

# **Mycology**

**– basis of diagnosis**

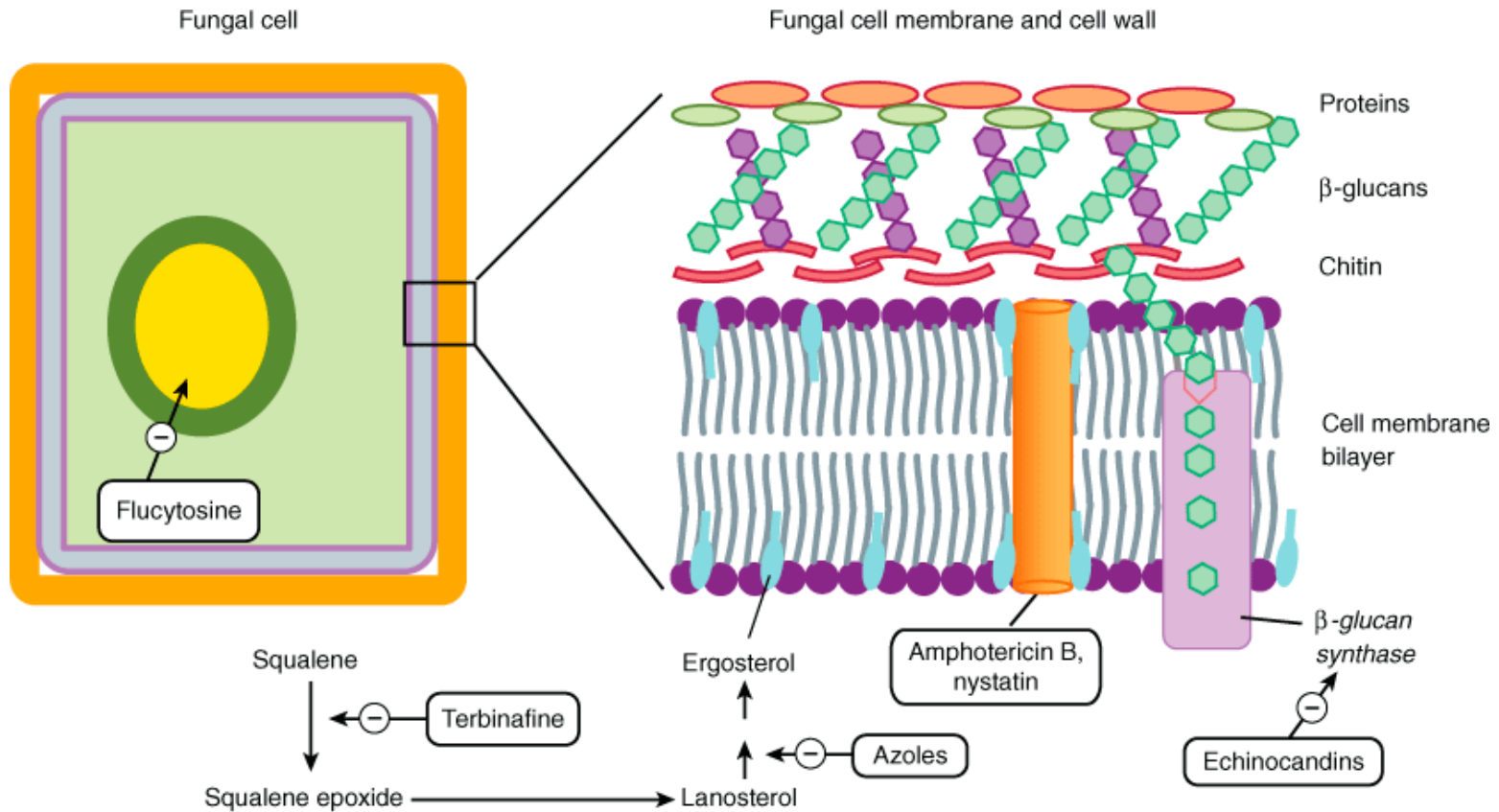
**mycology**  
**mycoses**  
**fungemia**  
**exo-antigen**  
**fungal antigenemia**  
**biomarker**  
**pre-emptive**  
**therapy**

# Fungi

	FUNGI	BACTERIA
nucleus	eukaryotes	prokaryotes
cell membrane	sterols (ergosterol)	-
cell wall	chitin, mannan, glucan, chitosan	murein, teichoic acid, proteins
oxygen	almost all strict aerobes	facultative and obligate aerobes and anaerobes,

- **heterotrophs** requiring organic carbon source for growth ( biotrophic, saprophyte)
- **extracellular enzymes**
- **host defense:** cell-mediated immunity (role of antibodies is minor) -> neutrophil phagocytosis and killing

# Antifungal agents- mode of action



Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology*, 11th Edition: <http://www.accessmedicine.com>

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- Polyenes (amphotericinB, nystatins, pimarcin)
- Azoles (ketokonazole, itraconazole, fluconazole, vericonazole, posaconazole)
- Echinocandins (caspofungin, mikafungin, anidulafungin )
- Nucleoside analogs(antimetabolites : (5 fluorocytosine)
- Allylamines: (tebinafine)

# Fungal morphotypes

Unicellular form (Yeast)

**Yeasts**

spherical or ellipsoid fungal cells  
reproduce by budding

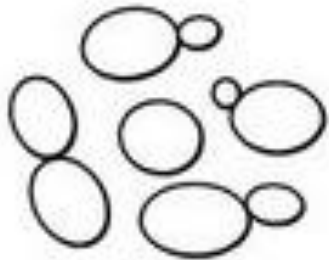
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Mycelial form : moulds, dermatophytes

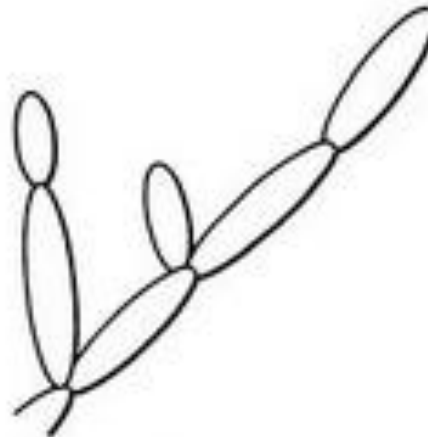
**Molds**

hyphal or mycelial form of growth  
branching filaments (filamentous)

# Fungal morphotypes



Blastospores



Pseudohypha

Unicellular form (Yeast)

# FUNGUS FAMILY

YEAST

MOLDS & dermatophytes

*Candida, Cryptococcus,  
Malessezia, Geotrichum,  
Trichosporon, Rodotorula  
etc.*

**Dimorphic fungi**  
*Blastomyces, Coccidioides,  
Histoplasma, Paracoccidioides*

*Aspergillus, Penicillium,  
Mucor, Rhizopus, Fusarium,  
Cladosporium,  
Scopulariopsis*

or

Dimorphic fungi – have two growth forms: molds & yeast, which develop under different growth conditions

# SPORULATION

**sexual** by  
sexual spores:

- ascospores
- zygospores

non common

**asexual** by  
asexual spores

common

**CONIDIOSPORES**  
borne externally  
on  
AERIAL HYPHA

**SPORANGIOSPORES**  
borne in a sac or ascus  
on  
AERIAL HYPHA

**ARTROSPORES**  
fragmentation  
of  
VEGETATIVE  
HYPHA

size of fungal spores ranges from 2–3  $\mu\text{m}$  (*Cladosporium*, *Aspergillus*, *Penicillium*) up to 160  $\mu\text{m}$  (*Helminthosporium*)



# clinically important fungi

## Yeasts

Basidiomycetes

**Glucan +**

- *Candida*
- *Geotrichum*
- *Sacharomyces*

**Glucan -**

- *Cryptococcus*
- *Trichosporon*
- *Rhodotorula*
- *Malassezia*

## filamentous fungi

**Glucan +**

Ascomycetes

Hyphomycetes

- *Aspergillus*
- *Acremonium*
- *Penicillium*
- *Paecilomyces*
- *Scedosporium*
- *Scopulariopsis*
- *Fusarium*

Dermatophytes

- *Trichophyton*
- *Epidermophyton*
- *Microsporum*

Pheohyphomycete

- *Alternaria*
- *Culvularia*
- *Phialophora*
- *Cladophialophora*
- *Bipolaris*
- *Exophiala*
- *Rhinocladiella*

**Glucan -**

Mucormycetes  
formerly: zygomycete

- Rhizopus*
- Mucor*
- Rhizomucor*
- Lichtheimia*
- Cunninghamella*
- Apophysomyces*

# Pathogenicity factors

- Adhesion
- Change of antigenic surface structure
- Dimorphism
- extracellular fungal products:
  - enzymes : proteinases, phospholipases
  - mycotoxins

# Mycoses

- 1. superficial** affect outermost layers
- 2. cutaneous** affect deeper layers (dermatophytes)
- 3. subcutaneous** subcutaneous tissue,  
connective tissue, muscle, fascia
- 4. systemic**
  - A) systemic primary – dimorphi fungi
  - B) systemic opportunistic (exogenous/endogenous NF)
- 5. allergic mycoses** affects lungs or sinuses

# 1. Superficial mycoses

- pityriasis versicolor (*Malassezia furfur*)
- tinea nigra (*Hortea werneckii*)
- black piedra (*Piedraia hortae*)
- white piedra (genous *Trichosporon*)

Infections of the STRATUM CORNEUM or hair shaft

# Malassezia furfur – lipophilic yeast

Considered part of microbial flora previously known as *Pityrosporum ovale*

## Pityriasis versicolor

chronic infection

occur as macular patches of discolored skin  
inflammation, scaling, irritation are minimal  
lesions fluoresce under Wood's lamp

opportunistic fungemia in patients receiving total parenteral nutrition (contamination of the lipid emulsion)

folliculitis – rarely  
contributor to dandruff and seborrheic dermatitis

## TINEA NIGRA – *Hortaea werneckii*

appear as a dark discoloration often on the palm

## PIEDRA - endemic in tropical countries

Black piedra - *Piedra hortae*: nodular infection of the hair shaft

White piedra - *Trichosporon spp.*: large, soft, yellowish nodules on the hair

Disease	Causative organisms	Incidence
Pityriasis versicolor Seborrhoeic dermatitis including Dandruff and Follicular pityriasis	<i>Malassezia</i> spp. (a lipophilic yeast)	Common
Tinea nigra	<i>Hortaea werneckii</i>	Rare
White piedra	<i>Trichosporon</i> spp.	Common
Black piedra	<i>Piedraia hortae</i>	Rare

# Cutaneous mycoses - dermatophytoses

- fungi that infect only the superficial keratinized tissue (nails, skin, hair)
- unable to grow in 37°C
- unable to grow in the presence of serum = no systemic spread

genera:

- *Trichophyton*
- *Epidermophyton*
- *Microsporum*

identification, based on morphological criteria (macroconidia and microconidia)

# skin, nails, hair

All 3 organisms infect attack skin BUT...

- > Microsporum does not infect nails
- > Epidermophyton does not infect hair



# Dermatophytes

## antropophilic

- relatively mild and chronic infections in human
- may be difficult to eradicate

## zoophilic or geophilic

- more acute inflammatory inf.
- tend to resolve more quickly

# Onychomycosis = fungal infections of the nail

Dermatophytes  
>80%

Candida  
10%

nondermatophytic molds  
6%

Dermatophytids - an allergic reaction to the fungus = fungus-free skin lesions

Ringworm infection may cause skin lesions in a part of the body that is remote from the actual infection. Such lesions are called "dermatophytid". The lesions themselves are fungus-free, and normally disappear upon treatment of the actual infection.

# Dermatophyte nail mycosis - risk factors

- weighting of the nail (jogging, tennis, badminton, climbing, marches)
- beauty treatments (pedicure, manicure)
- occupation requiring wearing footwear industry
- underlying diseases (diabetes, Cushing's syndrome, hypothyroidism, AIDS, cancer)



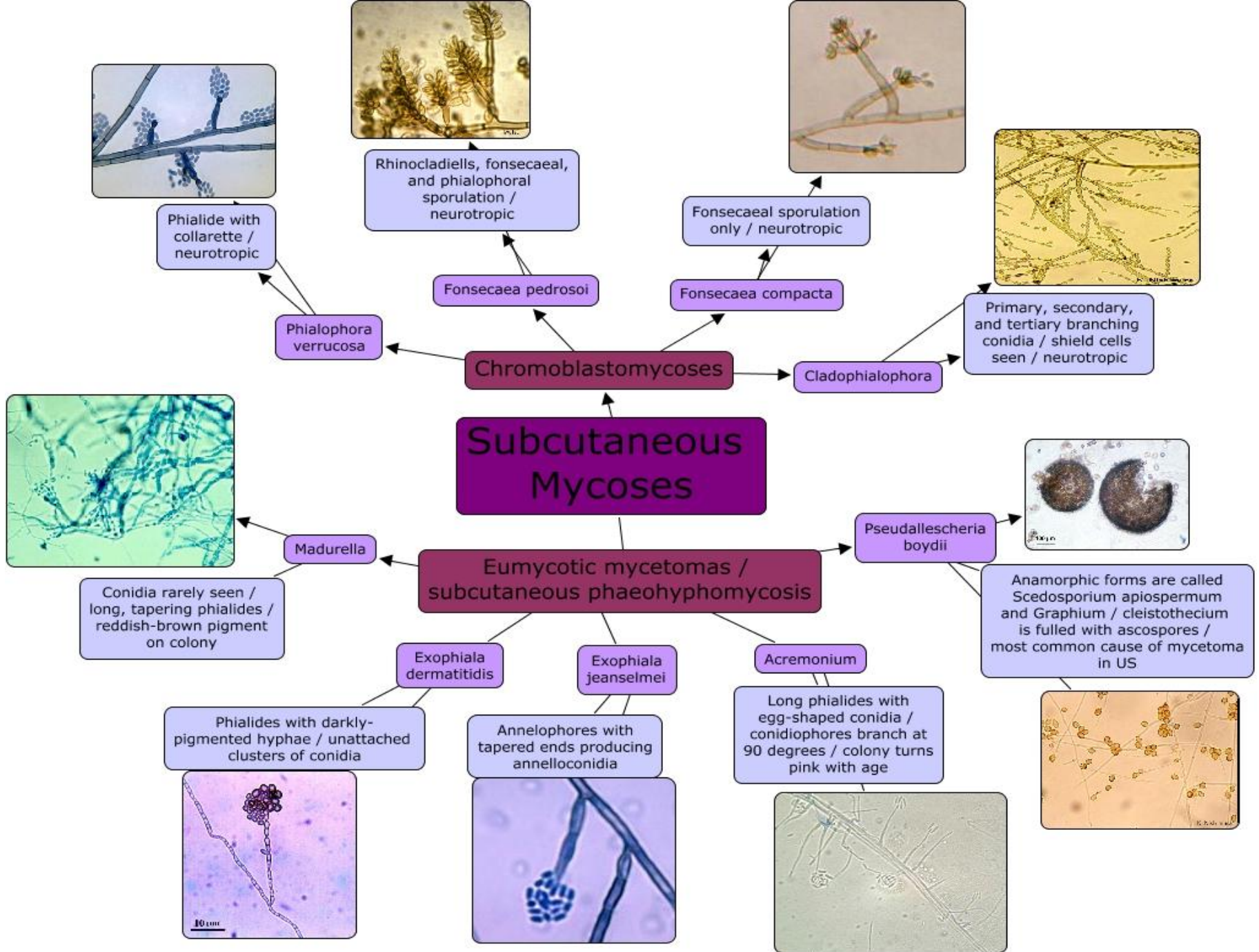
- it occurs in adults, especially the elderly. Rare in children
- nail infections are usually secondary to athlete's foot
- shoes are often „incubator” for fungi

# Prevention tinea pedis and unguium

- Decontaminated footwear and textiles
  - "15 minutes"
  - eliminate "the effect of the plastic bag"
  - examination of the patient's family
  - cure "pet" - zoofilne dermatophytes

# SUBCUTANEOUS MYCOSES

- acquired through traumatic lacerations or puncture wounds to enter
- usually confined to tropics and subtropics with exception of Sporotrichosis
- common among those who work with soil and vegetation and have little protective clothing



# Sporothrix scheneckii

Dimorphic: mycelial in nature, yeast in tissue

Raised skin lesions with proximal spread along lymphatic channels

Causative agent of sporotrichosis ("rose gardener's disease")

- Cutaneous sporotrichosis
- Extracutaneous sporotrichoses
- Central nervous system sporotrichosis

Risk groups: gardeners, forestry workers, miners, laboratory workers, veterinarians

Transmission : traumatically introduced into the skin typically by a thorn

# primary systemic mycoses – dimorphi fungi

- mycelial in nature, yeast in tissue
- all of primary systemic fungal pathogens are agents of respiratory infections

Endemic

blastomycosis

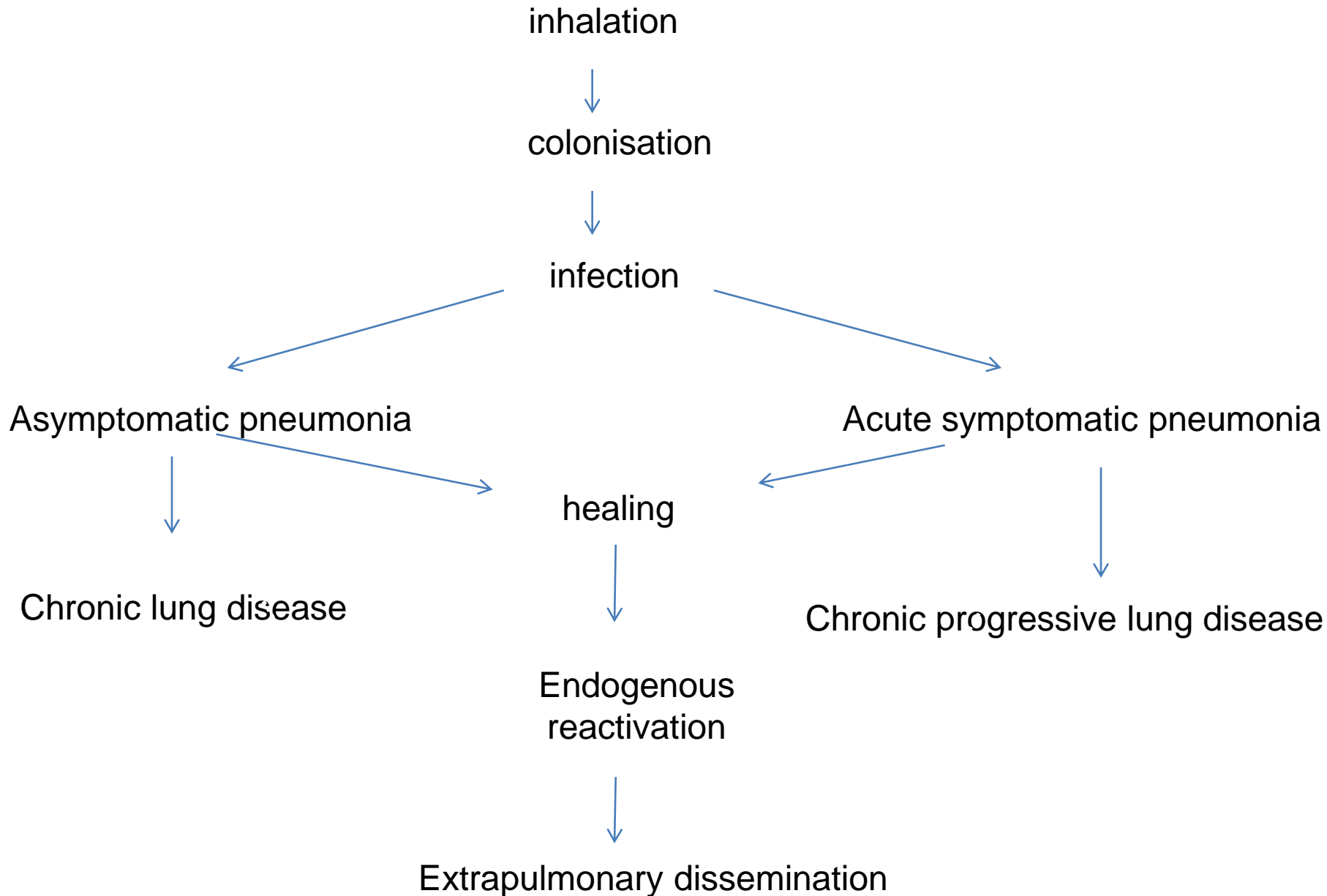
coccidiomycosis

histoplasmosis

paracoccidioidomycosis



# Possible clinical courses of mycosis by dimorphic fungi



dimorphi fungi	disease	endemic area	virulence
<i>Blastomyces dermatidis</i>	blastomycosis	Ohio-;Mississippi River Valley	Modification of cell wall composition (escape recognition by macrophages)
<i>Coccidioides immitis</i>	coccidiomycosis	deserts of Mexico regions, Central and South America	generate an alkaline microenvironment that helps to survive intracellularly within the phagosome
<i>Histoplasma capsulatum</i>	histoplasmosis	Latin America, parts of Asia, Middle East, eastern half of USA	survive and proliferate within phagosome ( unknown mechanism )
<i>Paracoccidioides brasiliensis</i>	Paracoccidioidomycosis	Central and South America	Hormonal influences on infection Estrogen inhibits transition from conidia to the yeast form this fungi

# Opportunistic systemic mycoses

- Candidiasis ( *Candida albicans*, *Candida* spp.)
- Cryptococcosis (*Cryptococcus neoformans*)
- Aspergillosis (*Aspergillus fumigatus*, *Aspergillus* spp.)
- Mucormycosis (*Rhizopus*, *Mucor*, *Absidia*)
- Hyalohyphomycosis (*Fusarium*, *Scopulariopsis*, *Beauveria*)
- Pheohyphomycosis (*Cladosporium*, *Bipolaris*, *Curvularia*)

## clinical groups and predisposing factors for invasive candidiasis

- broad-spectrum antibiotic therapy
  - corticosteroid therapy
  - pregnancy
  - oral contraceptive use
  - systemic disease ( diabetes mellitis etc.)
- 
- neutropenia (especially >7 days)
  - hematological & solid tumor malignancy
- 
- postsurgical intensive care patients
  - prolonged intravenous catheterization
  - parental nutrition
  - severe burns
  - neonates

## Clinical manifestations of *Candida* infections:

### Causative agents:

*Candida albicans*

*Candida parapsilosis*

*Candida glabrata*

*Candida tropicalis*

- Oral candidiasis (including thrush, glossitis, stomatitis)
- *Candida* vulvovaginitis
- Cutaneous candidiasis (including diaper candidiasis, paronychia, onychomycosis)
- Candiduria
  - ➔ Candidemia and disseminated candidiasis

# Candida albicans virulence factors

- enzymes (proteinases, phospholipases)
- composition of the cell surface/ hydrophobicity
- ability to undergo the yeast-to-hypha transformation  
(regulated by both pH and temperature)
- thigmotropism

# Cryptococcus neoformans

*C. neoformans* var *neoformans*

reservoir: bird droppings

host predisposition: immunocompromised

*C. neoformans* var *gatti*

reservoir: eucalyptus trees

host predisposition: mostly healthy people

endemic in Australia, Papua New Guinea, parts of Africa, the Mediterranean region, India, south-east Asia, Mexico, Brazil, Paraguay and Southern California

# Cryptococcus neoformans

## **Cryptococcosis :**

- pulmonary infections
- CNS infections (cryptococcal meningoencephalitis)
- rare infect other body sides
- cryptococcosis is an AIDS- defining illness in patients with HIV

### **virulence:**

- polysaccharide capsule
- phenoloxidase (enzyme that converts hydroxybenzoic substances to melanin;  
protect against oxidative host defense)
- ability to grow in 37 °C



# *Aspergillus*

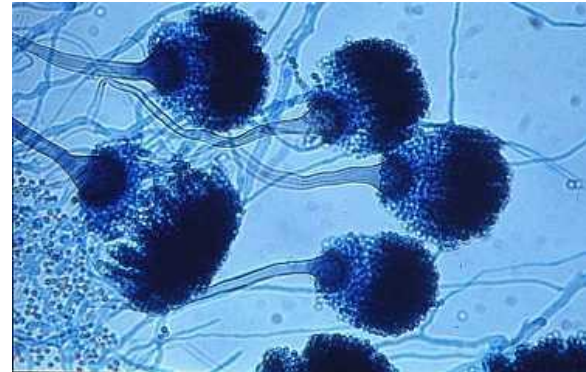
*A. fumigatus*

*A. flavus*

*A. niger*

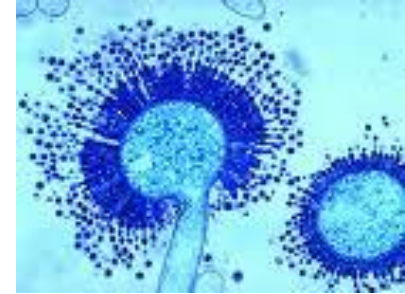
*A. terreus*

- have a global distribution
- small spore size
- thermo-tolerance allowing growth at human body temperature
- resistance to oxidative killing
- produce metabolites and enzymes with proteolytic and immunosuppressive activity



# Aspergillus spp. infections

- Invasive pulmonary aspergillosis
- aspergilloma (fungus ball )
- disseminated aspergillosis
- allergic bronchopulmonary aspergillosis
- Farmer's lung
- aspergillus sinusitis



## Well-know risk factor for invasive aspergillosis:

- macrophage function reduced
- phagocytosis or cellular killing reduced

# Clinical Infection = manifestation

Current diagnostic methods

Empirical/targeted therapy

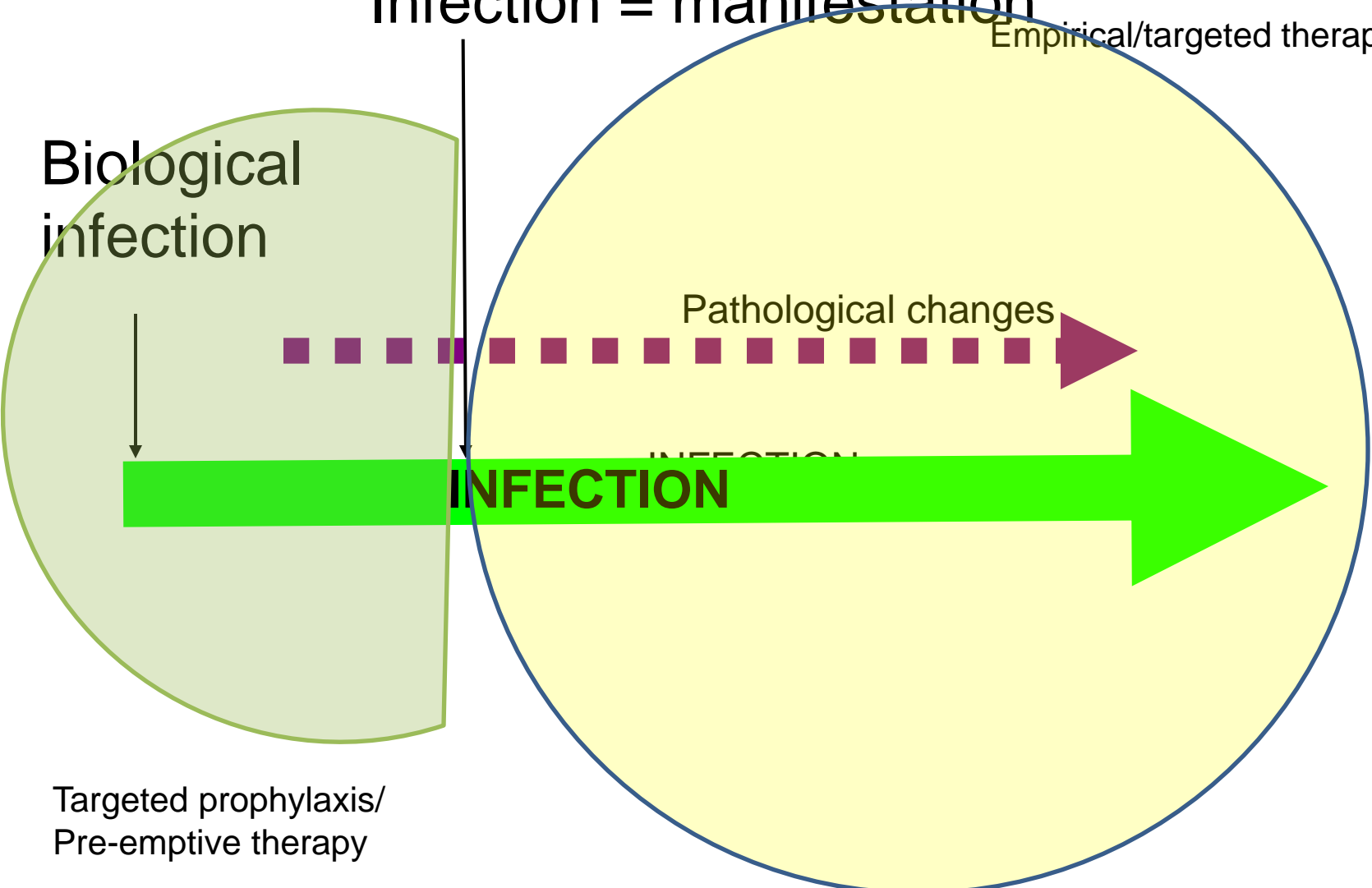
Biological  
infection

Pathological changes

**INFECTION**

Targeted prophylaxis/  
Pre-emptive therapy

PCR  
Antigen detection



# Diagnosis of invasive Aspergillosis

## Galactomannan (GM)

- polysaccharide component of the cell wall
- highly immunogenic antigen
- exo-antigen that can be detected in serum, BAL or CSF
  - monitoring of GM during antifungal therapy allows progression of treatment to be measured

## (1→3)-β-D-glucan

- exo-antigen
- present in molds, yeast, bacteria, plants
- may also be used in diagnosis of candidiasis or fusariosis
- absent in *Cryptococcus* species, zygomycetes and humans
- may be used as a complementary test to GM

## Invasive Candidiasis

Based on detection of antigen:

- >  $\beta$ -glucan
- > Mannan

### **Mannan**

- polysaccharide component of the cell wall of *Candida* spp.
- highly immunogenic antigen
- immunologically more active than  $\beta$ -glucan
- positive results may be obtained 2-15 days before positive blood cultures
- negative results of the tests do not exclude infection

## Invasive Cryptococcosis

Only based on detection of capsular polysaccharide  
(**glucuronoxylomannan**) antigen  
detection in serum, BAL or CSF

# Invasive fungal infections

biomarker	Best detection method	speciment	disease
(1→3)-β-D-glucan	Enzimetic fungitell	Blood serum	Invasive Candidiasis Fusariosis Aspergillosis
Mannan/ anty-mannan	EIA	Blood serum	Specific for Candidiasis 2x - = no IFI
galactomannan	EIA	Blood serum BAL CSF	Invasive Aspergillosis ! + in 50% fusariosis ! Cross reaction with Geotrichum
<b>glucuronoxylomannan</b>	EIA Latex agglutination	Blood serum CSF urine	Invasive Cryptococcosis

# Mycotoxins & mycotoxicoses

- secondary metabolites produced by fungi
- impair the immune system
- neurotoxic, mutagenic, carcinogenic and teratogenic effects.
- toxic effects depends on the type of mycotoxin, the duration and dose of exposure and the age, health and nutritional status of the individual affected

aflatoxin (*Aspergillus flavus* and *A. parasiticus*)

ergot alkaloids (*Claviceps spp.*, *A. fumigatus* and *Penicillium chermesinum*)

ochratoxins (*A. ochraceus* , *A. alliaceus* , *A. terreus* , *P. niger* and *P. viridicatum*)

Chronic exposure to mycotoxins causes immunosuppression of varying extent.

# Fungal Allergy

- majority of allergy-causing molds belong to the divisions of ascomycota or basidiomycota (*Alternaria* , *Aspergillus*, *Bipolaris* , *Cladosporium* , *Curvularia* ,*Penicillium*)
- outdoor spore concentration ranges from 230 to  $10^6$  spores/m<sup>3</sup>
- immunological mechanisms underlying mold allergies are hypersensitivity reactions of types I, II, III and IV

## Clinical Manifestations of fungal allergy:

- Allergic Rhinitis
- Allergic Asthma
- Atopic Dermatitis
- Allergic Bronchopulmonary Mycoses
- Allergic Sinusitis
- Hypersensitivity Pneumonitis



# *Pneumocystis jiroveci* (formally *P. carinii*)

## 1. **Pneumocystis pneumonia (PCP)**

- AIDS defining illness
- probably transmission from person to person
- CD4 level = predicting risk factor for developing PCP

CD4 count of <200 cells/mm<sup>3</sup> (90% AIDS patient develop PCP)

### SYMPTOMS:

- shortness of breath (especially with exertion)
- nonproductive cough
- fever

## 2. **Extrapulmonary infections** are rare (may infect any area of body)

occur in <3% of patients

*P. jiroveci* does not contain ergosterol and has not been cultured

# polyenes (amphotericinB, nystatines, pimarcin)

<b>Mechanism of action</b>	Formation of complexes with ergosterol in fungal cell membranes, resulting in membrane damage and leakage
<b>Spectrum of activity</b>	yeasts + molds (also Mucormycetes*) + dimorphic fungi
<b>INTRINSIC resistance</b>	yeasts: <i>Trichosporon spp</i>  hialopyphomycetes: - <i>A. terreus</i> - <i>Fusarium spp.</i> - <i>Scedosporium apiosperum</i>
<b>ACQUIRED resistance</b>	Rare ; if-> may be present in <i>Candida spp.</i>
other	<ul style="list-style-type: none"><li>➤ most effective drug for severe</li><li>➤ cidal</li><li>➤ insoluble in water</li><li>➤ nephrotoxicity (new formulations with liposomes)</li><li>➤ widely distributed in tissues, poor in body fluids</li><li>➤ half-life &gt;15 days</li></ul> <p style="text-align: right;">* drug of choice</p>

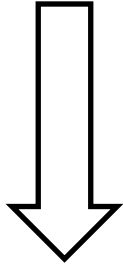
# azoles

(ketokonazole, itraconazole, fluconazole, voriconazole, posaconazole)

<b>Mechanism of action</b>	interfer with the synthesis of ergosterol <del>AmB + azoles</del>
<b>Spectrum of activity</b>	Antifungal spectrum different for each agent
<b>INTRINSIC resistance</b>	<i>Aspergillus spp.</i> - resistant to fluconazole <i>C. krusei</i> - resistant to fluconazole 50% <i>C. glabrata</i> intermediate for fluconazole
<b>ACQUIRED resistance</b>	<ul style="list-style-type: none"><li>➤ overproduction of enzyme (demethylation of lanosterol)</li><li>➤ efflux pumps</li><li>➤ less permeability for antifungal agent</li></ul>
other	static : <i>Candida spp.</i> cidal : II generation of triazoles for <i>Aspergillus</i> Supplanted AmB in less severe mycoses because are less toxic & can be administered orally

# azoles

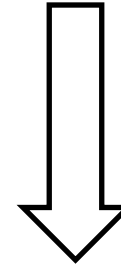
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## **IMIDAZOLES**

- Ketoconazole
- Miconazole
- Clotrimazole

usually localized fungal infections  
(topical agents)  
exc. ketoconazole -> oral  
administration for systemic inf.



## **TRIAZOLES**

### **I generation:**

- Fluconazole
- Itraconazole

### **II generation:**

- Voriconazole
- Posaconazole
- Isavuconazole

# echinocandins ( caspofungin, micafungin, anidulafungin)

## Mechanism of action

Perturb the synthesis of  $\beta$ -glucan

## Spectrum of activity

*Candida* spp., *Aspergillus*, spp. dimorphic fungi

## INTRINSIC resistance

Mucormycetes, *Cryptococcus* spp., *Trichosporon* spp.  
*C. parapsilosis* (high MIC)  
*Fusarium* (!)

## ACQUIRED resistance

*C. glabrata* - MDR

## other

cidial – *Candida*  
static – *Aspergillus* -> MEC (minimal effective concentration)  
Echinocandins not for UTI  
poor penetration for CNS

# antimetabolites (5- fluorocytosine)

## Mechanism of action

Interferes with DNA synthesis

## Spectrum of activity

- *Candida* spp.
- *Cryptococcus* spp.
- some of phaeohyphomycetes

## INTRINSIC resistance

## ACQUIRED resistance

because resistance develops quickly flucytosine is never used alone

## other

- penetrate well into all tissues, including CSF
- dose-related bone marrow suppression and hepatotoxicity, hair loss
- synergistic effect:
  - Candida : AmB + 5'FC
  - Cryptococcus: Fluconazol + 5'FC