*/M \times 100$

7. Recent advancements

a. Within a few weeks of storage much activity of potential spray dried probiotic is lost. It is caused by stress that is brought on by phase transitions, changes in temperature, and dryness, all of which have the potential to damage cell membranes and proteins. Thermoprotectants like adnitol, trehalose, non-fat milk solids, as well as growth-promoting agents such different probiotic/prebiotic combos and granular starch, have been added by researchers to the medium before drying as one way to improve probiotic viability. Despite being a well-established method, microencapsulation by spray drying is used very less for cell immobilization due to substantial mortality caused by simultaneous drying and thermal inactivating microorganisms. Instead, a low-cost method that uses continuous two-step spray drying and emulsification that coat milk fat droplets that contain freeze-dried bacteria along with whey protein polymers. Cryoprotectants have also been used to overcome inactivation during drying and stabilize cultures during storage when those are freeze dried. After entrapment those become easy to handle compared to in a suspension or in slurry. The matrix beads/microcapsules can then have another surface coating added after drying. This top layer may be functional, adding an additional layer of protection for the cells, as well as being employed to change the product's esthetic and sensory qualities (Anal and Singh, 2007).

b. Ultrasound aided spray drying has been widely used in food industry to improve the stability of a wide range of compounds that are sensitive. There are several advantages over conventional methods including improved size distribution, no nozzle clogging, lower velocity atomization, relatively energy efficient, no need for huge drying chamber etc. (Khaire and Gogate, 2021).

c. Some bioactive compounds have an unpleasant flavour, which limits their use in food items, particularly at large doses. Yeast cells are known as a novel carrier for bioactive compounds having benefits like- controlled release, biodegradable, provide photochemical and oxidative stability, thermal stability, no effect on sensory properties. *Saccharomyces cerevisiae* was abundantly used as a suitable carrier for food ingredients as it is easily available and encapsulating by yeast cells are easier to perform (Dadkhodazade *et al*., 2021).

d. Combined microencapsulation technologies for encapsulating bioactive compounds is also a novel approach in dairy sector. Encapsulation of fish oil was performed using spray drying followed by spray chilling. Firstly after spray drying it showed a pronounced fish oil taste but had no unpleasant odour. This restriction was removed by a second shell created using spray chilling (Fadini *et al*., 2018).

e. High efficiency (75%) vibrating technique was used to microencapsulate nisin in an alginate matrix, and the microcapsules were noted for their homogeneity in surface morphology and shape. On *Brochothrix thermosphacta* 7R1, the microcapsules' antibacterial activity was assessed. The experiment showed that the microcapsules maintained their antibacterial action while being stored at 4 °C and pH 6.0 (Maresca *et al*., 2016).

8. Future opportunities

This method is becoming popular among customers due to its integral benefits in the administration of active chemicals, related health benefits, and rising awareness. The

nutritional value of simple, ready-to-eat food products are enhanced by adding a variety of functional bioactive substances.

The market for microencapsulation is projected to increase at a CAGR of 12.9% from an estimated value of USD 8.5 billion in 2020 to USD 15.5 billion by 2025. Some of the factors propelling the growth of the microencapsulation market include the expanding uses of microencapsulated products across numerous industries, the rising need for agrochemical products, pharmaceutical and for functional food products.

As diseases like obesity and diabetes are becoming more common, people are paying more attention to what they eat and drink. The market is experiencing a rise in demand for fresh, flavourful food products as a result of consumer health concern. In the coming years, it is projected that developing countries like China and India will experience higher growth. The industrial uses and technology of starch processing are changing quickly in the Asia-Pacific area, especially in India. As a result of government initiatives to encourage the consumption of functional foods there in order to reduce malnutrition, the food encapsulation sector has been expanding in these countries ("Microencapsulation Market Size, Share, Trends and Industry Analysis," 5546).

8.1 Co-microencapsulation

Co-microencapsulation is a rising technique in the food business since it permits the manufacture of microcapsules with the same basics as microencapsulation with a longer shelf life, employing fewer encapsulating materials and fewer active chemicals, but having greater beneficial action.

Over the well-known microencapsulation, combining two or more active compounds that work in sync with one another and the encasing materials offers an advantage. Polyphenols fatty acids, probiotics, prebiotics are a few of the key active substances employed in this technique. Probiotics are typically coupled with one of the other active ingredients to increase their benefits (Niño-Vásquez *et al*., 2022).

Co-microencapsulation is a flexible method with room for expansion in the future. One can utilize almost any GRAS (generally recognized as safe) food-grade ingredient in it as an coating material or as an active component, including arabic gum, dextrans, maltodextrin, starch, persian gum, galacto-oligosaccharides, glucose, sucrose, lactose, vitamins, and egg white (Raddatz and Menezes, 2021).

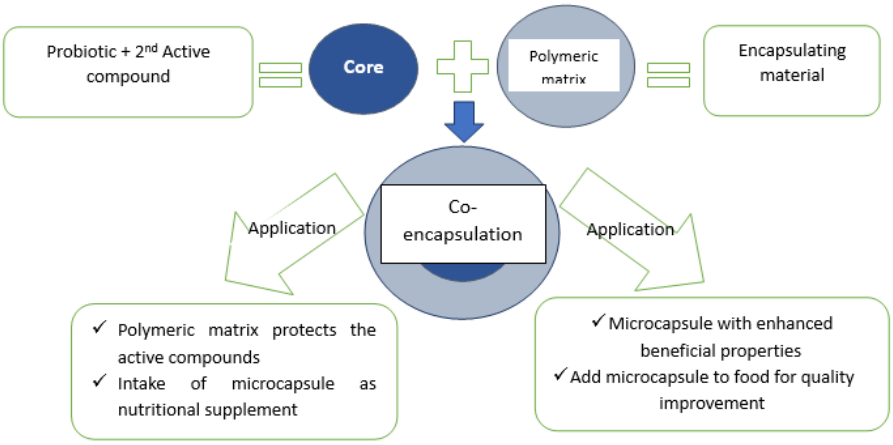


Figure 2. Co-encapsulation and its uses (Niño-Vásquez et al., 2022)

Summary
Encapsulation is a useful technique for enclosing an active substance with a protective wall material and as a result, it has many benefits. Microencapsulated foods and other substances have a wide range of applications since they are a powerful and crucial instrument for safeguarding food and other items from the most aggressive processing techniques while preserving various bacteria, nutritional components, enzymes, colours, etc. Every new use for microencapsulation has a different set of difficulties. These puzzles demand knowledge of numerous technology, aptitude, and experience to solve. We will be able to enhance the nutritional qualities and health advantages of food molecules by creating new methods and using cutting-edge stabilizing techniques.

9 References

Abdel-Rahman, M. A., Sadek, Z. I., Azab, M. S., Darwesh, O. M., & Hassan, M. S. (2019). Incorporation of microencapsulated lactobacillus rhamnosus into infant-foods inhibit proliferation of toxicogenic *Bacillus cereus* strains. *Biocatalysis and Agricultural Biotechnology*, *18*, 101013. https://doi.org/10.1016/j.bcab.2019.01.051

Afzaal, M., Saeed, F., Ateeq, H., Ahmed, A., Ahmad, A., Tufail, T., Ismail, Z., & Anjum, F. M. (2020). Encapsulation of *Bifidobacterium bifidum* by internal gelation method to access the viability in cheddar cheese and under simulated gastrointestinal conditions. *Food Science & Nutrition*, *8*(6), 2739-2747. https://doi.org/10.1002/fsn3.1562

Ajlouni, S., Ranadheera, C. S., & Chua, E. L. (2020). Encapsulation increases the *in vitro* bioaccessibility of probiotics in yoghurt. *International Journal of Dairy Technology*, *74*(1), 118-127. https://doi.org/10.1111/1471-0307.12746

Akgün, D., Gültekin-Özgüven, M., Yücetepe, A., Altin, G., Gibis, M., Weiss, J., & Özçelik, B. (2020). Stirred-type yoghurt incorporated with sour cherry extract in chitosan-coated liposomes. *Food Hydrocolloids*, *101*, 105532. https://doi.org/10.1016/j.foodhyd.2019.105532

Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in food science & technology*, *18*(5), 240-251. https://doi.org/10.1016/j.tifs.2007.01.004

Arab, M., Razavi, S. H., Hosseini, S. M., Nayebzadeh, K., Meybodi, N. M., Khanniri, E., Mardi, P., & Mortazavian, A. M. (2019). Production and characterization of functional flavored milk and flavored fermented milk using microencapsulated canthaxanthin. *LWT*, *114*, 108373. https://doi.org/10.1016/j.lwt.2019.108373

Arenas-Jal, M., Suñé-Negre, J. M., & García-Montoya, E. (2020). An overview of microencapsulation in the food industry: Opportunities, challenges, and innovations. *European Food Research and Technology*, *246*(7), 1371-1382. https://doi.org/10.1007/s00217-020-03496-x

Augustin, M. A. (2003). The role of microencapsulation in the development of functional dairy foods. *Australian Journal of Dairy Technology*, *58*(2), 156.

Augustin, M. A., & Udabage, P. (2007). Influence of processing on functionality of milk and dairy proteins. *Advances in Food and Nutrition Research*, *53*, 1-38. https://doi.org/10.1016/S1043-4526(07)53001-9

Bakry, A. M., Chen, Y. Q., & Liang, L. (2019). Developing a mint yogurt enriched with omega-3 oil: Physiochemical, microbiological, rheological, and sensorial characteristics. *Journal of Food Processing and Preservation*, *43*(12), e14287. https://doi.org/10.1111/jfpp.14287

Caleja, C., Ribeiro, A., Barros, L., Barreira, J. C., Antonio, A. L., Beatriz P.P. Oliveira, M., Barreiro, M. F., & Ferreira, I. C. (2016). Cottage cheeses functionalized with fennel and chamomile extracts: Comparative performance between free and microencapsulated forms. *Food Chemistry*, *199*, 720-726. https://doi.org/10.1016/j.foodchem.2015.12.085

Çam, M., İçyer, N. C., & Erdoğan, F. (2014). Pomegranate peel phenolics: Microencapsulation, storage stability and potential ingredient for functional food development. *LWT-Food Science and Technology*, *55*(1), 117-123. https://doi.org/10.1016/j.lwt.2013.09.011

Champagne, C. P., Gardner, N. J., & Roy, D. (2005). Challenges in the addition of probiotic cultures to foods. *Critical Reviews in Food Science and Nutrition*, *45*(1), 61-84. https://doi.org/10.1080/10408690590900144

Champagne, C., & Fustier, P. (2007). Microencapsulation for delivery of probiotics and other ingredients in functional dairy products. *Functional Dairy Products*, 404-426. https://doi.org/10.1533/9781845693107.3.404

Characterization of microcapsules. (n.d.). Share and Discover Knowledge on SlideShare. https://www.slideshare.net/kashf14/characterization-of-microcapsules-201961939

Chen, F., Liang, L., Zhang, Z., Deng, Z., Decker, E. A., & McClements, D. J. (2017). Inhibition of lipid oxidation in nanoemulsions and filled microgels fortified with omega-3 fatty acids using casein as a natural antioxidant. *Food Hydrocolloids*, *63*, 240-248. https://doi.org/10.1016/j.foodhyd.2016.09.001

Chobert, J. M., Sitohy, M. Z., & Whitaker, J. R. (1988). Solubility and emulsifying properties of caseins modified enzymatically by Staphylococcus aureus V8 protease. *Journal of Agricultural and Food Chemistry*, *36*(1), 220-224. https://doi.org/10.1021/jf00079a055

Choi, H. J., Ahn, J., Kim, N. C., & Kwak, H. S. (2006). The effects of microencapsulated chitooligosaccharide on physical and sensory properties of the milk. *Asian-Australasian Journal of Animal Sciences*, *19*(9), 1347-1353. https://doi.org/10.5713/ajas.2006.1347

Coronel-Aguilera, C. P., & San Martín-González, M. F. (2015). Encapsulation of spray dried β-carotene emulsion by fluidized bed coating technology. *LWT-Food Science and Technology*, *62*(1), 187-193. https://doi.org/10.1016/j.lwt.2014.12.036

Corredig, M., & Dalgleish, D. G. (1998). Characterization of the interface of an oil-in-water emulsion stabilized by milk fat globule membrane material. *Journal*

of Dairy Research, *65*(3), 465-477. https://doi.org/10.1017/S0022029998002982

Corredig, M. (2009). *Dairy-derived ingredients: Food and nutraceutical uses*. Woodhead Publishing.

Dadkhodazade, E., Khanniri, E., Khorshidian, N., Hosseini, S. M., Mortazavian, A. M., & Moghaddas Kia, E. (2021). Yeast cells for encapsulation of bioactive compounds in food products: A review. *Biotechnology Progress*, *37*(4), e3138. https://doi.org/10.1002/btpr.3138

De Moura, S. C., Schettini, G. N., Garcia, A. O., Gallina, D. A., Alvim, I. D., & Hubinger, M. D. (2019). Stability of hibiscus extract encapsulated by Ionic gelation incorporated in yogurt. *Food and Bioprocess Technology*, *12*(9), 1500-1515. https://doi.org/10.1007/s11947-019-02308-9

Donhowe, E. G., Flores, F. P., Kerr, W. L., Wicker, L., & Kong, F. (2014). Characterization and in vitro bioavailability of β-carotene: Effects of microencapsulation method and food matrix. *LWT-Food Science and Technology*, *57*(1), 42-48. https://doi.org/10.1016/j.lwt.2013.12.037

Duhan, N., Sahu, J. K., Mohapatra, A., & Naik, S. N. (2021). Microencapsulation of ghee flavorants with whey protein concentrate and guar gum using spray drying. *Journal of Food Processing and Preservation*, *45*(6), e15537. https://doi.org/10.1111/jfpp.15537

Dziezak, J. D. (1988). Microencapsulation and encapsulated ingredients. *Food technology (Chicago)*, *42*(4), 136-153.

El-Messery, T. M., El-Said, M. M., Shahein, N. M., El-Din, H. M., & Farrag, A. (2019). Functional yoghurt supplemented with extract orange peel encapsulated using coacervation technique. *Pakistan Journal of Biological Sciences*, *22*(5), 231-238. https://doi.org/10.3923/pjbs.2019.231.238

Encapsulation Methods and Properties of Capsules, LLS Health CDMO. (2019, October 28). LLS Health CDMO. https://lubrizolcdmo.com/technical-briefs/encapsulation/

Euston, S. R., Finnigan, S. R., & Hirst, R. L. (2001). Heat-induced destabilization of oil-in-water emulsions formed from hydrolyzed whey protein. *Journal of Agricultural and Food Chemistry*, *49*(11), 5576-5583. https://doi.org/10.1021/jf0102620

Fadini, A. L., Alvim, I. D., Ribeiro, I. P., Ruzene, L. G., Silva, L. B., Queiroz, M. B., Miguel, A. M., Chaves, F. C., & Rodrigues, R. A. (2018). Innovative strategy based on combined microencapsulation technologies for food application and the influence of wall material composition. *LWT*, *91*, 345-352. https://doi.org/10.1016/j.lwt.2018.01.071

Farrag, A. F., Zahran, H., Al-Okaby, M. F., El-Sheikh, M. M., & Soliman, T. N. (2020). Physicochemical properties of white soft cheese supplemented with encapsulated olive phenolic compounds. *Egyptian Journal of Chemistry*, *63*(8), 2921-2931. https://dx.doi.org/10.21608/ejchem.2020.23381.2388

García-Burgos, M., Moreno-Fernández, J., Alférez, M. J., Díaz-Castro, J., & López-Aliaga, I. (2020). New perspectives in fermented dairy products and their health relevance. *Journal of Functional Foods*, *72*, 104059. https://doi.org/10.1016/j.jff.2020.104059

Ghandehari Yazdi, A. P., Barzegar, M., Ahmadi Gavlighi, H., Sahari, M. A., & Mohammadian, A. H. (2020). Physicochemical properties and organoleptic aspects of ice cream enriched with microencapsulated pistachio peel extract. *International Journal of Dairy Technology*, *73*(3), 570-577. https://doi.org/10.1111/1471-0307.12698

GilliardNkhata, S., Ustunol, Z., & Menevseoglu, A. (2015). Iron Fortification of Yogurt and Pasteurized Milk. *Journal of Nutritional Health & Food Science*, *3*(3), 1-12. http://dx. doi. org/10.15226/jnhfs

González-Sánchez, F., Azaola, A., Gutiérrez-López, G. F., & Hernández-Sánchez, H. (2010). Viability of microencapsulated Bifidobacterium animalis ssp. lactis BB12 in kefir during refrigerated storage. *International Journal of Dairy Technology*, *63*(3), 431-436. https://doi.org/10.1111/j.1471-0307.2010.00604.x

Gowda, A., Sharma, V., Goyal, A., Singh, A. K., & Arora, S. (2018). Process optimization and oxidative stability of omega-3 ice cream fortified with flaxseed oil microcapsules. *Journal of Food Science and Technology*, *55*(5), 1705-1715. https://doi.org/10.1007/s13197-018-3083-4

Gruskiene, R., Bockuviene, A., & Sereikaite, J. (2021). Microencapsulation of Bioactive Ingredients for Their Delivery into Fermented Milk Products: A Review. *Molecules*, *26*(15), 4601. https://doi.org/10.3390/molecules26154601

Haratifar, S., & Corredig, M. (2014). Interactions between tea catechins and casein micelles and their impact on renneting functionality. *Food Chemistry*, *143*, 27-32. https://doi.org/10.1016/j.foodchem.2013.07.092

Heidebach, T., Först, P., & Kulozik, U. (2009). Transglutaminase-induced caseinate gelation for the microencapsulation of probiotic cells. *International Dairy Journal*, *19*(2), 77-84. https://doi.org/10.1016/j.idairyj.2008.08.003

Homayouni, A., Azizi, A., Ehsani, M. R., Yarmand, M. S., & Razavi, S. H. (2008). Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry*, *111*(1), 50-55. https://doi.org/10.1016/j.foodchem.2008.03.036

İnanç Horuz, T., & Belibağli, K. B. (2019). Encapsulation of tomato peel extract into nanofibers and its application in model food. *Journal of Food Processing and Preservation*, *43*(9). https://doi.org/10.1111/jfpp.14090

Ipar, V. S., Singhal, R. S., & Devarajan, P. V. (2022). An innovative approach using microencapsulated turmeric oleoresin to develop ready-to-use turmeric milk powder with enhanced oral bioavailability. *Food Chemistry*, *373*, 131400. https://doi.org/10.1016/j.foodchem.2021.131400

Jeong, H., Lee, Y., Ganesan, P., Kwak, H., & Chang, Y. H. (2017). Physicochemical, microbial, and sensory properties of Queso Blanco cheese supplemented with

powdered microcapsules of tomato extracts. *Korean Journal for Food Science of Animal Resources*, *37*(3), 342-350. https://doi.org/10.5851/kosfa.2017.37.3.342

Jung, J., Kil, J., Kim, S., Jeon, J., & Park, K. (2007). Survival of double-microencapsulated Bifidobacterium breve in milk in simulated gastric and small intestinal conditions. *Preventive Nutrition and Food Science*, *12*(1), 58-63. https://doi.org/10.3746/jfn.2007.12.1.058

Kailasapathy, K., & Lam, S. (2005). Application of encapsulated enzymes to accelerate cheese ripening. *International Dairy Journal*, *15*(6-9), 929-939. https://doi.org/10.1016/j.idairyj.2004.11.006

Kamyshny, A., & Magdassi, S. (2016). Microencapsulation. *Encyclopedia of Surface and Colloid Science, Third Edition*, 4636-4648. https://doi.org/10.1081/e-escs3-120023308

Karel, M., & Langer, R. (1988). Controlled release of food ingredients. *Flavor Encapsulation. American Chemical Society. Washington DC*, 177-191.

Khaire, R. A., & Gogate, P. R. (2021). Novel approaches based on ultrasound for spray drying of food and bioactive compounds. *Drying Technology*, *39*(12), 1832-1853. https://doi.org/10.1080/07373937.2020.1804926

Kim, H. H. Y., & Baianu, I. C. (1991). Novel liposome microencapsulation techniques for food applications. *Trends in Food Science & Technology*, *2*, 55-61. https://doi.org/10.1016/0924-2244(91)90622-P

Kim, N. C., Kim, J. B., & Kwak, H. S. (2008). Microencapsulation of Korean mistletoe (Viscum album var. coloratum) extract and its application into milk. *Asian-Australasian Journal of Animal Sciences*, *21*(2), 299-306. https://doi.org/10.5713/ajas.2008.70362

Kim, S. J., Ahn, J., Seok, J. S., & Kwak, H. S. (2003). Microencapsulated iron for drink yogurt fortification. *Asian-Australasian Journal of Animal Sciences*, *16*(4), 581-587. https://doi.org/10.5713/ajas.2003.581

Kontopidis, G., Holt, C., & Sawyer, L. (2004). Invited review: β-lactoglobulin: binding properties, structure, and function. *Journal of Dairy Science*, *87*(4), 785-796. https://doi.org/10.3168/jds.S0022-0302(04)73222-1

Koupantsis, T., Pavlidou, E., & Paraskevopoulou, A. (2014). Flavour encapsulation in milk proteins–CMC coacervate-type complexes. *Food Hydrocolloids*, *37*, 134-142. https://doi.org/10.1016/j.foodhyd.2013.10.031

Kwak, H. S., Chimed, C., Yoo, S. H., & Chang, Y. H. (2016). Physicochemical and sensory properties of appenzeller cheese supplemented with powdered microcapsule of tomato extract during ripening. *Korean Journal for Food Science of Animal Resources*, *36*(2), 244. https://doi.org/10.5851%2Fkosfa.2016.36.2.244

Lee, J. B., Ahn, J., Lee, J., & Kwak, H. S. (2004). L-ascorbic acid microencapsulated with polyacylglycerol monostearate for milk fortification. *Bioscience, Biotechnology and Biochemistry*, *68*(3), 495-500. https://doi.org/10.1271/bbb.68.495

Leskauskaite, D., Jasutiene, I., Malinauskyte, E., Kersiene, M., & Matusevicius, P. (2016). Fortification of dairy products with vitamin D3. *International Journal of Dairy Technology*, *69*(2), 177-183. https://doi.org/10.1111/1471-0307.12242

Li, C., Wang, C. L., Sun, Y., Li, A. L., Liu, F., & Meng, X. C. (2016). Microencapsulation of *Lactobacillus rhamnosus* GG by transglutaminase cross-linked soy protein isolate to improve survival in simulated gastrointestinal conditions and yoghurt. *Journal of Food Science*, *81*(7), M1726-M1734. https://doi.org/10.1111/1750-3841.13337

Lopes, D. C., Silvestre, M. P., Chiarini-Garcia, H., Garcia, E. S., Morais, H. A., & Silva, M. R. (2011). Evaluation of conjugated linoleic acid addition to a chocolate milk drink. *International Journal of Food Engineering*, *7*(2). https://doi.org/10.2202/1556-3758.1842

Magee Jr, E. L., Olson, N. F., & Lindsay, R. C. (1981). Microencapsulation of cheese ripening systems: Production of diacetyl and acetoin in cheese by encapsulated bacterial cell-free extract. *Journal of Dairy Science*, *64*(4), 616-621. https://doi.org/10.3168/jds.S0022-0302(81)82620-3

Maresca, D., De Prisco, A., La Storia, A., Cirillo, T., Esposito, F., & Mauriello, G. (2016). Microencapsulation of nisin in alginate beads by vibrating technology: Preliminary investigation. *LWT-Food Science and Technology*, *66*, 436-443. https://doi.org/10.1016/j.lwt.2015.10.062

Markman, G., & Livney, Y. D. (2012). Maillard-conjugate based core–shell co-assemblies for nanoencapsulation of hydrophobic nutraceuticals in clear beverages. *Food & Function*, *3*(3), 262-270. https://doi.org/10.1039/c1fo10220f

McClements, D. J. (2018). Recent developments in encapsulation and release of functional food ingredients: delivery by design. *Current Opinion in Food Science*, *23*, 80-84. https://doi.org/10.1016/j.cofs.2018.06.008

Mehansho, H. (2006). Iron fortification technology development: new approaches. *The Journal of Nutrition*, *136*(4), 1059-1063. https://doi.org/10.1093/jn/136.4.1059

Microencapsulation market size, share, trends and industry analysis. (n.d.). MarketsandMarkets. https://www.marketsandmarkets.com/Market-Reports/microencapsulation-market-83597438.html

Microencapsulation Market Size, Share, Trends and Industry Analysis. (5546, January 1). Retrieved from https://www.marketsandmarkets.com/Market-Reports/microencapsulation-market-83597438.html

Mishra, M. (2015). *Handbook of encapsulation and controlled release*. CRC Press.

Muñoz-Tébar, N., De la Vara, J., Ortiz de Elguea-Culebras, G., Cano, E., Molina, A., Carmona, M., & Berruga, M. (2019). Enrichment of sheep cheese with chia (*Salvia hispanica* L) oil as a source of omega-3. *LWT*, *108*, 407-415. https://doi.org/10.1016/j.lwt.2019.03.092.

Niño-Vásquez, I. A., Muñiz-Márquez, D., Ascacio-Valdés, J. A., Contreras-Esquivel, J. C., Aguilar, C. N., Rodríguez-Herrera, R., & Flores-Gallegos, A. C. (2022).

Co-microencapsulation: a promising multi-approach technique for enhancement of functional properties. *Bioengineered*, *13*(3), 5168-5189. https://doi.org/10.1080/21655979.2022.2037363

Otte, J., Ju, Z. Y., Faergemand, M., Lomholt, S. B., & Qvist, K. B. (1996). Protease-induced aggregation and gelation of whey proteins. *Journal of Food Science*, *61*(5), 911-916. https://doi.org/10.1111/j.1365-2621.1996.tb10900.x

Patrignani, F., Siroli, L., Serrazanetti, D. I., Braschi, G., Betoret, E., Reinheimer, J. A., & Lanciotti, R. (2017). Microencapsulation of functional strains by high pressure homogenization for a potential use in fermented milk. *Food Research International*, *97*, 250-257. https://doi.org/10.1016/j.foodres.2017.04.020

Puyol, P., Perez, M. D., Peiro, J. M., & Calvo, M. (1994). Effect of binding of retinol and palmitic acid to bovine β-lactoglobulin on its resistance to thermal denaturation. *Journal of Dairy Science*, *77*(6), 1494-1502. https://doi.org/10.3168/jds.S0022-0302(94)77088-0

Raddatz, G. C., & Menezes, C. R. (2021). Microencapsulation and Co-encapsulation of bioactive compounds for application in food: challenges and perspectives. *Ciência Rural*, *51*(3). https://doi.org/10.1590/0103-8478cr20200616

Radulović, Z., Miočinović, J., Mirković, N., Mirković, M., Paunović, D., Ivanović, M., & Seratlić, S. (2017). Survival of spray-dried and free-cells of potential probiotic Lactobacillus plantarum 564 in soft goat cheese. *Animal Science Journal*, *88*(11), 1849-1854. https://doi.org/10.1111/asj.12802

Relkin, P., Yung, J. M., Kalnin, D., & Ollivon, M. (2008). Structural behaviour of lipid droplets in protein-stabilized nano-emulsions and stability of α-tocopherol. *Food Biophysics*, *3*(2), 163-168. https://doi.org/10.1007/s11483-008-9064-9

Ribeiro, A., Caleja, C., Barros, L., Santos-Buelga, C., Barreiro, M. F., & Ferreira, I. C. (2016). Rosemary extracts in functional foods: Extraction, chemical characterization and incorporation of free and microencapsulated forms in cottage cheese. *Food & Function*, *7*(5), 2185-2196. https://doi.org/10.1039/C6FO00270F

Ribeiro, M. C. E., Chaves, K. S., Gebara, C., Infante, F. N., Grosso, C. R., & Gigante, M. L. (2014). Effect of microencapsulation of Lactobacillus acidophilus LA-5 on physicochemical, sensory and microbiological characteristics of stirred probiotic yoghurt. *Food Research International*, *66*, 424-431. https://doi.org/10.1016/j.foodres.2014.10.019

Rodriguez-Concepcion, M., Avalos, J., Bonet, M. L., Boronat, A., Gomez-Gomez, L., Hornero-Mendez, D., Limon, M. C., Meléndez-Martínez, A. J., Olmedilla-Alonso, B., Palou, A., Ribot, J., Rodrigo, M. J., Zacarias, L., & Zhu, C. (2018). A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. *Progress in Lipid Research*, *70*, 62-93. https://doi.org/10.1016/j.plipres.2018.04.004

Rosenberg, M., Kopelman, I. J., & Talmon, Y. (1985). A scanning electron microscopy study of microencapsulation. *Journal of Food Science*, *50*(1), 139-144. https://doi.org/10.1111/j.1365-2621.1985.tb13295.x

Šeregelj, V., Tumbas Šaponjac, V., Lević, S., Kalušević, A., Ćetković, G., Čanadanović-Brunet, J., Nedović, V., Stajčić, S., Vulić, J., & Vidaković, A. (2019). Application of encapsulated natural bioactive compounds from red pepper waste in yogurt. *Journal of Microencapsulation*, *36*(8), 704-714. https://doi.org/10.1080/02652048.2019.1668488

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of Functional Foods*, *18*, 820-897. https://doi.org/10.1016/j.jff.2015.06.018

Shahidi, F., & Han, X. Q. (1993). Encapsulation of food ingredients. *Critical Reviews in Food Science & Nutrition*, *33*(6), 501-547. https://doi.org/10.1080/10408399309527645

Sharaf, O. M., El-Shafei, K., Ibrahim, G. A., El-Sayed, H. S., Kassem, J. M., Assem, F. M., Tawfek, N.F., Effat, B., Abd El-Khalek, A., & Dabiza, N. (2015). Preparation, Properties and Evaluation of Folate and Riboflavin Enriched Six Functional Cereal-Fermented Milk Beverages Using Encapsulated *Lactobacillus plantarum* or *Streptococcus thermophilus*. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, *6*(4), 1724-1735.

Sharifan, P., Khoshakhlagh, M., Khorasanchi, Z., Darroudi, S., Rezaie, M., Safarian, M., Vatanparast, H., Afshari, A., Ferns, G., Ghazizadeh, H., & Ghayour Mobarhan, M. (2020). Efficacy of low-fat milk and yogurt fortified with encapsulated vitamin D 3 on improvement in symptoms of insomnia and quality of life: Evidence from the SUVINA trial. *Food Science & Nutrition*, *8*(8), 4484-4490. https://doi.org/10.1002/fsn3.1750

Sharifi, S., Rezazad-Bari, M., Alizadeh, M., Almasi, H., & Amiri, S. (2021). Use of whey protein isolate and gum Arabic for the co-encapsulation of probiotic Lactobacillus plantarum and phytosterols by complex coacervation: Enhanced viability of probiotic in Iranian white cheese. *Food Hydrocolloids*, *113*, 106496. https://doi.org/10.1016/j.foodhyd.2020.106496

Shoji, A. S., Oliveira, A. C., Balieiro, J. C. D. C., Freitas, O. D., Thomazini, M., Heinemann, R. J. B., Okuro, P.K., & Fávaro-Trindade, C. S. (2013). Viability of *L. acidophilus* microcapsules and their application to buffalo milk yoghurt. *Food and Bioproducts Processing*, *91*(2), 83-88. https://doi.org/10.1016/j.fbp.2012.08.009

Tamjidi, F., Nasirpour, A., & Shahedi, M. (2012). Physicochemical and sensory properties of yogurt enriched with microencapsulated fish oil. *Food Science and Technology International*, *18*(4), 381-390. https://doi.org/10.1177/1082013211428212

Thompson, A. K., Haisman, D., & Singh, H. (2006). Physical stability of liposomes prepared from milk fat globule membrane and soya phospholipids. *Journal of Agricultural and Food Chemistry*, *54*(17), 6390-6397. https://doi.org/10.1021/jf0605695

Thompson, A. K., Hindmarsh, J. P., Haisman, D., Rades, T., & Singh, H. (2006). Comparison of the structure and properties of liposomes prepared from milk fat globule membrane and soy phospholipids. *Journal of Agricultural and Food Chemistry*, *54*(10), 3704-3711. https://doi.org/10.1021/jf052859b

Thu, T. T. M., & Krasaekoopt, W. (2016). Encapsulation of protease from *Aspergillus oryzae* and lipase from *Thermomyces lanuginoseus* using alginate and different copolymer types. *Agriculture and Natural Resources*, *50*(3), 155-161. https://doi.org/10.1016/j.anres.2016.06.002

Tidona, F., Charfi, I., Povolo, M., Pelizzola, V., Carminati, D., Contarini, G., & Giraffa, G. (2015). Fermented beverage emulsion based on donkey milk with sunflower oil. *International Journal of Food Science & Technology*, *50*(12), 2644-2652. https://doi.org/10.1111/ijfs.12936

Yadav, K., Bajaj, R. K., Mandal, S., Saha, P., & Mann, B. (2018). Evaluation of total phenol content and antioxidant properties of encapsulated grape seed extract in yoghurt. *International Journal of Dairy Technology*, *71*(1), 96-104. https://doi.org/10.1111/1471-0307.12464

Yilmaz, M. T., Taylan, O., Karakas, C. Y., & Dertli, E. (2020). An alternative way to encapsulate probiotics within electrospun alginate nanofibers as monitored under simulated gastrointestinal conditions and in kefir. *Carbohydrate Polymers*, *244*, 116447. https://doi.org/10.1016/j.carbpol.2020.116447

Yüksel-Bilsel, A., & Şahin-Yeşilçubuk, N. (2019). Production of probiotic kefir fortified with encapsulated structured lipids and investigation of matrix effects by means of oxidation and in vitro digestion studies. *Food Chemistry*, *296*, 17-22. https://doi.org/10.1016/j.foodchem.2019.05.181

To Cite This Chapter:
Das, K., et al. (2023). **Importance of microencapsulation in value added dairy products.** In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 207-212). ISRES Publishing**.**

BIOLOGICAL ATTRIBUTES OF LIVESTOCK AS AFFECTED BY HEAT STRESS IN TROPICAL AND SUB-TROPICAL REGIONS

Arooj FAITMA
Zarva AHSAN
Omazia NASIR
Faiz-Ul-HASSAN
Rana Muhammad BILAL
Muhammad JUNAID
Muhammad SADAR
Umair YOUNAS

Introduction

Heat stress is a foremost problem for animals particularly tropical in addition to subtropical areas where, relative humidity and ambient temperature levels are high. Various livestock animals including cattle, sheep along poultry; all have to adjust physiologically and behaviorally to acclimatize against these harsh weather conditions. some oof the phenomenons as a consequence of heat stress are alteration in hormone release pattern, lower feed intake, slow growth rates, and impair reproductive function. Proper management methods, such as providing shade, ventilation, and accessibility to clean water, are critical for mitigating the adverse impacts of high-temperature stress while maintaining the health of animals and production. Heat stress have a significant influence on dairy cow milk output. High temperatures lead to increased metabolic heat generation. These variables all contribute to reduced milk supply, milk composition alterations, and poor udder health. To deal with the heat stress, dairy cows frequently shift energy beyond milk production, which leads to economic losses to dairy farmers. To ensure maximum production of milk also nutritional value during heat stress times, effective heat abatement methods like as cooling systems, and sprinklers, and suitable barn architecture are in dire need of farmers. Heat stress also reduces meat output in poultry and livestock animals. Altered nutritional utilization, and increased thermoregulation energy expenditure are other feed back of high environmental temperatures. These elements all contribute to ineffective feed conversion, and poor carcass quality. Heat-stressed animals may also suffer from muscular degeneration and a greater vulnerability to illness. Implementing cooling methods, optimizingoptimizing nutrition, and changing management practices are critical to reducing meat production losses during heat stress events. There is a significant influence on animal reproductive performance, affecting both male along female fertility. Elevated temperatures can cause hormonal disruption, resulting in decreased quantity as well as quality of sperm within males. Heat stress in females can induce irregular estrous cycles, lower ovulation rates, and impaired embryo development. As a result, conception rates, pregnancy rates, along overall reproductive efficiency fall. Scheduled breeding, artificial insemination, as well as better heat abatement measures, are crucial to maintaining reproductive success in heat-stressed animals. Heat stress causes complicated hormonal changes in cattle. To deal with the challenge, stress hormones such as cortisol and adrenaline are produced, influencing both energy and metabolism allocation. Growth hormones, thyroid hormones, sex hormones, (testosterone and estrogen), and reproductive hormones (LH, FSH, prolactin) experience disruptions. These hormonal changes can lead to altered metabolic rates, growth suppression, decreased fertility, and changes in milk production. Understanding these hormonal fluctuations helps develop targeted strategies for

minimizing heat stress impacts on livestock health in addition to productivity. Mitigating heat stress within livestock involves a multi-faceted approach. Providing adequate shade, proper ventilation, and cooling mechanisms like misting systems or fans helps regulate body temperature. Ensuring access to clean and cool water is essential for hydration. Nutritional adjustments, such as formulating balanced diets and using feed additives, can aid in reducing the metabolic heat load.

Heat Stress in Tropical and Subtropical Region

Heat stress in tropical in addition to subtropical regions is a critical environmental issue that affects both humans and animals, where high temperatures and humidity levels create harsh living conditions(Sejian et al., 2021). This phenomenon occurs when an animal's capability to dissipate heat becomes overwhelmed, leading to potentially life-threatening consequences(Levine, 1997). As temperatures soar in these regions, the impact of heat stress on animal populations becomes increasingly significant, affecting a diverse range of species across various ecosystems (Kays et al., 2015). The equatorial location of tropical regions and the proximity to the Tropics of Cancer and Capricorn in subtropical areas expose them to intense solar radiation, contributing to elevated heat levels (Bradley & Diaz, 2021). The resulting heat index, combining temperature and humidity, exacerbates the feeling of heat, making it even more challenging for animals to cope (Herbut et al., 2018).

Heat stress affects animals of all sizes, from tiny insects to large mammals, and can disrupt the delicate balance of ecosystems. Wildlife and domesticated animals alike face physiological challenges in regulating their body temperature and altering their behaviors and reproductive cycles (Colditz & Hine, 2016). Heat stress has an influence on individual animals as well as biodiversity and overall ecological stability within these areas. Understanding the effect of heat stress on animal populations in tropical and subtropical weather is critical for conserving biodiversity, sustaining ecosystems, and assuring the survival of both wild and domesticated animals (Haubrock et al., 2021). There is an immense need for investigating the environmental elements that put up to heat stress, their physiological and behavioralbehavioral consequences on livestock animals, and their potential consequences for interactions between animals and the larger ecosystem. We can take proactive actions to lessen the effects of heat stress and preserve the vast diversity of species that thrive in these hard temperatures by bringing this critical problem to light (Folke et al., 2021).

Various types of stress are applied to livestock, impacting their output, reproduction, and health. Environmentally induced heat stress is currently a major source of concern due to its negative consequences, particularly for high-productive animals. Elevated ambient temperature is a primary factor threatening animal productivity in tropical, subtropical, and desert environments.

Various types of stress are applied to livestock, impacting their output, reproduction, and health. Environmentally persuaded heat stress is currently a main source of distress due to its negative consequences, particularly for high-productive animals. Elevated ambient temperature is a primary aspect threatening animal productivity in subtropical, tropical, and desert environments.

Homoeothermic animals possess a thermoneutral zone in which the temperature of their body is constant besides their energy consumption is modest. The effectiveness of temperature in the environment rises over the thermo-neutral zone of animals due

to high ambient temperature, sunlight, also wind speed. As a result, the body temperature of the creature exceeds the range recommended for its thermo-neutral zone, therefore the overall heat load surpasses the animal's heat dissipation capability (Bernabucci et al., 2014). As a result, the body temperature of the creature exceeds the range recommended for its thermo-neutral zone, therefore the overall heat load surpasses the animal's heat dissipation capability (Marai et al., 2007; Robinson et al., 2000).

Heat stress worsens health issues besides raises death rates, especially in high-productive in addition to heat-intolerant animals. During an extreme heat wave in California in 2006, over 30 000 cows who produced milk died. Over 4,000 beef cattle perishing within Iowa for exactly the same cause. The effects of heat stress may cause 100% mortality within the broilers in Nigeria (Belhadj Slimen et al., 2016).

Indeed, persistent heat stress has been related to reduced synthesis of milk. A meta-analytic technique would utilizedutilized to examine the influence of high ambient temperatures affect the production of milk, in addition to it was found that the lower milk yield may be due to the cumulative impact of heat stress upon feed intake, physiology, and metabolism of dairy cattle (Belhadj Slimen et al., 2016). Selection for greater milk production has been validated to diminish heat tolerance in dairy sheep (Riggio et al., 2007).

In addition, heat stress has been demonstrated to have a deleterious influence on animal growth performance in subtropical along with tropical locations throughout the world. lower weight for their bodies, average daily increase, then growth rate within Broilers (He et al., 2018), rabbits (Ayyat & Marai, 1997), lambs (Padua et al., 1997) plus beef cattle (Hahn, 1999). After modest or brief heat stress, beef cattle showed compensatory growth (Alsharif, 2022).

Furthermore, heat stress has an impact on almost every element of males as well as females' reproductive functions, including frequency of pregnancy, estrous activity, embryonic and sperm motility, and spermatozoa mortality also abnormalities. Several reviews discuss how heat stress affects reproductive features (Marai & Haeeb, 2010).

The livestock's species and physiological state determine the extent of the animal's sensitivity to higher ambient temperatures. Goats are determined to be the most resistant to high ambient temperatures among livestock animals (Silanikove, 2000). Equally, indigenous animals raised within tropical as well as desert environments were more suited to hot climates than folks raised in temperate climates environments (Marai et al., 2007). Ruminants that are pregnant or nursing are more vulnerable to extreme heat stress than those that are not pregnant or lactating (Conte et al., 2018). Animals chosen for greater production ability are less heat tolerant than animals selected for low production potential(El-Saadony et al., 2021).

Heat stress has been proposed as the reason for oxidative stress within animals living on farm throughout the summer (Belhadj Slimen et al., 2016). Numerous types of research had found that heat increases ROS production in addition to causing oxidative stress, which could lead to cytotoxicity. Furthermore, heat stress would considered to be related with oxidative stress due to similarities within the genes expressed with heat exposure (such as genes encoding heat-shock proteins as well as antioxidant enzymes) and that are expressed after oxidant agent exposures (Belhadj

Slimen et al., 2016). Various researchers like (Salo et al., 1991) and (Mujahid et al., 2005) showed a heat-induced rise in ROS production, particularly superoxide anion.

High humidity and temperature levels are common in these areas, which can have a variety of health and environmental repercussions. Here's more information about heat stress in these areas:

1. Environmental Conditions: Hot and humid weather characterises tropical and subtropical locations. The tropical position and direct light exposure result in high solar radiation, which amplifies the heat felt by organisms (McMichael, 2003).
2. Heat Index: The combination of humidity in addition to high temperature produces the heat index, which is an estimate of how hot it feels when sweat evaporation is taken into consideration. Heat-related diseases are more likely when the heat index is high (Zune et al., 2020).
3. Physiological Effects in Animals: Thermoregulation is difficult for animals in these areas. To discharge excess heat, they may display physiological reactions that consist of panting, sweating, and even increased blood flow to peripheral locations (Mota-Rojas et al., 2021).
4. Impact on Wildlife: Heat stress affects animals of all sizes, from insects to huge mammals. Endangered species include those who have restricted access to water, such as desert dwellers, and animals having thick fur coats, such as certain monkeys (Godde et al., 2021).
5. Heat-Related Illnesses: Heat exhaustion along with heatstroke can take place in animals as a consequence of heat stress. Heat exhaustion is characterized by dehydration, and weakness, including lethargy, but heatstroke is a potentially fatal illness characterized by symptoms such as fast breathing, convulsions, and collapse (D'Alton & Hofmeyr, 2017).
6. Effects on Reproduction and Behavior: Heat stress can affect animal reproductive cycles, resulting in lower conception rates. It can also cause changes in behavioral patterns, with certain species being less active during the warmest times of the day (Levy et al., 2019).
7. Livestock and Agriculture: Heat stress could influence an animal output along with agricultural efficiency in tropical as well as subtropical climates. Cattle, poultryand are especially vulnerable to heat-related problems (Thornton et al., 2009).
8. Biodiversity and Ecosystems: Heat stress has a domino effect on ecosystems. It can modify species distribution, disrupts food webs, and endanger the existence of particular creatures, which could contribute to a loss of biodiversity (Yang et al., 2021).
9. Climate Change Amplification: Heat stress in these areas is exacerbated by climate change. Rising global temperatures can cause more frequent and violent heatwaves, putting animal populations and habitats under even more strain (Soravia et al., 2021).
10. Mitigation StrategiesProactive actions are required to combat heat stress across tropical and subtropical locations. These may include the creation of shady habitats, the provision of water supplies, the preservation of natural ecosystems, and the implementation of heat measures for domesticated animals.

11. Human Health: Animal heat stress can have an indirect impact on human health. Heat-induced changes in ecosystems, for example, may influence transmission of disease patterns or food availability (Ali et al., 2020).
12. Research and Awareness: More research is needed to understand the special effects of heat stress on animals plus ecosystems. Spreading awareness about the need for heat stress mitigation and its larger ramifications is also critical for conservation initiatives (Hassan et al., 2021).

Finally, heat stress is a chief problem in tropical along subtropical areas, harming animals, livestock, and ecosystems. To protect the delicate balance of these unique and valued settings, mitigating heat stress and its impacts needs a multifaceted strategy that includes scientific study, conservation activities, and community participation.

Heat Stress Affecting Milk Production

Heat stress can have a major influence on the production of milk within animals, particularly dairy cows. As temperatures rise, dairy cows face various challenges in maintaining their normal physiological processes, leading to reduced milk yield and quality. Here's a detailed note on how heat stress affects milk production in animals:

Physiological Changes: Heat stress disrupts the thermoregulatory mechanisms of dairy cows. To cope with elevated temperatures, cows redirect blood flow towards the skin's surface for heat dissipation, reducing blood flow to vital organs. This redistribution affects the efficiency of nutrient uptake and metabolism, impacting milk synthesis (Burhans et al., 2022).

Reduced Feed Intake: High temperatures often lead to decreased feed intake in dairy cows. This can result from decreased appetite due to discomfort or reduced grazing time to avoid exposure to the heat. The reduced intake leads to lower energy and nutrient availability for milk production. (Sarangi, 2018)

Dehydration: Heat stress causes increased water loss through sweating and respiration. As cows try to cool themselves, they become dehydrated, leading to a reduction in milk volume(Burhans et al., 2022).

Hormonal Changes: Heat stress modifies hormone levels within dairy cows. The release of prolactin, the hormone responsible for milk production, decreases during heat stress, further impacting milk synthesis (Ouellet et al., 2020).

Behavioral Changes: Heat-stressed cows may change their behavior to avoid heat exposure. They may spend more time seeking shade or lying down, reducing their time spent eating or ruminating, which further affects nutrient absorption and milk production(Herbut et al., 2021).

Milk Composition: Besides reducing milk volume, heat stress can alter milk composition. The concentration of milk constituents such as protein, lactose, and fat may fluctuate, affecting the overall quality of the milk (Gao et al., 2019).

Reproductive Challenges: Heat stress can also negatively impact cow fertility and reproduction. Reduced milk production may lead to delayed estrus cycles, decreased conception rates, and longer calving intervals, impacting overall herd productivity (Walsh et al., 2011).

Heat Stress Management: To moderate the impact of heat stress on milk production, dairy farmers implement various management practices. Providing shaded areas, cooling systems, and access to ample clean water can help alleviate heat stress and maintain cow comfort. Additionally, adjusting feeding strategies and offering nutritionally balanced diets can aid in sustaining milk production during hot periods (Ekine-Dzivenu et al., 2020).

Genetic Selection: Some dairy farmers focus on breeding heat-tolerant cow breeds or using crossbreeding programs to improve resilience to heat stress. These strategies aim to select animals with better heat adaptation traits (Cheruiyot et al., 2022).

Climate Change Implications: With the increasing frequency and intensity of heatwaves because of changes within climate, heat stress on dairy cows is becoming more challenging for farmers to manage. Climate change adaptation plus moderation strategies are crucial to sustain milk production also maintain the commercial viability of dairy operations in the face of rising temperatures (Chang-Fung-Martel et al., 2021).

In conclusion, heat stress suggestively affects milk production in animals, particularly in dairy cows. It causes physiological, hormonal, and behavioral changes that result in reduced yield of milk as well as alterations within milk composition. Implementing heat stress management practices and exploring genetic selection for heat tolerance are vital steps to support animal welfare and maintain dairy productivity in a warming climate.

Losses in Meat Production

Heat stress can have substantial impacts on meat production in animals, particularly in livestock species for example small ruminants, cattleand poultry. As temperatures rise, these animals face numerous challenges that can lead to reduced growth rates, decreased feed efficiency, and overall economic losses for farmers and the meat industry (Rahimi et al., 2021). Here's a detailed note on the losses in meat production in animals because of heat stress.

Reduce in Feed Intake: Heat stress often leads to decreased appetite within livestock. Animals eat less during hot weather, resulting in reduced nutrient intake, which negatively affects their growth and meat production potential (F. Batool et al., 2023).

Lower Weight Gain: High temperatures can hinder an animal's ability to convert feed into body weight. As a result, livestock may experience slower growth rates, leading to a delay in reaching market weight (Nardone et al., 2010).

Poor Feed Efficiency: Heat-stressed animals may have impaired digestion and nutrient absorption, leading to lower feed conversion efficacy. This means a large amount of feed is compulsory for the production of the same amount of meat, increasing production costs (Abdel-Moneim et al., 2021).

Water Loss and Dehydration: Heat stress causes animals to lose water through panting and sweating. Dehydration can lead to weight loss, muscle cramps, and reduced overall health, affecting meat quality and yield (Pandey, 2008).

Changes in Carcass Quality: Heat-stressed animals may experience changes in carcass characteristics. For instance, increased fat deposition in some species can negatively impact meat quality and trim yield (Schumacher et al., 2022).

Reduce in Performance of Reproduction: Heat stress could affect reproductive performance in livestock. It may result in reduced conception rates, higher embryonic mortality, and extended calving or farrowing intervals, further impacting herd productivity (Andreu-Vázquez et al., 2012).

Heat-Related Mortality: Extreme heat episodes can cause heat-related mortality in animals. Heatstroke or comparable heat-related disorders can kill animals who are unable to deal with the heat (Yezli, 2023).

Behavioral Changes: Heat-stressed animals may change their behavior in order to avoid being exposed to high temperatures. They may limit their level of physical activity and spend longer seeking shade to cool down, resulting in less time spent eating and growing (Sejian et al., 2021).

Stress and Immune Suppression: Heat stress can stress animals, causing immune system suppression. This increases the susceptibility of animals to illnesses and infections, thus influencing meat output and quality (Chauhan et al., 2021).

Economic Impact: Heat stress-related meat production losses have major economic ramifications for farmers and the meat industry. Reduced productivity and higher production costs might result in lower profitability and financial hardship for manufacturers (Summer et al., 2019).

To mitigate the damages in meat production triggered due to heat stress, farmers employ several management strategies:

- Providing shaded areas and adequate ventilation to keep animals cool.
- Ensuring access to clean and cool drinking water at all times.
- Adjusting feeding regimes and using heat stress-tolerant feed ingredients.
- Implementing cooling systems like fans, sprinklers, or misters in livestock facilities.
- Selecting heat-tolerant animal breeds or genetic lines that can better cope with high temperatures.
- Scheduling feeding and other farm activities during cooler periods of the day.

In conclusion, heat stress poses significant challenges to meat production in animals, resulting in reduced growth rates, poorer feed efficiency, changes in carcass quality, and economic losses for farmers. Implementing heat stress management practices is crucial to maintaining livestock productivity and ensuring the sustainability of the meat industry in the face the rising global temperatures.

Heat Stress effects on reproductive performance

The dairy industry is especially vulnerable to climate change because heat stress increases the prevalence of viral as well as metabolic illnesses, decreases milk output, and over time decreases the fertility of lactating cows (Gauly & Ammer, 2020). Dairy cows become anxious when temperatures over the limit of their thermoneutral zone (16 to 25 ^{o}C) are present. Increased temperatures and high relative humidity can make cows more prone to heat exhaustion (Owuor, 2021).

The first sign of heat exhaustion is behavioural changes. The behaviors of seeking shade and lying down are impacted in this group. As the degree of heat stress increases, dairy cows spend more time standing than lying down. An animal's attempt to release heat through convection and evaporation could account for this tendency. This changing behavior worsens welfare and puts cows at risk for health issues, output

issues, and fertility issues (Health et al., 2022). Lactating cows consume more water and less dry matter when they are under heat stress. Due to water loss during the summer from evaporation, perspiration, panting, and urination, which causes blood hyperosmolarity, water consumption can increase by two to four times. On the other hand, the intake of dry matter is greatly reduced, which results in a negative energy balance and a large reduction in milk production as well as a reduction in fertility (F. Zhang et al., 2020).

Cows under heat stress consume less dry matter, which results in a negative energy balance. It is widely known that during times of low energy balance availability, which frequently happen throughout the hot seasons of the year, GnRH neurons are sensitive to changes in glucose and different metabolic signaling molecules (insulin, leptin, IGF-1, melanocortin, kisspeptin). Acylated ghrelin concentrations, which regulate gonadotrophin production, are influenced differentially in the summer in nursing cows and pregnant heifers (Dovolou et al., 2023).

The production and function of male and female gametes, the growth of the embryo, and the growth of the fetus are all impacted by the effects of heat exhaustion on reproductive function(Ahmad Para et al., 2020). High-producing cows are more likely to experience these side effects than low-producing cows and heifers that aren't nursing. This procedure might have an immediate or delayed effect on different reproductive organs and reproductive processes (E. Ribeiro et al., 2018).

Lower estrus detection rates were found to be a sign of reproductive issues in dairy cows under heat stress. shrinkage of the ovarian follicle in size, altered follicular fluid composition, and aberrant ovarian steroid concentrations, Heat stress alters the relative position, shape, and function of ooplasmic organelles, particularly mitochondria, and these changes are particularly noticeable in oocytes of Bos taurus taurus ancestry(Ye et al., 2014). effects that last for up to 50 days after being exposed to heat stress, increased morphological defects, decreased sperm concentration and motility abnormal chromatin condensation in sperm, and embryonic death before birth (Robinson et al., 2023).

Semen quality may be affected by the intensity and duration of testicular warmth brought on by heat wave's direct effects on semen production. An increase in testicular temperature has the potential to change the morphology of sperm, even if they may continue to function normally for a few days if the epididymal spermatozoa are not considerably damaged. Gradually emerging sperm with defective morphology will then follow (Rahman et al., 2011).

High ambient temperatures make it more difficult for cows to engage in normal mating behavior because they shorten and weaken estrous expression. It has been proposed that decreased DMI and its associated effects on hormone synthesis cause a decrease in estrous behavior (Sammad, Umer, et al., 2020). Some claim that the decrease in the cow's natural selection for seasonal breeding is the result of improved feed quality and availability, improved health monitoring, and care for the calf. These factors include the need to express various components necessary for successful reproduction (health of uterus, quality of embryo, concentration of hormone), which is why the natural selection of cows for seasonal breeding has been decreased. However, year-round breeding remains a challenge for farmers due to the pervasive result of heat stress on the reproductive system, which linger and get worse over the summer. (López-Gatius, 2012). Physical lethargy brought on by heat stress serves as a coping mechanism that

prevents the animal's internal heat production from increasing further after it has already increased due to estrus-related activity (Becker et al., 2020).

The percentage of embryos that can continue to develop declines as temperature rises. Additionally, the physiological adjustments that homeotherms make in response to heat stress may jeopardize other vital physiological systems(Joseph et al., 2023). Thermal stress causes the blood to move from the viscera to the periphery. This adaptive response slows fetal growth while simultaneously increasing the disposal of body heat to the surrounding space. Additionally, it lessens the placental vascular bed's blood passage (Manaig et al., 2022).

Through direct effects on reproduction as well as indirect effects on giving milk to newborns, production of milk, energy balance, and heat exhaustion, which leads to lower Luteinizing hormone secretion and a smaller dominant follicle width in the puerperal period, heat stress has an impact on reproductive function (Ghaffari, 2022). Since a sustained negative energy balance is one of the main causes of the absence of ovulation in dairy cows, especially during the early puerperal period, any deterioration of energy balance over the summer would impair fertility in dairy cows at this time (Maqhashu, 2019).

Low fertility in dairy cows that are nursing is largely caused by summer heat stress. It is a widespread issue that causes significant economic losses and has an impact on around 60% of the world's cattle population (Rovelli et al., 2020). Depending on how severe the temperature stress is, The summertime sees a drop in conception rates from the cooler months' average of 40–60% to just 10–20%. Since heat directly affects and damages the cellular activities of numerous sections of the reproductive system, its multifactorial nature is the most salient feature of summer infertility (Baruselli et al., 2020). Additionally, subjecting cattle to heat stress causes indirect reactions that may affect the reproductive system. These reactions include changes in how blood is distributed throughout the body's organs, a decrease in food intake, respiratory alkalosis, etc (Das et al., 2016).

The increase in body temperature brought on by heat exhaustion or the physical adjustments cows make to lessen the severity of high body temperature directly causes the effects of heat exhaustion on the reproductive system and other physiological activities. Therefore, altering thermoregulatory systems to lessen the rise in body temperature that occurs in response to heat stress is one method for lowering the adverse impacts of heat stress on production (Roth, 2020).

Hormonal Variation due to Heat Stress

Hormonal variation because of heat stress within livestock is an important topic. When animals are exposed to high temperatures, their bodies undergo physiological changes to cope with the stress (González-Tokman et al., 2020). Heat stress triggers the discharge of stress-related hormones for example cortisol in addition to adrenaline(Getabalew et al., 2020). These hormones help mobilize energy reserves and prepare the animal body for the “fight or flight” response(Adamo, 2014). Additionally, heat stress can impact reproductive hormones like luteinizing hormone (LH) plus follicle-stimulating hormone (FSH), leading to disruptions within the reproductive cycle(Qiang et al., 2022). This can result in reduced fertility, decreased conception rates, and irregular estrous cycles in female animals. Furthermore, heat stress affects thyroid hormones, which take a part in regulating metabolism. Elevated temperatures can reduce the change of the inactive thyroid hormone (T4) into the

active form (T3), slowing down metabolic processes(Bianco et al., 2019). The release of prolactin, a hormone that stimulates milk production, can also be affected. High temperatures might lead to decreased prolactin levels, impacting milk yield and quality in dairy animals(Das et al., 2016). In summary, heat stress induces a cascade of hormonal changes in livestock, affecting stress, reproductive, thyroid, and milk-related hormones. These alterations collectively impact the overall health, reproduction, and productivity of the animals. Proper management strategies, for example providing shade, ventilation, and adequate water, are crucial to mitigate the adverse effects of heat stress on hormonal regulation in livestock (David Renaudeau et al., 2012).

1. Stress Hormones (Cortisol and Adrenaline): Similar to humans, heat stress triggers the release of stress hormones like cortisol and adrenaline in livestock. These hormones help animals respond to stressful situations by increasing the rate of heart, redirecting the flow of blood toward essential organs, then releasing energy stores for a “fight or flight” response (M. N. Ribeiro et al., 2018).
2. Thyroid Hormones: Heat stress can disrupt thyroid function in livestock as well. Thyroid hormones regulate metabolism, which is critical for energy production and overall health. Heat stress can lead to the altered amount of thyroxine (T4) as well as triiodothyronine (T3), potentially affecting the rate of metabolism in livestock(Gupta & Mondal, 2021).
3. Reproductive Hormones: Heat stress has a profound impact on reproductive hormones in livestock. For instance, in dairy cattle, high temperatures can lead to decreased concentrations of luteinizing hormone (LH) in addition to follicle-stimulating hormone (FSH), which are crucial for the estrous cycle plus ovulation. This can result in reduced fertility, irregular cycles, and lower conception rates (Wolfenson & Roth, 2019).
4. Growth Hormone: Heat stress can disrupt the secretion of growth hormone in livestock. Growth hormone influences growth and development, as well as nutrient utilization. Heat stress might lead to reduced growth rates and suboptimal feed efficiency due to altered growth hormone levels (Ahmad et al., 2022).
5. Testosterone and Estrogen: Heat stress could affect the reproductive hormones of males as well as females livestock. In males, there might be a decrease in testosterone levels, impacting sperm production and fertility. In females, heat stress can lead to irregular estrous cycles, reduced ovulation rates, and decreased estrogen production (Abdelnour et al., 2020).
6. Prolactin: Heat stress can also influence prolactin, a hormone important for milk production along with maternal behavior. High temperatures might lead to decreased prolactin levels, impacting milk production in dairy animals (Sammad, Wang, et al., 2020).
7. Renin-Aldosterone System: Heat stress can influence the renin-aldosterone system, which regulates electrolyte balance and blood pressure. Livestock might experience alterations in aldosterone secretion, leading to imbalances in sodium and potassium levels. This can lead to dehydration along with heat-related illnesses (Burhans et al., 2022).
8. Insulin and Glucagon: Heat stress has been shown to affect insulin sensitivity and glucagon secretion in animals. This can have an impact on glucose control and energy metabolism, potentially leading to blood sugar abnormalities (G. Wang et al., 2021).

9. Melatonin: Melatonin release in animals can be disrupted by heat stress, impacting their circadian cycles and sleep habits. Sleep-wake cycles that are disrupted can influence eating behaviour and general well-being (Lunn et al., 2017).
10. Vasopressin: Heat stress can affect vasopressin, a hormone that regulates water balance along with blood pressure. Animals may produce more vasopressin to preserve water, although dehydration can lead to consequences such as heat exhaustion (ROBERTSON, 1977).

Individual characteristics like as breed, age, and health state also play a role in how various livestock species adapt to heat stress. Providing adequate shade, ventilation, and access to clean water is essential to mitigate the impact of heat stress on livestock. Additionally, nutritional adjustments and management practices can help minimize hormonal disruptions and maintain optimal animal health and production.

Alleviation of Heat stress

Heat stress is understood to be a serious issue for the productivity of animals. Many methods of lowering heat exhaustion in farm animals exposed to brief or prolonged periods of high environmental temperature and humidity have been studied and developed (West, 2003). The three kinds of successful heat stress management techniques include those that increase the intake of feed or reduce heat produced through metabolic activity, those that improve the capability of heat loss, and those that involve genetic selection for heat tolerance (D Renaudeau et al., 2012). Improvements in the composition of a diet that either encourage an increase in intake by reducing diet-induced thermogenesis (contain diet having low protein and fibre) or compensate for the low consumption of feed by offering diets having high energy and protein should be able to increase production under heat stress. To meet the unique demands of heat-stressed animals, dietary adjustments should include the addition of minerals, vitamins, electrolytes, amino acids, or other supplements (Renaudeau et al., 2010).

Environment's climatic characteristics: The animal's climatic environment is intricate, particularly while it's outside. Practically speaking, in such circumstances, relative humidity, sun radiation, and wind speed must also be taken into account (Porter & Gates, 1969). Air alone cannot be a representative estimate of the thermal environment. The ability or inability of an animal to effectively deal with acute or ongoing hot conditions can be determined indirectly by looking at animal performance, such as growth rate, egg, and milk production, or directly by looking at physical measurements, such as the temperature of rectum, cloaca and skin, pulse rate, panting, and production of heat (D Renaudeau et al., 2012). Recently, several indexes created from meteorological measures have been examined. These indices range from a straightforward measurement to one that considers the impact of air temperature, relative humidity, radiations of sun, and speed of wind. For instance, using the dry bulb temperature, direct and indirect radiations, a wet or dry bulb temperature, or temperature, and humidity indices, it was possible to determine the optimaler temperature for grazing animals (Erell et al., 2014).

Effects of thermoregulatory responses on the performance of animals: Animal productivity and health suffer as a result of the physiological and metabolic changes brought on by the thermoregulatory responses to thermal stress. Under warm weather, heat stress's direct and indirect impacts on lower feed intake account for a major portion of the decreased animal performance (Bernabucci et al., 2010; Silanikove,

2000). The decrease in heat output associated with feed consumption and metabolic utilization in ad libitum-fed animals is a crucial mechanism for keeping Tb at a physiologically safe range. The drop in meat, milk, and egg production during heat stress is mostly explained by the associated reduced calorie and nutritional intakes (Wasti et al., 2020). The direct impact of higher temperature on the physiology of the reproductive system, health, energy metabolism, and deposition of protein and fat, however, accounts for some of the decreased performance (Mayorga et al., 2020; David Renaudeau et al., 2012).

When temperatures and humidity are high, cattle on pasture and those in feedlots both experience heat stress, which slows down and inefficiently produces beef. Ruminant animals are frequently raised outdoors, either entirely or in part, around the world, where they are constantly exposed to the elements (Seré et al., 1996). In these circumstances, a combination of environmental factors (temperature, relative humidity, radiations of sun, movement of air, and precipitation) leads to heat stress. As a result, for ruminant species, the black globe heat index is frequently employed to determine how thermally stressed an area is (Habeeb et al., 2018).

In addition to being highly susceptible to harsh thermal climatic circumstances, beef cattle are also particularly vulnerable to abrupt climate change. Generally speaking, cattle on pastures are less prone to heat stress than cattle in feedlots (Godde et al., 2021). While feedlot cattle are more susceptible to the radiating heat from surfaces like dirt or concrete, pastured cattle can seek out shade, water, and air circulation to stay cool. Feedlot cattle are more prone to heat exhaustion depending on the severity and length of thermal challenge as well as elements specific to the animal (breed, BW, growth stage, sanitary and nutritional state; (Mader & Davis, 2004).

Environmental changes: It is possible to reduce the negative impacts of hot temperatures by utilizing a wide range of environmental and technological solutions. However, if factors affecting animal performance such as diet, illness control, or breeding are not optimal, applications to the environment that aim to lessen heat stress in farm animals are unacceptable. For instance, in warm temperatures, stocking density for laying hens should be lowered to prevent the buildup of heat exchange between the animals and unnecessary heat stress. Animals should not be subjected to additional stress during the hottest hours of the day. Consequently, to prevent stress-related mortality, animals shouldn't be handled when it's hot outside. Finally, heat can be reduced by using straightforward design principles for the animal (such as form, inclination, thermophysical properties of building materials, ventilation, and facility openings)(Das et al., 2016).

The main methods for lowering adverse environmental effects on animal production and dairy profitability in cattle would be physical environment change or genetic improvement for more heat-tolerant animals (Bernabucci, 2019). These techniques can be classified into two categories: those that enhance the exchange of heat between an environment and an animal in an effort to minimize or lessen the level of heat stress to which the animals are subjected (David Renaudeau et al., 2012).

Alleviation of heat stress: To maintain output during periods of high thermal-heat loads, adequate mitigation methods must be put in place given the detrimental consequences of heat stress on livestock that have been stated. Depending on the region, resources (economic and ecological), and species, different mitigation measures may be used. They can range from management techniques (such as the usage of cooling technologies), which stand in for the principal method of reducing

heat stress, to genetic advancements and dietary supplements. Additionally, reducing the harmful effects of hyperthermia can protect appropriate animal welfare standards and lessen the stress that animals experience (Ali & Al Nsairat, 2009).

Management techniques: Species, geography, and resource considerations can affect the management of heat stress. Sprinkler systems with air-circulating fans are useful in decreasing heat load in dairy cattle species, especially in environments with low humidity. Additionally, you can save cattle from dying from heat exhaustion by giving them shade, avoiding vaccines, constraints, and transport during the warmest times of the day. In many parts of the world, shade systems that shield livestock from direct solar radiation have shown to be an efficient and cost-effective method of managing heat stress. Systems that block radiant heat transfer are efficient in any environment and can lessen the adverse effects of heat exhaustion on livestock's body temperatures. The final factor that might significantly affect animal health during periods of heat stress is building design (Gunn et al., 2019).

Nutritional control: Ruminant species must consume enough fiber to sustain a healthy ruminal pH and engage in rumination. Because of this, while increasing calorie density to lower the heat increment of diets may help to maintain euthermia during heat stress, this must be balanced with the proper amount of fiber in ruminant diets to preserve health and productivity (Brown-Brandl, 2018). Furthermore, it is believed that one of the essential elements of successfully adjusting to and surviving a heat load is adequate insulin function. Therefore, increasing insulin sensitivity with the inclusion of food additives that increase insulin sensitivity, like chromium, lipoic acid, or thiazolidinediones, may enhance animal performance under heat stress (Johnson, 2018).

Hypoxia and GI tract injury result when heat-stressed animals redirect blood flow to the periphery to boost heat-dissipation capability. Nutritional measures such as the addition of L-glutamine, zinc, or betaine may be used to lessen this damage and encourage healing. L-glutamine is an amino acid that is only partially required but is a primary source of energy for cells that divide quickly, such as lymphocytes and enterocytes (Adedokun & Olojede, 2019). It also acts as an immunomodulator by inhibiting the release of cytokines that promote inflammation. Supplemental L-glutamine enhances immunological response, and oxidative defence, prevents intestinal atrophy, improves antibacterial activity, and increases nutritional absorption to improve intestinal barrier function and health. L-glutamine also increases milk output in dairy animals under heat stress (Johnson, 2018).

Managing water: For livestock animals, water is a vital nutrient, particularly when they are under temperature stress. Water intake during heat stress is a limiting factor for survival and performance since it is essential for temperature regulation and maintaining hydration balance in the heat exchange system. Regardless of the species, a lack of water exacerbates the negative consequences of heat stress on the performance of animals. Water losses rise in hot weather (evaporation from perspiration and panting) while metabolism produces less water than usual. Therefore, an animal under heat stress needs to drink more drinking water to meet their needs. Having a plentiful and pristine source of drinking water adjacent to the feeding area is a crucial aspect of good husbandry in warm climates (David Renaudeau et al., 2012).

Farm animals undergo heat stress when it is too hot outside, but with high relative humidity, the severity of the stress increases since there is less heat loss through

evaporative cooling and the animal has a harder time controlling its core body temperature. When the environment is at or above an animal's body temperature, the sensible methods of heat loss through radiation, convection, and conduction become inefficient, and the only remaining option is evaporation, which is largely regulated by the air's humidity. Deleterious effects of extreme heat and humidity that lead to immune system suppression, increased mortality, poor disease resistance, and poor growth (McCafferty et al., 2017).

The visceral organs, such as the heart and lungs, which are crucial for thermoregulation, did not develop in size in tandem with genetic selection for faster growth. Animal thermotolerance can be increased by altering breeding practices in tropical settings. Low heritability genetic features are those that are responsible for adaptation, and the genetics of heat stress are still poorly known (David Renaudeau et al., 2012). To enhance livestock performance while preserving essential adaptability, more research is required. The ability of animals to adapt depends on key characteristics that enable their survival. A set of genes might help animals better adapt to humid, tropical habitats (Dumont et al., 2013).

Additionally, it has been discovered that choosing an animal's coat color can increase its ability to withstand hot, humid climates. Compared to animals with light coat hues, those with dark coat colors absorb more heat (Oke et al., 2021).

By using relevant scientific techniques, such as physical environment modifications and genetic development of heat-stress-tolerant breeds, reduced reproductive productivity is possible while under heat stress. Furthermore, using cutting-edge reproductive technologies like hormone therapy, ovulation synchronization followed by fixed-time artificial insemination, and embryo transfer to increase the likelihood of pregnancy can ensure reproductive success during the summer (Yadav et al., 2022). Summertime conception rates are 22% lower than wintertime rates, indicating that heat stress is fatal and cannot be treated using standard methods, necessitating special attention (Ellis, 1972). High-volume, low-speed fans, tunnel ventilation, and low-volume, high-speed fans are the three main techniques used to lessen the effects of heat stress (Khan et al., 2023).

Summary

High-producing cows eat more and generate more heat and may begin to experience heat stress in well-ventilated barns at air temperatures as low as 65°F. However, in order to lessen losses to the cattle industry, it is possible to limit the effects of heat stress on the overall efficiency of cattle by providing effective measures like shade and cooling strategies (fans, Showers, foggers, misters, tunnel ventilation) during the hot summer months. Production of milk and conception rates are used to gauge how effective these cooling techniques are. Providing clean water is important, especially during warm weather and it is essential to monitor ventilation systems to ensure that they are running properly.

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

Acknowledgments
The authors concede the support of the Cholistan University of Veterinary & Animal Sciences-Bahawalpur, Pakistan, during the write-up.

Conflict of Interest
The authors declare no conflict of interest.

References

Abdel-Moneim, A.-M. E., *et al*. (2021). "Nutritional manipulation to combat heat stress in poultry–A comprehensive review." Journal of Thermal Biology **98**: 102915.

Abdelnour, S. A., *et al*. (2020). "Useful impacts of royal jelly on reproductive sides, fertility rate and sperm traits of animals." Journal of animal physiology and animal nutrition **104**(6): 1798-1808.

Adamo, S. A. (2014). The effects of stress hormones on immune function may be vital for the adaptive reconfiguration of the immune system during fight-or-flight behavior, The Society for Integrative and Comparative Biology.

Adedokun, S. A. and O. C. Olojede (2019). "Optimizing gastrointestinal integrity in poultry: the role of nutrients and feed additives." Frontiers in Veterinary Science **5**: 348.

Ahmad Para, I., *et al*. (2020). "Impact of heat stress on the reproduction of farm animals and strategies to ameliorate it." Biological Rhythm Research **51**(4): 616-632.

Ahmad, R., *et al*. (2022). "Influence of heat stress on poultry growth performance, intestinal inflammation, and immune function and potential mitigation by probiotics." Animals **12**(17): 2297.

Ali, H. H. and S. F. Al Nsairat (2009). "Developing a green building assessment tool for developing countries–Case of Jordan." Building and Environment **44**(5): 1053-1064.

Ali, M. Z., *et al*. (2020). "Impact of global climate change on livestock health: Bangladesh perspective." Open veterinary journal **10**(2): 178–188-178–188.

Alsharif, I. (2022). "Comprehensive exploration of the molecular response, clinical signs, and histological aspects of heat stress in animals." Journal of Thermal Biology: 103346.

Andreu-Vázquez, C., *et al*. (2012). "Effects of twinning on the subsequent reproductive performance and productive lifespan of high-producing dairy cows." Theriogenology **78**(9): 2061-2070.

Ayyat, M. and I. Marai (1997). "Effects of heat stress on growth, carcass traits and blood components of New Zealand White rabbits fed various dietary energy–fibre levels, under Egyptian conditions." Journal of Arid Environments **37**(3): 557-568.

Baruselli, P. S., *et al*. (2020). "Use of embryo transfer to alleviate infertility caused by heat stress." Theriogenology **155**: 1-11.

Batool, F., *et al*. (2023). "An updated review on behavior of domestic quail with reference to the negative effect of heat stress." Animal Biotechnology **34**(2): 424-437.

Becker, C., *et al*. (2020). "Invited review: Physiological and behavioral effects of heat stress in dairy cows." Journal of Dairy Science **103**(8): 6751-6770.

Belhadj Slimen, I., *et al*. (2016). "Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review." Journal of animal physiology and animal nutrition **100**(3): 401-412.

Bernabucci, U. (2019). Climate change: impact on livestock and how can we adapt, Oxford University Press US. **9:** 3-5.

Bernabucci, U., *et al*. (2014). "The effects of heat stress in Italian Holstein dairy cattle." Journal of Dairy Science **97**(1): 471-486.

Bernabucci, U., *et al*. (2010). "Metabolic and hormonal acclimation to heat stress in domesticated ruminants." Animal **4**(7): 1167-1183.

Bianco, A. C., *et al*. (2019). "Paradigms of dynamic control of thyroid hormone signaling." Endocrine reviews **40**(4): 1000-1047.

Bradley, R. S. and H. F. Diaz (2021). "Late Quaternary Abrupt Climate Change in the Tropics and Sub-Tropics: The Continental Signal of Tropical Hydroclimatic Events (THEs)." Reviews of Geophysics **59**(4): e2020RG000732.

Brown-Brandl, T. M. (2018). "Understanding heat stress in beef cattle." Revista Brasileira de Zootecnia **47**.

Burhans, W., *et al*. (2022). "Invited review: Lethal heat stress: The putative pathophysiology of a deadly disorder in dairy cattle." Journal of Dairy Science **105**(5): 3716-3735.

Chang-Fung-Martel, J., *et al*. (2021). "Negative relationship between dry matter intake and the temperature-humidity index with increasing heat stress in cattle: a global meta-analysis." International journal of biometeorology **65**(12): 2099-2109.

Chauhan, S. S., *et al*. (2021). "Impacts of heat stress on immune responses and oxidative stress in farm animals and nutritional strategies for amelioration." International journal of biometeorology **65**: 1231-1244.

Cheruiyot, E. K., *et al*. (2022). "Improving genomic selection for heat tolerance in dairy cattle: Current opportunities and future directions." Frontiers in Genetics **13**: 894067.

Colditz, I. G. and B. C. Hine (2016). "Resilience in farm animals: biology, management, breeding and implications for animal welfare." Animal Production Science **56**(12): 1961-1983.

Conte, G., *et al*. (2018). "Feeding and nutrition management of heat-stressed dairy ruminants." Italian Journal of Animal Science **17**(3): 604-620.

D'Alton, C. and R. Hofmeyr (2017). "Heat-related illness in the African wilderness." South African Medical Journal **107**(8): 664-668.

Das, R., *et al*. (2016). "Impact of heat stress on health and performance of dairy animals: A review." Veterinary world **9**(3): 260.

Dovolou, E., *et al*. (2023). "Heat Stress: A Serious Disruptor of the Reproductive Physiology of Dairy Cows." Animals **13**(11): 1846.

Dumont, B., *et al*. (2013). "Prospects from agroecology and industrial ecology for animal production in the 21st century." Animal **7**(6): 1028-1043.

Ekine-Dzivenu, C. C., *et al*. (2020). "Evaluating the impact of heat stress as measured by temperature-humidity index (THI) on test-day milk yield of small holder dairy cattle in a sub-Sahara African climate." Livestock science **242**: 104314.

El-Saadony, M. T., *et al*. (2021). "Selenium nanoparticles from Lactobacillus paracasei HM1 capable of antagonizing animal pathogenic fungi as a new source from human breast milk." Saudi Journal of Biological Sciences **28**(12): 6782-6794.

Ellis, F. (1972). "Mortality from heat illness and heat-aggravated illness in the United States." Environmental Research **5**(1): 1-58.

Erell, E., *et al*. (2014). "Effect of high-albedo materials on pedestrian heat stress in urban street canyons." Urban climate **10**: 367-386.

Folke, C., *et al*. (2021). "Our future in the Anthropocene biosphere." Ambio **50**: 834-869.

Gao, S., *et al*. (2019). "Heat stress negatively affects the transcriptome related to overall metabolism and milk protein synthesis in mammary tissue of midlactating dairy cows." Physiological genomics **51**(8): 400-409.

Gauly, M. and S. Ammer (2020). "Challenges for dairy cow production systems arising from climate changes." Animal **14**(S1): s196-s203.

Getabalew, M., *et al*. (2020). "Review on hormonal metabolic adaptations of farm animals." stress **8**(9).

Ghaffari, M. H. (2022). "Developmental programming: Prenatal and postnatal consequences of hyperthermia in dairy cows and calves." Domestic Animal Endocrinology **80**: 106723.

Godde, C. M., *et al*. (2021). "Impacts of climate change on the livestock food supply chain; a review of the evidence." Global food security **28**: 100488.

González-Tokman, D., *et al*. (2020). "Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world." Biological Reviews **95**(3): 802-821.

Gunn, K. M., *et al*. (2019). "Projected heat stress challenges and abatement opportunities for US milk production." PLoS One **14**(3): e0214665.

Gupta, M. and T. Mondal (2021). "Heat stress and thermoregulatory responses of goats: a review." Biological Rhythm Research **52**(3): 407-433.

Habeeb, A. A., *et al*. (2018). "Temperature-humidity indices as indicators to heat stress of climatic conditions with relation to production and reproduction of farm animals." International Journal of Biotechnology and Recent Advances **1**(1): 35-50.

Hahn, G. L. (1999). "Dynamic responses of cattle to thermal heat loads." Journal of animal science **77**(suppl_2): 10-20.

Hassan, M. U., *et al*. (2021). "Heat stress in cultivated plants: Nature, impact, mechanisms, and mitigation strategies—A review." Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology **155**(2): 211-234.

Haubrock, P. J., *et al*. (2021). "The redclaw crayfish: A prominent aquaculture species with invasive potential in tropical and subtropical biodiversity hotspots." Reviews in Aquaculture **13**(3): 1488-1530.

He, S., *et al*. (2018). "Impact of heat stress and nutritional interventions on poultry production." World's Poultry Science Journal **74**(4): 647-664.

Health, E. P. o. A., *et al*. (2022). "Welfare of equidae during transport." EFSA Journal **20**(9): e07444.

Herbut, P., *et al*. (2018). "Environmental parameters to assessing of heat stress in dairy cattle—a review." International journal of biometeorology **62**: 2089-2097.

Herbut, P., *et al*. (2021). "The effects of heat stress on the behaviour of dairy cows–a review." Annals of Animal Science **21**(2): 385-402.

Johnson, J. S. (2018). "Heat stress: impact on livestock well-being and productivity and mitigation strategies to alleviate the negative effects." Animal Production Science **58**(8): 1404-1413.

Joseph, J., *et al*. (2023). "Impacts of climate change on animal welfare." CABI Reviews(2023).

Kays, R., *et al*. (2015). "Terrestrial animal tracking as an eye on life and planet." Science **348**(6240): aaa2478.

Khan, I., *et al*. (2023). "Heat Stress as a Barrier to Successful Reproduction and Potential Alleviation Strategies in Cattle." Animals **13**(14): 2359.

Levine, P. A. (1997). Waking the tiger: Healing trauma: The innate capacity to transform overwhelming experiences, North Atlantic Books.

Levy, O., *et al*. (2019). "Time and ecological resilience: can diurnal animals compensate for climate change by shifting to nocturnal activity?" Ecological Monographs **89**(1): e01334.

López-Gatius, F. (2012). "Factors of a noninfectious nature affecting fertility after artificial insemination in lactating dairy cows. A review." Theriogenology **77**(6): 1029-1041.

Lunn, R. M., *et al*. (2017). "Health consequences of electric lighting practices in the modern world: A report on the National Toxicology Program's workshop on shift work at night, artificial light at night, and circadian disruption." Science of the Total Environment **607**: 1073-1084.

Mader, T. and M. Davis (2004). "Effect of management strategies on reducing heat stress of feedlot cattle: feed and water intake." Journal of animal science **82**(10): 3077-3087.

Manaig, M. M., *et al*. (2022). "Heat Stress Management Strategies using Plant Extracts in Poultry." Intl J Agric Biol **28**: 235-245.

Maqhashu, A. (2019). Characterization and evaluation of reproductive performance in Bapedi sheep breed, University of the Free State.

Marai, I., *et al*. (2007). "Physiological traits as affected by heat stress in sheep—a review." Small ruminant research **71**(1-3): 1-12.

Marai, I. and A. Haeeb (2010). "Buffalo's biological functions as affected by heat stress—A review." Livestock Science **127**(2-3): 89-109.

Mayorga, E., *et al*. (2020). "Biology of heat stress; the nexus between intestinal hyperpermeability and swine reproduction." Theriogenology **154**: 73-83.

McCafferty, D., *et al*. (2017). "Animal thermoregulation: A review of insulation, physiology and behaviour relevant to temperature control in buildings." Bioinspiration & biomimetics **13**(1): 011001.

McMichael, A. J. (2003). Climate change and human health: risks and responses, World Health Organization.

Mota-Rojas, D., *et al*. (2021). "Physiological and behavioral mechanisms of thermoregulation in mammals." Animals **11**(6): 1733.

Mujahid, A., *et al*. (2005). "Superoxide radical production in chicken skeletal muscle induced by acute heat stress." Poultry science **84**(2): 307-314.

Nardone, A., *et al*. (2010). "Effects of climate changes on animal production and sustainability of livestock systems." Livestock Science **130**(1-3): 57-69.

Oke, O., *et al*. (2021). "Environmental stress and livestock productivity in hot-humid tropics: Alleviation and future perspectives." Journal of Thermal Biology **100**: 103077.

Ouellet, V., *et al*. (2020). "Late gestation heat stress in dairy cows: Effects on dam and daughter." Theriogenology **150**: 471-479.

Owuor, S. A. (2021). Origin, Genetic Diversity and HSP 70 Gene Polymorphisms of Domesticated Rabbits of Kenya, JKUAT-IBR.

Padua, J., *et al*. (1997). Effect of high environmental temperature on weight gain and food intake of Suffolk lambs reared in a tropical environment. Proceedings of 5th international symposium, Bloomington, Minnesota, USA.

Pandey, V. (2008). "Management of heat stress in dairy cattle and buffaloes for optimum productivity." Journal of Agrometeorology (Special issue-Part 2) **365**: 368.

Porter, W. P. and D. M. Gates (1969). "Thermodynamic equilibria of animals with environment." Ecological monographs **39**(3): 227-244.

Qiang, J., *et al*. (2022). "Effects of heat stress on follicular development and atresia in Nile tilapia (Oreochromis niloticus) during one reproductive cycle and its potential regulation by autophagy and apoptosis." Aquaculture **555**: 738171.

Rahimi, J., *et al*. (2021). "Heat stress will detrimentally impact future livestock production in East Africa." Nature Food **2**(2): 88-96.

Rahman, M. B., *et al*. (2011). "Scrotal insulation and its relationship to abnormal morphology, chromatin protamination and nuclear shape of spermatozoa in Holstein-Friesian and Belgian Blue bulls." Theriogenology **76**(7): 1246-1257.

Renaudeau, D., *et al*. (2012). "Adaptation to hot climate and strategies to alleviate heat stress in livestock production." Animal **6**(5): 707-728.

Renaudeau, D., *et al*. (2010). "Adaptation to tropical climate and research strategies to alleviate heat stress in livestock production." Advances in Animal Biosciences **1**(2): 378-379.

Renaudeau, D., *et al*. (2012). Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal 6 (5): 707–728.

Ribeiro, E., *et al*. (2018). "Economic aspects of applying reproductive technologies to dairy herds." Animal Reproduction (AR) **9**(3): 370-387.

Ribeiro, M. N., *et al*. (2018). "Physiological and biochemical blood variables of goats subjected to heat stress–a review." Journal of Applied Animal Research **46**(1): 1036-1041.

Riggio, V., *et al*. (2007). "Genetic parameters for milk somatic cell score and relationships with production traits in primiparous dairy sheep." Journal of Dairy Science **90**(4): 1998-2003.

ROBERTSON, G. L. (1977). The regulation of vasopressin function in health and disease. Proceedings of the 1976 Laurentian Hormone Conference, Elsevier.

Robinson, B. R., *et al*. (2023). "Testicular heat stress, a historical perspective and two postulates for why male germ cells are heat sensitive." Biological Reviews **98**(2): 603-622.

Robinson, D. R., *et al*. (2000). "The protein tyrosine kinase family of the human genome." Oncogene **19**(49): 5548-5557.

Roth, Z. (2020). "Reproductive physiology and endocrinology responses of cows exposed to environmental heat stress-Experiences from the past and lessons for the present." Theriogenology **155**: 150-156.

Rovelli, G., *et al*. (2020). "The genetics of phenotypic plasticity in livestock in the era of climate change: a review." Italian Journal of Animal Science **19**(1): 997-1014.

Salo, D. C., *et al*. (1991). "HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise." Free Radical Biology and Medicine **11**(3): 239-246.

Sammad, A., *et al*. (2020). "Dairy cow reproduction under the influence of heat stress." Journal of animal physiology and animal nutrition **104**(4): 978-986.

Sammad, A., *et al*. (2020). "Nutritional physiology and biochemistry of dairy cattle under the influence of heat stress: Consequences and opportunities." Animals **10**(5): 793.

Sarangi, S. (2018). "Adaptability of goats to heat stress: A review." The Pharma Innovation Journal **7**(4): 1114-1126.

Schumacher, M., *et al*. (2022). "Fat deposition and fat effects on meat quality—A Review." Animals **12**(12): 1550.

Sejian, V., *et al*. (2021). "Heat stress and goat welfare: Adaptation and production considerations." Animals **11**(4): 1021.

Seré, C., *et al*. (1996). World livestock production systems, Food and Agriculture Organization of the United Nations.

Silanikove, N. (2000). "Effects of heat stress on the welfare of extensively managed domestic ruminants." Livestock production science **67**(1-2): 1-18.

Soravia, C., *et al*. (2021). "The impacts of heat stress on animal cognition: Implications for adaptation to a changing climate." Wiley Interdisciplinary Reviews: Climate Change **12**(4): e713.

Summer, A., *et al*. (2019). "Impact of heat stress on milk and meat production." Animal Frontiers **9**(1): 39-46.

Thornton, P. K., *et al*. (2009). "The impacts of climate change on livestock and livestock systems in developing countries: A review of what we know and what we need to know." Agricultural systems **101**(3): 113-127.

Walsh, S., *et al*. (2011). "A review of the causes of poor fertility in high milk producing dairy cows." Animal reproduction science **123**(3-4): 127-138.

Wang, G., *et al*. (2021). "Effects of heat stress on gut-microbial metabolites, gastrointestinal peptides, glycolipid metabolism, and performance of broilers." Animals **11**(5): 1286.

Wasti, S., *et al*. (2020). "Impact of heat stress on poultry health and performances, and potential mitigation strategies." Animals **10**(8): 1266.

West, J. W. (2003). "Effects of heat-stress on production in dairy cattle." Journal of Dairy Science **86**(6): 2131-2144.

Wolfenson, D. and Z. Roth (2019). "Impact of heat stress on cow reproduction and fertility." Animal Frontiers **9**(1): 32-38.

Yadav, M. R., *et al*. (2022). "Impacts, tolerance, adaptation, and mitigation of heat stress on wheat under changing climates." International Journal of Molecular Sciences **23**(5): 2838.

Yang, L. H., *et al*. (2021). "The complexity of global change and its effects on insects." Current Opinion in Insect Science **47**: 90-102.

Ye, Y.-H., *et al*. (2014). "Physiology of Gametogenesis." Gamete and Embryo-fetal Origins of Adult Diseases: 1-38.

Yezli, S. (2023). "Risk factors for heat-related illnesses during the Hajj mass gathering: an expert review." Reviews on environmental health **38**(1): 33-43.

Zhang, F., *et al*. (2020). "Effects of propylene glycol on negative energy balance of postpartum dairy cows." Animals **10**(9): 1526.

Zune, M., *et al*. (2020). "The vulnerability of homes to overheating in Myanmar today and in the future: A heat index analysis of measured and simulated data." Energy and Buildings **223**: 110201

About The Authors

Arooj FATIMA earned Bachelor in microbiology from Cholistan University of Veterinary and Animal Sciences (CUVAS) Bahawalpur Pakistan. Currently she is doing M.phil in Microbiology from her parent institute (CUVAS) Bahawalpur Pakistan. Her research interest in Microbial genetics and food quality and its application. She has published more than 3 articles in national and international journals.
E-mail: aroojfatima31202@gmail.com, ORCID:0000-0002-4709-4177

Zarva AHSAN earned Doctor of Veterinary Medicine (DVM) from Riphah College of Veterinary Sciences Lahore Pakistan. She is doing M.phil in livestock management from Cholistan University Of Veterinary and Animal Sciences (CUVAS) Bahawalpur, Pakistan. Her research interests are managemental practices in livestock farm, milk quality, milk supply chain and heat stress.
E-mail: dr.zarva.ahsan@gmail.com, ORCID: 0009-0004-3473-7454

Omazia NASIR a dedicated research scholar, is currently pursuing her MPhil in Microbiology at Quaid-e-Azam University Islamabad, Pakistan. She completed her Bachelor's Degree in Applied Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur, Pakistan. Her profound research interests span Microbiology, Molecular Biology, and Environmental Microbiology. Notably, she has contributed to academia by publishing review papers in national and international journals. Outside the scope of publications, Omazia actively engages in academic discourse as a speaker and participant at both national and international conferences. Her commitment to continuous learning is evident through her participation in numerous workshops tailored to her research expertise.
Email: omazia106@gmail.com, ORCID: 0000-0002-5985-409X

Dr. Faiz-ul HASSAN, received his BSc. (Hons) Animal Husbandry in 2001 from University of Agriculture, Faisalabad with distinction (Gold Medal). After that he secured his MSc (Hons) in 2004 and PhD degree in 2012 in the field of Animal Breeding and Genetics from same university. Recently, Dr Hassan has completed two-year postdoctrate (2018-2020) in field of Nutri-genomics" under 'Talented Young Scientist' fellowship of the Ministry of Science and Technology, China. Dr. Hassan recently (December, 2022) joined as a Professor in Department of Breeding and Genetics, Cholistan University of Veterinary and Animals Sciences, Bahawalpur. He has published more than 70 peer reviewed articles in well-known SCI international journals with a cumulative impact factor of >215. He has been keenly involved in National Centre for Livestock Breeding, Genetics and Genomics (NCLBGG) sub-centre UAF for genome wide association studies for milk productions traits in Nili-Ravi buffaloes.
Email: faizabg@gmail.com, ORCID: 0000-0002-8781-0510

Dr. Rana Muhammad BİLAL is a PhD in Animal nutrition with a major research in poultry nutrition. He has also earned his M phil by working on rumen digestion kinetics. Currently, he working as associate professor in animal nutrition department in Cholistan university of veterinary and animal sciences(CUVAS) Bahawalpur Pakistan. His research area is primarily on the poultry and nutriton by exploring novel feed ingrendusts that may replace AGPs. Moreover, he has also feed manufacturing experience . He has publish various paper in peer review journals.
E-mail: Muhammad.bilal@cuvas.edu.pk, ORCID: 0000-0002-1760-0841

Dr. Muhammad JUNAİD serves as a lecturer at University of Veterinary and Animal Sciences in the Department of Dairy Technology. His academic record include DVM, M.Phil., and Ph.D in Dairy Technology. His expertise encompasses product development, value-added dairy products, and dairy value chain management. With 14 research papers published in national and international journals, Dr. Muhammad Junaid has demonstrated a commitment to advancing knowledge in his field. His professional contributions extend to the Livestock & Dairy Development Department, Government of Punjab, where he serves as the member of Livestock Advisory Committee. Additionally, since 2017, he has contributed his expertise to the Punjab Food Authority as a valuable member of the scientific panel.
Email: mjunaid@uvas.edu.pk, ORCID: 0000-0003-1493-4070

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkiye. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 68 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.
E-mail: msafdar@cuvas.edu.pk , ORCID: 0000 0002 3720 2090

Dr. Umair YOUNAS received his DVM and Mphil (Livestock Management) degree from the University of Veterinary and Animal Sciences, Lahore. During M.Phil, Dr. Umair got training from PTC+ institute (Netherlands) on dairy farm practices and milk processing. After completing M.Phil., Dr. Umair secured his Ph.D (Livestock

Management) degree from same university along research work from the University of Florida as Sabbatical. Dr. Umair younas has vast experience in academics and since 2008 he has been working on various academic posts. Dr. Umair has more than 35 publications and Currently, he is working as Head of Department (Livestock Management) in CUVAS as well as ORIC member and Deputy director QEC (Quality Enhancement Cell).
Email: umairyounas@cuvas.edu.pk, ORCID: 0000-0002-5107-9391

To Cite This Chapter:
Fatima, A., et al. (2023). Biological Attributes of Livestock as Affected by Heat Stress in Tropical and Sub-tropical Regions. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 409-431). ISRES Publishing

BREAKTHROUGHS IN DISEASE PREVENTION AND TREATMENT

Muhammad RAMEEZ
Aman Ullah KHAN
Ali RAZA

Introduction

Disease prevention and treatment stand at the forefront of global health priorities, serving as the cornerstone of enhanced well-being and improved quality of life for individuals and communities worldwide. In an era of remarkable scientific progress and technological advancement, the field of healthcare has witnessed transformative breakthroughs that have redefined our approach to combating diseases.

This chapter delves into the monumental strides achieved in disease prevention and treatment, casting a spotlight on the innovative methodologies, strategies, and technologies that have reshaped the landscape of global health. As we navigate the complexities of modern healthcare, understanding these breakthroughs becomes not just a matter of academic interest but a crucial element in safeguarding public health. Within these pages, we will explore the historical milestones that have paved the way for our present-day understanding of disease prevention. From the remarkable success stories of vaccination campaigns to the nuanced approaches of targeted interventions, the impact of these efforts resonates across generations. Likewise, the advent of precision medicine and personalized therapies has revolutionized disease treatment, steering us toward individualized care based on genetic, environmental, and lifestyle factors.

As we embark on this journey, we shall delve into the integration of cutting-edge fields like genomics and artificial intelligence, uncovering how they are revolutionizing disease diagnostics and therapy development. The ethical considerations surrounding these breakthroughs shall not be overlooked, as we examine the need for equitable access and responsible innovation.
In an era of rapid change, we must also peer into the future, exploring emerging trends such as gene editing and immunotherapy. By fostering a deepened understanding of these breakthroughs, we can collectively contribute to the advancement of global health and the mitigation of disease burdens. This chapter invites us to embrace knowledge as a force for positive change, propelling us toward a healthier and more equitable world.

Historical Milestones in Disease Prevention

Throughout history, humanity has waged a tireless battle against infectious diseases, seeking ways to prevent their spread and mitigate their devastating impact. Key among these efforts is the triumphs of vaccination campaigns, which have not only shaped the course of public health but also paved the way for modern disease prevention strategies.

One of the most iconic success stories is the eradication of smallpox. In 1796, Edward Jenner's pioneering work laid the foundation for immunization by introducing the concept of vaccination (Plotkin, 2012). His discovery that exposure to cowpox could confer immunity to smallpox set the stage for a global campaign. Over a century later, in 1980, the World Health Assembly declared smallpox eradicated – a monumental

achievement that showcased the power of vaccination to eliminate a disease that had plagued humanity for centuries.

Similarly, the introduction of the polio vaccine marked a watershed moment in disease prevention. Polio, a paralyzing and often deadly disease, once wreaked havoc on communities worldwide. The development of effective vaccines in the mid-20th century, including the oral polio vaccine, spearheaded by Albert Sabin, and the inactivated polio vaccine, championed by Jonas Salk, led to a dramatic reduction in polio cases (Rose, 2016). While complete global eradication remains a challenge, these vaccines have significantly diminished polio's prevalence and impact.

Vaccination campaigns have also transformed the landscape of diseases like measles, mumps, and rubella. The introduction of the measles vaccine in the 1960s marked a turning point in disease prevention (Hendriks, 2013). Before its widespread use, measles was a major cause of childhood mortality. The combined measles, mumps, and rubella (MMR) vaccine further bolstered these efforts, leading to substantial declines in the incidence of these once-common diseases.

The impact of these vaccination campaigns reverberates across generations. By interrupting the transmission of pathogens, vaccines not only protect individuals but also contribute to herd immunity, safeguarding vulnerable populations unable to receive vaccines. The success of these efforts underscores the potential for disease prevention to reshape the global health landscape.

In sum, historical milestones in disease prevention, epitomized by successful vaccination campaigns, have profoundly shaped the course of human health. These victories remind us that by harnessing scientific innovation, collaborative efforts, and a commitment to public health, we can achieve remarkable feats in the pursuit of disease eradication and reduction.

Innovative Strategies for Disease Prevention

In the quest to further advance disease prevention, modern healthcare has embraced innovative strategies that encompass both targeted interventions and large-scale public health campaigns. These approaches leverage scientific knowledge, technology, and community engagement to mitigate the impact of diseases that have long posed significant threats to global well-being.

Targeted Interventions: Precision in Prevention

One of the hallmarks of modern disease prevention is the move toward precision and specificity. Rather than adopting a one-size-fits-all approach, targeted interventions focus on populations at the highest risk, maximizing the impact of preventive measures (Ammar, 2006). This approach is particularly effective in the control of vector-borne diseases like malaria. Through the distribution of insecticide-treated bed nets, indoor residual spraying, and prompt diagnosis and treatment, malaria incidence has been significantly reduced in many endemic regions. This precision targeting not only optimizes resource allocation but also accelerates progress toward disease elimination.

Public Health Campaigns: A Collaborative Endeavor

Public health campaigns play a pivotal role in raising awareness, mobilizing communities, and effecting behavior change on a large scale. A standout example is

the global effort to combat HIV/AIDS. Collaborative initiatives have promoted safe sex practices, increased access to antiretroviral therapy, and destigmatized the disease. These campaigns have led to a decline in new infections and improved quality of life for those living with HIV (Cheever, 2011). The success of these efforts demonstrates the power of coordinated action and underscores the importance of comprehensive, multifaceted approaches to disease prevention.

Case Studies in Successful Prevention Strategies

Malaria Control: A Multi-Faceted Approach

Malaria, a mosquito-borne disease, has plagued humanity for centuries. However, innovative strategies have brought about remarkable progress. In addition to targeted interventions like bed nets and indoor spraying, community engagement, and education have been instrumental. By involving local communities in the implementation and maintenance of prevention measures, programs have achieved greater sustainability and impact (Alshuwaikhat, 2008). Countries like Rwanda and Ethiopia have witnessed substantial reductions in malaria cases through a combination of vector control, improved diagnosis, and community involvement.

HIV Prevention: Breaking Barriers

The global response to HIV/AIDS serves as a model for successful prevention strategies. Beyond medical interventions, such as pre-exposure prophylaxis (PrEP) and treatment as prevention (TasP), initiatives have sought to address social and structural barriers (Bernays, 2021). These include advocating for the rights of marginalized populations, reducing stigma, and providing comprehensive sexual education. Countries like Thailand and Uganda have demonstrated that a combination of medical breakthroughs and community-based approaches can lead to substantial declines in new HIV infections (Kurth, 2011).

The landscape of disease prevention continues to evolve, shaped by a dynamic interplay of science, technology, and community engagement. The innovative strategies discussed here represent a departure from traditional approaches, emphasizing precision, collaboration, and comprehensive action. As we navigate the challenges posed by emerging infectious diseases and changing health landscapes, the lessons learned from successful prevention strategies serve as beacons of hope, guiding us toward a healthier and more resilient future.

Precision Medicine and Personalized Therapies

In an era characterized by unprecedented advances in medical research and technology, the paradigm of healthcare has shifted from a generalized approach to one that embraces the individuality of each patient. This transformation is encapsulated in the concept of precision medicine, a revolutionary approach to disease treatment that tailors interventions to the unique characteristics of each individual. Precision medicine recognizes that no two individuals are alike, and this understanding has paved the way for a new frontier in healthcare (Sneha, 2016).

The Essence of Precision Medicine

At its core, precision medicine seeks to unravel the intricacies of diseases by delving into the genetic, environmental, and lifestyle factors that influence an individual's health trajectory (Farina, 2023). By harnessing the power of genomics, proteomics, and other -omics technologies, healthcare professionals gain insights into the molecular underpinnings of diseases (Yao, 2014). This knowledge allows for the

identification of specific mutations, biomarkers, and molecular pathways associated with particular conditions.

Genetic, Environmental, and Lifestyle Factors in Personalized Therapies

The journey toward personalized therapies begins with a thorough understanding of genetic makeup. Genetic information, gleaned through techniques like DNA sequencing, provides a blueprint of an individual's susceptibility to certain diseases and their potential responses to various treatments (Rexroad, 2019). Genetic factors may determine how an individual metabolizes medications, their risk of developing specific diseases, and their likelihood of experiencing adverse reactions to treatments.

Beyond genetics, environmental influences play a pivotal role. Exposures to toxins, pollutants, and lifestyle choices can modulate gene expression and contribute to disease development (Tiffon, 2018). Consider lung cancer, for instance. Precision medicine takes into account factors like smoking history, exposure to carcinogens, and genetic predispositions to tailor interventions that maximize the chances of successful treatment and recovery.

Lifestyle factors round out the trifecta of personalized therapies. Diet, exercise, stress levels, and social determinants of health collectively impact disease progression and treatment outcomes (Wilkinson, 2003). For instance, in managing chronic conditions like diabetes, precision medicine considers not only genetic factors influencing insulin sensitivity but also the patient's dietary preferences and activity levels.

Promising Examples of Precision Medicine

Precision medicine has yielded promising results in several diseases, offering a glimpse of its potential impact on healthcare. In oncology, the use of targeted therapies based on genetic mutations has revolutionized cancer treatment. For instance, drugs like imatinib have transformed the prognosis of chronic myeloid leukemia by specifically targeting the abnormal protein driving the disease (Manley, 2020).

In the realm of cardiovascular health, genetic testing has enabled the identification of individuals at heightened risk of inherited cardiac conditions. Armed with this knowledge, medical professionals can implement preventative measures and personalized treatment plans to mitigate the risk of adverse cardiac events.

Neurological disorders, too, have witnessed the dawn of precision medicine. In treating epilepsy, genetic testing aids in identifying the most effective antiepileptic medications based on an individual's genetic makeup (Löscher, 2017). This approach minimizes the trial-and-error process associated with drug selection, enhancing patient well-being.

Precision medicine marks a transformative departure from traditional healthcare paradigms. By embracing the intricacies of genetics, environment, and lifestyle, it presents a new frontier in disease treatment and management. Through personalized therapies that account for individual variability, precision medicine empowers healthcare professionals to optimize interventions, improve patient outcomes, and usher in an era of healthcare that is as unique as each individual it serves.

Integration of Genomics and Diagnostics

The intersection of genomics and modern healthcare has ushered in a new era of disease diagnostics and treatment planning. Genomics, the study of an individual's complete set of DNA, has revolutionized our understanding of diseases at the molecular level (Hasin, 2017). This has not only enabled more accurate diagnoses but has also paved the way for tailored treatment strategies that leverage genetic information to improve patient outcomes.

Genomics: Decoding the Blueprint of Life

The human genome contains a vast wealth of information that holds the key to unraveling the mysteries of diseases. By sequencing an individual's DNA, scientists and healthcare professionals can identify specific genetic variations and mutations associated with diseases (Dewey, 2014). This knowledge offers insights into disease susceptibility, progression, and response to treatment, thus transforming the landscape of medical care.

Identifying Risk Factors and Predicting Susceptibility

Genomic information plays a crucial role in identifying individuals at heightened risk of developing certain diseases. Genetic risk factors, often involving the interplay of multiple genes, contribute to the predisposition of conditions such as cardiovascular diseases, diabetes, and certain cancers (Ordovás, 2010). Armed with this knowledge, healthcare providers can offer personalized preventive strategies, early screening, and interventions to mitigate the risk.

Predicting disease susceptibility is a pivotal application of genomics. For example, in breast cancer, mutations in the BRCA1 and BRCA2 genes significantly increase the risk of developing the disease (Parmigiani, 1998). Genetic testing empowers individuals with this information, enabling them to make informed decisions about risk reduction strategies and potential early interventions.

Guiding Treatment Decisions through Genomics

Perhaps one of the most transformative aspects of genomics is its influence on treatment decisions. By analyzing an individual's genetic profile, healthcare professionals can tailor therapies to maximize effectiveness and minimize adverse reactions. This approach is exemplified in the field of pharmacogenomics, which studies how genetic variations impact an individual's response to medications.

The integration of genomics into cancer treatment exemplifies this paradigm shift. In cases of non-small cell lung cancer, specific genetic mutations can be targeted with precision therapies like tyrosine kinase inhibitors (Zhu, 2020). These treatments are designed to inhibit the aberrant signaling pathways driving cancer, resulting in improved outcomes and reduced side effects compared to traditional chemotherapy.

Case Studies: Breakthroughs in Genomic-Based Treatments

Cystic Fibrosis: Personalized Approaches

Cystic fibrosis, a genetic disorder affecting the respiratory and digestive systems, has benefited significantly from genomics. Specific mutations in the CFTR gene lead to faulty chloride channel function, causing mucus buildup and impaired lung function (Döring, 2009). Genomic analysis allows for the identification of individual mutations, guiding treatment decisions. For instance, in patients with specific mutations, modulator therapies like ivacaftor can improve lung function by targeting the underlying genetic defect (Skov, 2019).

Precision Oncology: Transforming Cancer Care

Precision oncology has harnessed the power of genomics to revolutionize cancer treatment. In melanoma, for instance, the presence of BRAF gene mutations prompted the development of targeted therapies like vemurafenib, which inhibits the abnormal BRAF signaling driving cancer (Czarnecka, 2020). Similarly, HER2-positive breast cancer is treated with targeted therapies like trastuzumab, which specifically targets the HER2 protein overexpressed in these tumors (Oh, 2020).

The integration of genomics into diagnostics and treatment planning has unveiled a new frontier in healthcare, one where individualized care is driven by genetic insights. By leveraging the power of genomics to identify risk factors, predict susceptibility, and guide treatment decisions, medical professionals can offer tailored interventions that maximize efficacy and minimize adverse effects. As the field of genomics continues to advance, it holds the promise of unraveling the complexities of diseases, unlocking novel treatment avenues, and empowering patients with knowledge that can transform their healthcare journeys.

Artificial Intelligence in Disease Management

The fusion of artificial intelligence (AI) with healthcare has ushered in a new era of disease management, revolutionizing the way we diagnose, treat, and understand medical conditions. AI's capacity to process vast amounts of data, identify patterns, and make predictions has breathed new life into disease management, offering transformative capabilities that were once relegated to the realm of science fiction (Lawry, 2020).

AI's Role in Disease Diagnosis and Treatment

AI's impact on disease diagnosis and treatment is profound. In the realm of diagnostics, AI-driven algorithms analyze medical images with remarkable precision. This enhances the accuracy of radiological interpretations, allowing for early detection and more informed clinical decisions. In cardiology, for instance, AI can analyze cardiac images to identify subtle abnormalities that might elude human observers (Zhou, 2021). Such capabilities empower clinicians to intervene at earlier stages, improving patient outcomes.

Predictive modeling is another potent application of AI. By assimilating patient data and historical records, AI algorithms can forecast disease trajectories, enabling healthcare providers to anticipate complications and optimize treatment plans (Ahmed, 2020). In diabetes management, AI can predict blood glucose fluctuations, helping patients and healthcare teams adapt insulin regimens to maintain optimal glycemic control (Shifrin, 2020).

AI-Powered Applications: Image Analysis, Predictive Modeling, and Drug Discovery

Image Analysis: AI excels in analyzing medical images, from X-rays and MRIs to histopathology slides. Machine learning algorithms learn from vast datasets, detecting subtle patterns indicative of diseases. For instance, AI-driven mammography analysis can detect early signs of breast cancer, enhancing the accuracy of screenings (Tran, 2021). Similarly, AI's prowess in analyzing retinal images aids in diagnosing diabetic retinopathy, a leading cause of blindness (Cheng, 2020).

Predictive Modeling: AI's predictive prowess extends to identifying risk factors and disease progression. In cardiovascular health, AI can predict heart disease risk by assessing factors like age, lifestyle, and genetic predisposition (Lawry, 2020). Furthermore, AI algorithms can predict patient deterioration in intensive care units, providing critical early warnings and improving timely interventions.
Drug Discovery: AI's capacity to sift through immense datasets has accelerated drug discovery. AI-driven algorithms analyze biological data to identify potential drug candidates and predict their efficacy (Mak, 2022). This expedites the identification of molecules that could target diseases like cancer, Alzheimer's, and rare genetic disorders.

AI-Driven Breakthroughs in Medical Fields

Radiology: In radiology, AI assists in detecting anomalies that might elude human radiologists. For instance, AI algorithms can analyze lung CT scans to detect early signs of lung cancer, enhancing survival rates through early intervention (Tran, 2021).
Pathology: In pathology, AI streamlines the analysis of tissue samples. Algorithms can identify cancerous cells, quantify tumor aggressiveness, and guide treatment decisions (Mandair, 2023).
Neurology: In neurology, AI aids in diagnosing neurological conditions like Parkinson's and Alzheimer's disease (Gopinath, 2022). Algorithms analyze brain scans, identifying structural and functional anomalies.
Genomics: In genomics, AI analyzes vast genomic datasets to identify disease-causing mutations and predict disease susceptibility. This aids in personalized treatment strategies.

The integration of artificial intelligence into disease management transcends conventional boundaries, offering capabilities that enhance precision, speed, and efficacy. From improving disease diagnosis through image analysis and predictive modeling to accelerating drug discovery, AI has penetrated diverse medical fields, leaving an indelible mark on healthcare (Lawry, 2020). As AI continues to evolve, it holds the promise of unlocking novel insights, amplifying human expertise, and transforming the way we approach diseases, ultimately ushering in an era of more informed, effective, and personalized medical care.

Ethical Considerations and Equitable Access

As breakthroughs in disease management continue to reshape the healthcare landscape, they bring with them a host of ethical challenges that warrant thoughtful consideration. These challenges range from ensuring fair access to emerging treatments to navigating the ethical dilemmas posed by cutting-edge technologies. Addressing these concerns is not only essential for maintaining ethical integrity but also for promoting equitable and responsible healthcare practices.

Ethical Challenges in Breakthroughs

One of the foremost ethical challenges revolves around the potential disparities that could emerge from breakthroughs. As novel treatments and interventions become available, there is a risk that these advancements might disproportionately benefit privileged populations, further exacerbating existing health inequities (Pérez-Stable, 2019). Moreover, the pressure to adopt the latest technologies could inadvertently marginalize conventional and proven methods, hindering access for those who are unable to access or afford cutting-edge treatments.

Equitable Access: A Moral Imperative

Equitable access to breakthroughs in disease management is not merely an ethical aspiration—it is a moral imperative. As the healthcare community advances, it must be guided by the principle that every individual, regardless of their socioeconomic background, deserves access to high-quality care. This becomes even more pressing in the case of underserved populations, who are often at the greatest risk of disease burden and least likely to have access to advanced treatments.

Recognizing the importance of equitable access, strategies should be employed to ensure that breakthroughs reach those who need them most. This might involve targeted outreach and education programs, partnerships with community organizations, and policy initiatives that prioritize underserved populations (Horst, 2017). By actively working to bridge the gap between advancements and accessibility, the healthcare community can demonstrate its commitment to social justice and responsible medical practice.

Balancing Technological Advancement and Ethical Responsibility

The rapid pace of technological advancement in healthcare introduces ethical complexities that require careful navigation. On one hand, cutting-edge technologies have the potential to revolutionize disease management and improve patient outcomes. On the other hand, ethical concerns arise when innovations outpace the capacity for informed decision-making, potentially exposing patients to unknown risks.

Striking a balance between technological progress and ethical responsibility involves a multi-pronged approach. Rigorous research and testing are imperative to ensure that innovations are safe, effective, and ethically sound before widespread adoption (Earl, 2019). Additionally, clear communication with patients and informed consent processes become even more critical as the complexity of treatments and interventions increases.

Case in Point: CRISPR and Genetic Editing

The ethical implications of breakthroughs are perhaps nowhere more pronounced than in the field of genetic editing, exemplified by CRISPR technology (Deary, 2019). While CRISPR holds the potential to cure genetic diseases, the ability to manipulate the human genome raises profound ethical questions. Balancing the promise of eliminating hereditary disorders with the potential for unintended consequences and ethical transgressions underscores the need for rigorous ethical oversight and public discourse.

As the landscape of disease management transforms, it is incumbent upon the healthcare community to navigate the ethical terrain with care and consideration. Equitable access, responsible innovation, and a commitment to patient well-being must guide decision-making in the face of breakthroughs. By addressing ethical challenges head-on and striving for inclusivity and fairness, the healthcare community can forge a path that leads to better health outcomes for all, while upholding the highest ethical standards.

Emerging Trends and Future Directions

The landscape of disease prevention and treatment is in a state of constant evolution, driven by scientific discoveries, technological innovations, and a commitment to improving global health outcomes. As we peer into the future, several exciting trends

are poised to shape the way we approach diseases, offering both challenges and opportunities that will redefine healthcare as we know it.

Gene Editing: Precision Redefined

Gene editing technologies, particularly CRISPR-Cas9, are poised to revolutionize disease treatment at the genetic level (acinto, 2020). The ability to precisely modify DNA sequences holds promise for correcting genetic mutations responsible for hereditary diseases. From sickle cell anemia to cystic fibrosis, gene editing offers the potential to transform treatment strategies from managing symptoms to addressing the root cause (Wailoo, 2006). However, this innovation also raises ethical concerns and prompts discussions on the boundaries of genetic manipulation and unintended consequences.

Immunotherapy: Unleashing the Immune System

Immunotherapy has emerged as a groundbreaking approach to cancer treatment. By harnessing the body's immune system to target cancer cells, therapies like immune checkpoint inhibitors and CAR-T cell therapies have demonstrated remarkable success in some patients (Guedan, 2019). The personalized nature of immunotherapy aligns with the principles of precision medicine, although challenges remain in predicting patient responses and managing potential side effects. As research continues, immunotherapy could extend its reach to other diseases beyond cancer.

Digital Health Technologies: Empowering Patients

The integration of digital health technologies, including wearable devices, telemedicine, and health apps, promises to empower patients and transform disease management (Fan, 2022). These technologies facilitate real-time monitoring, personalized treatment plans, and remote patient-doctor interactions. Telemedicine, in particular, has gained prominence, offering convenient access to healthcare services, especially in remote or underserved areas (Chang, 2021). However, ensuring data privacy, addressing the digital divide, and maintaining the quality of virtual care remain critical considerations.

Challenges and Opportunities on the Horizon

Ethical Considerations: As science pushes the boundaries of what is possible, ethical questions become more complex. Balancing innovation with potential risks, like those posed by gene editing, requires robust ethical frameworks and international collaboration to ensure responsible scientific progress.

Equitable Access: The promise of cutting-edge treatments must be matched by a commitment to equitable access. Ensuring that breakthroughs are available and affordable for all, regardless of geographical location or socioeconomic status, remains a challenge that demands collaborative efforts from governments, industries, and the global health community (Lawry, 2020).

Regulatory Hurdles: Bringing novel treatments to market involves navigating intricate regulatory landscapes. Streamlining the regulatory process while maintaining rigorous safety standards is essential to ensure that breakthroughs reach patients promptly without compromising their well-being.

Integration of Data: The abundance of data generated by genomics, digital health, and other technologies presents opportunities for insights and innovation. However,

integrating and analyzing these diverse datasets poses challenges in terms of data privacy, interoperability, and ensuring data accuracy.

The emerging trends in disease prevention and treatment reflect a future where science and technology converge to redefine healthcare possibilities. Gene editing holds the promise of rewriting the genetic code of diseases, while immunotherapy harnesses the body's defenses to combat illness (Walters, 1997). Digital health technologies are democratizing access to care, placing patients at the center of their healthcare journeys.

Yet, with great promise comes great responsibility. The challenges of ethical dilemmas, equitable access, regulatory compliance, and data integration must be navigated with care. As we stand at the intersection of breakthroughs and ethics, collaboration between scientists, healthcare professionals, policymakers, and the public will be pivotal in shaping a future where innovation and ethical considerations harmoniously coexist. By embracing these trends and addressing their challenges, we have the potential to usher in an era of healthcare that transcends today's limitations and enhances the well-being of individuals and communities worldwide.

Summary

The chapter "Breakthroughs in Disease Prevention and Treatment" has illuminated the transformative advancements that have reshaped the landscape of healthcare. From historical milestones in disease prevention to the integration of genomics, artificial intelligence, and personalized therapies, this journey has showcased the power of innovation to enhance disease management. Ethical considerations, equitable access, and the balance between progress and responsibility have underscored the complex terrain we navigate.

As we conclude, it is evident that the pursuit of improved global health outcomes requires ongoing dedication to research, collaboration, and innovation. The successes highlighted herein stand as testaments to the potential of human ingenuity, inspiring us to push boundaries and challenge norms in our quest for better healthcare solutions.

The path forward demands a collective call to action. Let us continue to champion equitable access, ensuring that breakthroughs benefit all individuals, regardless of their background or location. Let us nurture ethical mindfulness as we explore the frontiers of technology and medicine, always placing the well-being of patients at the forefront. As we shape the future of disease management, our shared commitment to research-driven progress and compassionate care will define our success in improving global health outcomes for generations to come.

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

Acknowledgments

The authors concede the support of the University of Veterinary & Animal Sciences Lahore, Sub-Campus Jhang, Pakistan, during the write-up.

Conflict of Interest

The authors declare no conflict of interest.

References

Acinto, F. V. (2020). CRISPR/Cas9-mediated genome editing: From basic research to translational medicine. . Journal of cellular and molecular medicine, 24(7),, 3766-3778.

Ahmed, Z. M. (2020). Artificial intelligence with multi-functional machine learning platform development for better healthcare and precision medicine. Database, 2020, baaa010.v. Database, , baaa010.

Alshuwaikhat, H. M. (2008). An integrated approach to achieving campus sustainability: assessment of the current campus environmental management practices. Journal of cleaner production, 16(16),, 1777-1785.

Ammar, A. &. (2006). ne size fits all?: Recasts, prompts, and L2 learning. . Studies in second language acquisition, 28(4), , 543-574.

Bernays, S. B. (2021). Remaking HIV prevention: The promise of TasP, U= U and PrEP. Remaking HIV prevention in the 21st century: The promise of TasP, U= U and PrEP, , 1-18.

Chang, J. E. (2021). Rapid transition to telehealth and the digital divide: implications for primary care access and equity in a post-COVID era. . The Milbank Quarterly, 99(2), , 340-368.

Cheever, L. W. (2011). A model federal collaborative to increase patient access to buprenorphine treatment in HIV primary care. JAIDS Journal of Acquired Immune Deficiency Syndromes, 56, , S3-S6.

Cheng, C. Y. (2020). Big data in ophthalmology. . The Asia-Pacific Journal of Ophthalmology, 9(4), , 291-298.

Czarnecka, A. M. (2020). Targeted therapy in melanoma and mechanisms of resistance. International Journal of Molecular Sciences, 21(13), , 4576.

Deary, A. J. (2019). CRISPR V Culture. . UC Merced Undergraduate Research Journal, 11(1).

Dewey, F. E. (2014). Clinical interpretation and implications of whole-genome sequencing. . Jama, 311(10), , 1035-1045.1035-1045.

Döring, G. &. (2009). Cystic fibrosis and innate immunity: how chloride channel mutations provoke lung disease. . Cellular microbiology, 11(2), , 208-216.

Earl, J. (2019). Innovative practice, clinical research, and the ethical advancement of medicine. . The American Journal of Bioethics, 19(6),, 7-18.

Fan, K. &. (2022). Mobile health technology: a novel tool in chronic disease management. Intelligent Medicine, 2(1), , 41-47.

Farina, L. (2023). Systems Precision Medicine: Putting the Pieces Back Together. . Systems, 11(7), , 367.

Gopinath, N. (2022). Artificial intelligence and neuroscience: An update on fascinating relationships. Process Biochemistry.

Guedan, S. R. (2019). Emerging cellular therapies for cancer. . Annual review of immunology, 37, , 145-171.

Hasin, Y. S. (2017). Multi-omics approaches to disease. Genome biology, 18(1),, 1-15.

Hendriks, J. &. (2013). Measles vaccination before the measles-mumps-rubella vaccine. . American journal of public health, 103(8), , 1393-1401.

Horst, M. M. (2017). The intersection of planning, urban agriculture, and food justice: A review of the literature. . Journal of the American Planning Association, 83(3), , 277-295.

Kurth, A. E. (2011). Combination HIV prevention: significance, challenges, and opportunities. . Current Hiv/aids Reports, 8, , 62-72.

Lawry, T. (2020). AI in health: a leader's guide to winning in the new age of intelligent health systems. . CRC Press.
Löscher, W. (2017). Animal models of seizures and epilepsy: past, present, and future role for the discovery of antiseizure drugs. . Neurochemical research, 42, , 1873-1888.
Mak, K. K. (2022). Success stories of AI in drug discovery-where do things stand? Expert opinion on drug discovery, 17(1), , 79-92.
Mandair, D. R.-F. (2023). Biological insights and novel biomarker discovery through deep learning approaches in breast cancer histopathology. NPJ Breast Cancer, 9(1), , 21.
Manley, P. W.-J. (2020). The specificity of asciminib, a potential treatment for chronic myeloid leukemia, as a myristate-pocket binding ABL inhibitor and analysis of its interactions with mutant forms of BCR-ABL1 kinase. . Leukemia research, 98, , 106458.
Oh, D. Y. (2020). HER2-targeted therapies—a role beyond breast cancer. . Nature reviews Clinical oncology, 17(1),, 33-48.
Ordovás, J. M. (2010). Epigenetics and cardiovascular disease. . Nature Reviews Cardiology, 7(9), , 510-519.
Parmigiani, G. B. (1998). Determining carrier probabilities for breast cancer–susceptibility genes BRCA1 and BRCA2. The American Journal of Human Genetics, 62(1), , 145-158.
Pérez-Stable, E. J.-F. (2019). Leveraging advances in technology to promote health equity. . Medical care, 57, , S101-S103.
Plotkin, S. L. (2012). A short history of vaccination. Vaccines .
Rexroad, C. V. (2019). Genome to phenome: improving animal health, production, and well-being–a new USDA blueprint for animal genome research 2018–2027. . Frontiers in genetics, 10,, 327.
Rose, D. W. (2016). Friends and Partners: The Legacy of Franklin D. Roosevelt and Basil O'Connor in the History of Polio. . Academic Press.
Shifrin, M. &. (2020). Near-optimal insulin treatment for diabetes patients: a machine learning approach. . Artificial Intelligence in Medicine, 107, , 101917.
Skov, M. H. (2019). Cystic fibrosis–an example of personalized and precision medicine. . Apmis, 127(5),, 352-360.
Sneha, P. &. (2016). Molecular dynamics: new frontier in personalized medicine. . Advances in protein chemistry and structural biology, 102, , 181-224.
Tiffon, C. (2018). The impact of nutrition and environmental epigenetics on human health and disease. International journal of molecular sciences, 19(11), , 3425.
Tran, W. T.-N. (2021). Computational radiology in breast cancer screening and diagnosis using artificial intelligence. . Canadian Association of Radiologists Journal, 72(1), , 98-108.
Wailoo, K. &. (2006). The troubled dream of genetic medicine: ethnicity and innovation in Tay-Sachs, cystic fibrosis, and sickle cell disease. . JHU Press.
Walters, L. &. (1997). The ethics of human gene therapy. Oxford University Press, USA.
Wilkinson, R. G. (2003). Social determinants of health: the solid facts. . World Health Organization.
Yao, J. Y. (2014). Chemistry, biology, and medicine of fluorescent nanomaterials and related systems: new insights into biosensing, bioimaging, genomics, diagnostics, and therapy. . Chemical reviews, 114(12),, 6130-6178.
Zhou, J. D. (2021). Artificial intelligence in echocardiography: detection, functional evaluation, and disease diagnosis. Cardiovascular ultrasound, 19(1), , 1-11.

Zhu, C. Z. (2020). Frontiers of ctDNA, targeted therapies, and immunotherapy in non-small-cell lung cancer. . Translational lung cancer research, 9(1), , 111.

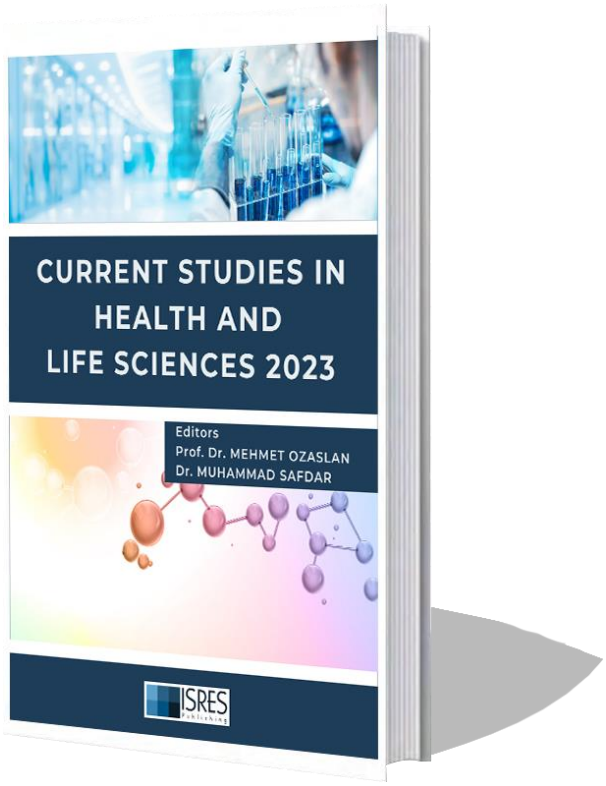
About The Authors

Muhammad Rameez is a diligent student pursuing a Doctor of Veterinary Medicine at UVAS, Lahore, slated to graduate in May 2024. He has actively pursued online courses, notably in epidemiology, cancer metastasis, and antimicrobial resistance. He has contributed to publications on poultry antibiotics, in vitro meat, and the Legalization of euthanasia. Engaging in conferences, workshops, and leadership roles, he exhibits strong organizational and interpersonal skills. His commitment to academic excellence and research positions him as a promising contributor to the field of health and life sciences.
E-mail: mrameez953@gmail.com, ORCID: 0009-0008-4822-3621

Dr. Aman Ullah Khan, a Ph.D. holder from Freie University Berlin, Germany, serves as a dedicated Lecturer in the Department of Microbiology at CVAS, Jhang, UVAS, Lahore, accumulating 11 years of teaching and research experience. With over 30 publications in high-impact journals and two authored book chapters, his expertise spans zoonotic pathogens' surveillance, molecular characterization, and combating antimicrobial resistance. Having initiated his career in 2011, Dr. Khan's international stint at Friedrich-Loeffler-Institut, Germany, focused on Brucellosis. Actively participating in 50+ conferences and supervising postgraduate students, he aspires to establish advanced diagnostic methods for zoonotic diseases, contributing to Microbiology and Public Health**.**
E-mail: amanullah.khan@uvas.edu.pk, ORCID: 0000-0002-5616-7833

Ali Raza, a current Doctor of Veterinary Medicine student at the University of Veterinary and Animal Sciences, Pakistan. His practical expertise from a Veterinarian Internship at Baig Stud Farm demonstrates proficiency in equine disease management. Actively engaged in workshops and international conferences, he is a dedicated member of various societies, showcasing leadership in organizing events. His language proficiency, laboratory skills, and accolades, underscore his commitment to excellence. Co-authoring articles in the Farmer Reformer journal his diverse skills contribute significantly to veterinary medicine.
E-mail: vetaliraza2546@gmail.com, ORCID: 0009-0007-1078-9817

To Cite This Chapter:
Rameez, M. & Khan, A. U. (2023). **Breakthroughs in disease prevention and treatment**. In M. Ozaslan & M. Safdar (Eds.), *Current Studies in Health and Life Sciences 2023*(pp. 431-443). ISRES Publishing



Current Studies in Health and Life Sciences 2023 is published annually from the selected papers invited by the editors.

This edition includes 3 sections and 21 papers from the field of Health and Life Sciences including Medical, Life, Veterinary and Animal.

All submissions are reviewed by at least two international reviewers. The purpose of the book is to provide the readers with the opportunity of a scholarly refereed publication in the field of Medical & Life Sciences.

Current Studies in Health and Life Sciences 2023 is published by ISRES Publishing.