Abstracts

ORAL PRESENTATIONS

Sunday, 11 September 2016

Monday, 12 September 2016

Tuesday, 12 September 2016

Wednesday, 13 September 2016

POSTER PRESENTATIONS

Monday, 12 September 2016 • 10.30-11.30

Postersession 01

Monday, 12 September 2016 • 17.00-18.00

Postersession 02

Tuesday, 13 September 2016 • 10.30-11.30

Postersession 03

Tuesday, 13 September 2016 • 17.00-18.00

Postersession 04

AUTHOR INDEX

KEY

Fachgruppe (FG), Ständige Arbeitsgemeinschaft (StAG), Kommission (Kom.)

Sections **Presentation/Poster-ID**

Antimicrobial Resistence and Drugs, Infection Prevention (FG PR) ..... PRV, PRP
Infektionsprävention und Antibiotikaresistenz in der Krankenhaushygiene

Clinical Microbiology and Infectious Diseases (StAG KM) ..... KMV, KMP
Klinische Mikrobiologie und Infektiologie

Diagnostic Microbiology (StAG DV) ..... DVV, DVP
Diagnostische Verfahren in der Mikrobiologie

Eukaryotic Pathogens (FG EK) ..... EKV, EKP
Eukaryontische Krankheitserreger

Food Microbiology and Food Hygiene (FG LM) ..... LMV, LMP
Lebensmittelmikrobiologie und -hygiene

Free Topics (FT) ..... FTV, FTP
Freie Themen

Gastrointestinal Pathogens (FG GI) ..... GIV, GIP
Gastrointestinale Infektionen

General and Hospital Hygiene (StAG HY) ..... HYV, HYP
Allgemeine- und Krankenhaushygiene

Infection Epidemiology and Population Genetics (FG MS) ..... MSV, MSP
Mikrobielle Systematik, Populationsgenetik und Infektionsepidemiologie

Infection Immunology (FG II) ..... IIV, IIP
Infektionsimmunologie

Microbial Pathogenesis (FG MP) ..... MPV, MPP
Mikrobielle Pathogenität

Microbiota, probiotics and host (FG PW) ..... PWV, PWP
Mikrobiota, Probiota und Wirt

National Reference Laboratories and Consiliary Laboratories (StAG RK) ..... RKV, RKP
Nationale Referenzzentren und Konsiliarlaboratorien

Quality Management in Diagnostic Microbiology (QS) ..... QSV, QSP
Qualitätssicherung

Zoonoses (ZO) ..... ZOV, ZOP
Zoonosen

DGHM-LECTURE: Bacterial toxins - How to outsmart the host
11 Sept. 2016 • 16.00–16.45

DGHM Lecture

Bacterial protein toxins - How to outsmart the host The paradigm of *Clostridium difficile* toxins

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Many bacterial protein toxins outsmart the host by hijacking crucial signalling pathways of the host target. To this end, several functional steps are needed, including binding of the toxin to the target cell, translocation into the cytosol and specific and highly efficient manipulation of key host factors to eventually control host cell behavior and environment.

Paradigmatic bacterial protein toxins are *Clostridium difficile* toxins A and B that cause of antibiotic-associated diarrhea and pseudomembranous colitis. Both toxins inactivate Rho GTPases of host cells by glycosylation thereby affecting numerous cellular pathways involved in host-pathogen interaction. In addition, some highly virulent strains produce the ADP-ribosylating toxin CDT that modifies actin resulting in major changes of the cytoskeleton of host cells involving microtubules and septins.

Recent studies by many laboratories largely extended our knowledge on the action of both clostridial glycosylating and ADP-ribosylating toxins. Genetic screening, crystal structure analyses, immunological and cell biological studies allowed novel insights into structure-function relationships, molecular mechanisms and pathogenesis of *Clostridium difficile* toxins.

The role of both toxin groups will be discussed in respect to structure-function relationships and interference with host signaling pathways important for pathogenesis.

Presentation: Sunday, 11 September 2016 from 16:00 – 16:45 in room Donausaal 3.

WORKSHOP 01
Compliance in Infection Control (FG PR)
12 Sept. 2016 • 08.30–10.00

001/PRV

Influenza vaccination and infection rates among health care workers: Results of a survey from a German university hospital, 2014 / 2015

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Introduction: Seasonal influenza causes acute and potentially severe infections in hospitalized patients and health care workers (HCW). Annual influenza vaccination of medical staff is recommended by the German standing commission on immunization (STIKO) in order to prevent nosocomial influenza infections. Vaccination rates in medical staff are lower than in other target groups in Germany [1, 2] and the motivation of HCW to receive the annual vaccine is a major challenge for hospital management. At the University Hospital of *Wuerzburg* (UKW), the strong 2014/2015 influenza season lead to high HCW dropout rates related to acute respiratory illnesses.

Aim: The aim of this survey was to analyze vaccination rates, attitudes towards vaccination and the frequency of acute respiratory infections among HCW of the UKW in the influenza season 2014/2015, in order to support future measures in influenza control within the hospital.

Materials and Methods: A retrospective cross-sectional study using anonymized questionnaires was conducted. Questionsaddressed socio-demographic data, information on vaccination and respiratory symptoms, and one open-ended question on reasons for vaccine refusal.

Result: The overall self-reported vaccination rate was 55%. Uptake was significantly higher in physicians (72%) than in nursing staff (45%), as well as in males (67%) compared to females (51%) ($p<0.05$). A high proportion of vaccines were administered by medical staff on the wards. Self-reported lost work-time due to adverse events after vaccination was low (0.6%). Vaccine uptake was highest in the pediatric clinic (70%) and lowest in the surgery department (37%). Eight categories for vaccine refusal were identified, whereof doubts about effectiveness and indication was the most relevant. Acute respiratory infection was reported by 24% and symptoms compatible with probable influenza by 10% of the participants. Laboratory testing was rare (5%) and influenza virus was confirmed in five individuals.

Conclusion: Future activities to stimulate vaccination in HCW should focus on nursing staff and departments with a low uptake rate. Educational measures need to highlight scientific evidence for the benefits of HCW vaccination. Administering vaccines directly on wards was a successful strategy and should be supported. Uncertainty of causative agents for respiratory infections impedes reliableinfluenza surveillance within the hospital.

References

[1] Böhmer, M. and D. Walter, *Grippeschutzimpfung in Deutschland: Ergebnisse des telefonischen Gesundheitssurveys GEDA 2009*, in *GBE kompakt*.2011, Robert Koch-Institut: Berlin. p. 1-3.

[2] Wiese-Posselt, M., et al., *Influenza vaccination coverage in adults belonging to defined target groups, Germany, 2003/2004.* Vaccine, 2006. **24**(14): p. 2560-6.

Presentation: Monday, 12 September 2016 from 8:30 – 8:45 in room Donausaal 3.

002/PRV

Are you vaccinated? Self-reported vaccination behaviour and attitudes towards vaccinating

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Introduction: Vaccinations are a key element in strengthening clinical preventive medicine and effective measures of maintenance of health. Despite an increasing acceptance of vaccines, mainly through national and primarily international vaccination programmes (e.g. World Health Organization [1]), there are still some doubts and uncertainties about the vaccine efficacy, which result in gaps in immunity.
Objective: To answer at least some of these questions about vaccination gaps in the population and conduct respective strategies, a central overview of possible causes would be needed. Main objective was to find out, what influence informational sessions will have on vaccination behaviour.
Method: After a lecture series titled *"Medizin für Jedermann*" (University Hospital Leipzig), participants were asked to think of their vaccination behaviour and attitudes towards vaccinating. A short developed questionnaire with eleven items was used (demographic data, vaccination status, barriers and self-reported vaccination behaviour).
Result: 204 participants were approached and 164 (80.4 %) participated. Mean age of the participants was 57.4 ± 18.3 years. Most of the respondents were women (64% of all respondents). Almost 100 percent of the respondents agree that they are vaccinated against tetanus, 83.3% against diphtheria and 67.8% against influenza. Meningococcal vaccine was at the bottom of the list of eight possible vaccinations suggested (14.4%). There is no significant difference in vaccination status by gender. 29% gave the justification that they have no medical recommendation for vaccination. Only a small share of the target group reported that they are against vaccination, in general (1.8%; no significant differences by gender). After the information session just over half of the respondents said that they re-examine (62%) and/or correct their vaccination behaviour (59.1%).
Conclusion: These data show that information sessions about benefits of vaccinations could be helpful for the behavioural change. The data gives an idea of potential barriers. To conduct a full assessment of attitudes against vaccinating, more information is needed onopportunities to improve the vaccination behaviour.

Reference

[1] Global Vaccine Action Plan 2011 - 2020, 2015 assessment report of the global vaccine action plan. strategic advisory group of experts on immunization: http://www.who.int/immunization/global_vaccine_action_plan/SAGE_GVAP_Assessment_Report_2015_EN.pdf?ua=1.

Presentation: Monday, 12 September 2016 from 8:45 – 9:00 in room Donausaal 3.

003/PRV

Compliance of infection control during surgical ward rounds: How is the compliance of surgeons in implementing post-operative infection prevention measures?

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Introduction: Surgical site infections (SSIs) are one of the most important causes of healthcare-associated infections. The prevention of SSIs has a growing importance in the surgical sector. The improvements in prevention according to the AWMF-guideline [1] ensures that the clinical problem of SSIs [2] will be approached. In this context, the direct observation of infection prevention measures is suitable for clarification and improvement of implementation challenges. During surgical ward rounds there are many opportunities for hand hygiene and the reduction of other avoidable risks.
Objective: The aim is to find out whether surgeons behave correctly in terms of post-operative infection prevention measures during surgical ward rounds. The emphases are on hand hygiene compliance (HHC), monitoring the actual condition of the devices and the performance of wound treatment and replacing of wound dressing.
Method: During surgical ward rounds the data was recorded by means of a specially-developed data collection sheet. However, only physicians were observed.
Result: As part of a four-week observation period 357 ward rounds were documented (in the Department of Visceral, Transplant and Thoracic Surgery at the University Hospital Leipzig): 129 ward rounds with isolated patients (WRIP) and 228 ward rounds with non-isolated patients (WRNIP). The medical team, which was observed, consisted of 3-4 physicians, the duration of ward rounds averaged 3.3 minutes. Furthermore, the overall result of HHC was 59% (715 opportunities for hand hygiene). There were significant differences between WRIP and WRNIP (42% vs. 69%; p<.001). For checking the vessel catheter there were no differences between WRIP and WRNIP (8% vs. 9%; p=.438). The evaluation of replacing wound dressing was observed more frequently in WRNIP (p=.031). In half of all cases bandage changing was observed (53%; no significant differences; p=.326).
Conclusion: The results of the observational study manifested quite clearly that the medical team has a significant need for improvement in implementing post-operative infection prevention measures during surgical ward rounds. Educational sessions for physicians should clarify the higher risk of the transmission of pathogens, from one patient to another during ward rounds.

References

[1] S1-Leitlinie „Strategien zur Prävention von postoperativen Wundinfektionen", AWMF-Registernummer029/031.http://www.awmf.org/uploads/tx_szleitlinien/029031l_S1_Postoperative_Wundinfektionen_Praevention_2014-01.pdf.
[2] Behnke M, Hansen S, Leistner R et al (2013) Nosokomiale Infektionen und Antibiotika-Anwendung: Zweite nationale Prävalenzstudie in Deutschland. DtschArztebl 110:627-633.

Presentation: Monday, 12 September 2016 from 9:00 – 9:15 in room Donausaal 3.

004/PRV

Patients' intention to speak up for health care providers' hand hygiene in inpatient diabetic foot wound treatment: a cross-sectional survey in diabetes outpatient centres in Lower Saxony, Germany

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Introduction: Hand hygiene in wound care by health care providers (HCPs) is a key principle in treating hospitalized patients with diabetic foot infections. At the same time, patient participation (PP) in promoting HCPs' hand hygiene has been recognized as an option to prevent nosocomial infections [1-2]. One measure is for patients to ask HCPs about their hand hygiene, or remind them of it (speak up). HCPs confronted with somatic problems make PP less likely [3], indicating potential barriers to PP in diabetic foot care. So far, no study has scrutinized PP-motivation in this context.
Objective: This study aimed to estimate the extent to which patients with type-2-diabetes (T2D) intend to speak up for HCPs'

hand hygiene during inpatient foot treatment, test whether this motivation increases in a scenario with an invitation by the hospital to speak up, and identify associations with socio-demographics, knowledge of HCPs' hand hygiene, and diabetes-related factors.
Method: A questionnaire survey was conducted in eight diabetes outpatient centres in Lower Saxony, Germany. Intention to speak up for HCPs' hand hygiene in inpatient foot treatment without and with institutional encouragement, knowledge about HCPs' hand hygiene during foot treatment, and diabetes-related variables were assessed. Analyses of variance were used, partly as repeated measures-models with intention-items as within-subject factor.
Result: N=473 patients participated (response rate: 77.4%). N=177 (41%) strongly intended to speak up. Envisioned encouragement by the hospital was associated with increased rates of strong (54%; p=.001) and higher mean intention to speak up (M=3.4 vs. 3.9 without vs. with encouragement [5-point-scales]; p<.001). In patients without diabetic foot syndrome, this effect was moderated in being limited to those with at least medium school education (p=.013) and knowledge on HCPs' hand hygiene (p=.047). Diabetes-related factors did not moderate the effect.
Conclusion: Most T2D-patients in diabetes outpatient centres intend to speak for HCPs' hand hygiene in inpatient foot treatment, and are receptive to institutional invitation. However, this encouragement presupposes at least medium education and knowledge about HCPs' hand hygiene. Scrutinized for inpatient diabetic foot wound treatment for the first time, these results emphasize that patient empowerment begins with knowledge [4].

References

[1] World Health Organization (WHO). WHO Guidelines on Hand Hygiene in Health Care. Geneva; WHO: 2009
[2] McGuckin M, Govednik J. Patient empowerment and hand hygiene, 1997-2012. J Hosp Infect 2013; 84(3): 191-199
[3] van den Brink-Muinen A, van Dulmen SM, de Haes HC, et al. Has patients' involvement in the decision-making process changed over time? Health Expect 2006; 9: 333-342
[4] McGuckin M, Govednik J. Patient empowerment begins with knowledge. Am J Infect Control 2014; 42(10): 1106-1108

Presentation: Monday, 12 September 2016 from 9:15 – 9:30 in room Donausaal 3.

005/PRV
European campaigns for a prudent use of antibiotics - a literature review

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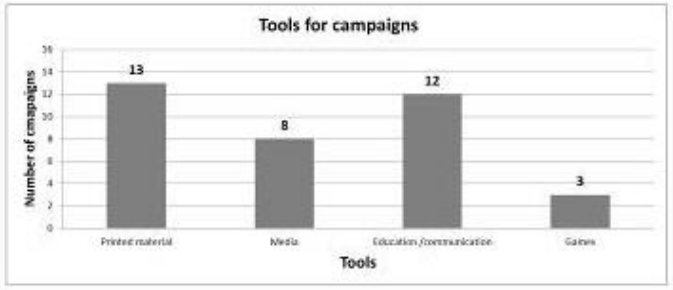
Background: Antibiotic resistance provides an alarming threat of global health and patient safety. Antibiotic misuse was identified as a key driver for the development of antibiotic resistance. This review aimed to assess interventions and campaigns for a prudent use of antibiotics in Europe implemented or evaluated after 2008.
Method: MED-LINE PubMed scientific database was searched from 1st January 2000 to 30th June 2014. All studies that described and evaluated campaigns / interventions for general public, patients, children and parents in Europe to achieve a prudent use of antibiotics were included. Data on name of the campaign, publications, setting, main message, organizing institution / funding, target population, intervention, methodology of evaluation / data collection, outcomes and effects were extracted. Quality of included interventions / campaigns was assessed using the quality assessment tool for quantitative studies by Effective Public Health Practice Project (EPHPP).
Result: The search strategy resulted in 35 publications representing 16 campaigns/interventions that addressed general public, patients, children and parents. European campaigns considerably differ in most parameters including target population, funding, tools (Figure 1), evaluation and methods for assessment of success. Campaigns for a prudent use of antibiotics in Europe were most effective, when they i) applied multi-faceted interventions with a wide media coverage, ii) provided specificity and personal communication to the target population and iii) evaluated the process of the campaign by measurement of surrogate parameters. Successful campaigns were primarily conducted in high income countries with a high antibiotic consumption (France, Belgium). Effects of interventions in Europe varied between no effects (English poster campaign) to 36 % reduction of antibiotic consumption in Belgium (mass media campaign).
Conclusion: In conclusion, prudent use of antibiotics may be achieved by tailored antibiotic resistance awareness programs using a high variety of tools including mass media.

Figure 1



Presentation: Monday, 12 September 2016 from 9:30 – 9:45 in room Donausaal 3.

006/PRV
Applying a newly developed evidence-based medicine framework to questions from infection prevention: An ongoing systematic review on the prognostic value of microbiological colonisation screening for gram-negative pathogens in neonates for sepsis prediction

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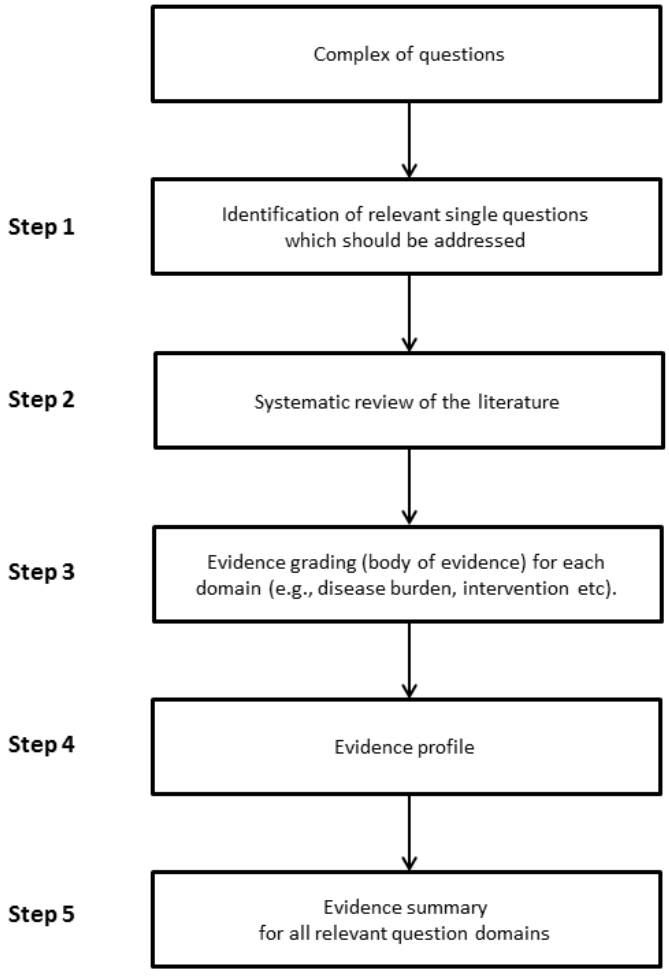
Introduction: At neonatal intensive care units (NICUs) sepsis due to gram-negative bacteria is an important cause of morbidity and mortality. Benefits of routine microbiological screening to predict and prevent sepsis are controversially discussed. To assess the scientific evidence for these measures, we currently conduct a systematic review, using the newly developed Project on a Framework for Rating Evidence in Public Health (PRECEPT) framework. PRECEPT is an ECDC (European Centre for Disease Prevention and Control)-funded project which provides a methodology for appraising evidence in the field of infectious disease prevention and control.
Objective: To pilot the PRECEPT methodology by performing a systematic review and assess the evidence on the prognostic value (sensitivity, specificity) of routine microbiological screening for colonisation of body surface of neonates admitted to NICUs by gram-negative pathogens for the prediction of sepsis.
Method: We developed a protocol for a systematic review according the PRECEPT guidance that comprises five steps

(Figure). Two of the authors searched independently for prognostic-accuracy-studies addressing routine microbiological screening in the literature databases EMBASE and Medline. We extracted data from identified studies accompanied by a risk-of-bias-analysis using the Quality-Assessment-of-Diagnostic-Accuracy-Studies (QUADAS) tool, meta-analysis and assessment quality of evidence using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach.

Result: We identified 3.258 publications, of which by 01/06/2016 nine were eligible and reported data to calculate sensitivity and specificity as well as number needed to screen to prevent one case of sepsis (preliminary results). The finally included studies allow the presentation of pooled sensitivity and specificity. Positive and negative predictive values critically depend on prevalence and are therefore not pooled but presented as a range. Confounding and loss-to-follow-up are not captured by the QUADAS tool and are addressed by an additional tool.

Conclusion: Neonates frequently develop sepsis which leads to increased morbidity and mortality. Sepsis prevention is an important public health task, but microbiological screening of neonates is costly and may even be harmful, e.g. unnecessary antibiotic treatment or barrier precautions. The PRECEPT framework served to identify relevant studies and to assess their quality of evidence. Additionally, further analysis will be used to show the utility of the PRECEPT framework itself. The results of this ongoing systematic review are needed for evidence-based recommendations in regard to routine microbiological screening for gram-negative bacteria to prevent sepsis in neonates.

Figure Legend: Workflow PRECEPT
Figure 1



Presentation: Monday, 12 September 2016 from 9:45 – 10.00 in room Donausaal 3.

WORKSHOP 02
Host-pathogen interactions of Gram-positive and Gram-negative bacteria (FG MP)
12 Sept. 2016 • 08.30–10.00

007/MPV

Platelet activation and aggregation provoked by *Staphylococcus aureus* secreted proteins

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Introduction: Platelets are small anucleate cells of the vascular system comprising indispensable importance in haemostasis but also connect the innate with the adaptive immunity. Infective endocarditis (IE) and disseminated intravascular coagulopathy (DIC) are vascular diseases with *Staphylococcus aureus* as the leading pathogen. Both clinical syndromes comprise an uncontrolled activation of both coagulation cascade and platelets resulting in thrombocytopenia (<150 x 10^9 platelets/L). The opportunistic human pathogen *S. aureus* evolved a broad range of strategies to interact with platelets which can occur either directly by binding an exposed platelet receptor or indirectly by recruitment of serum components.
Aim: This study focused on the identification of new staphylococcal proteins with platelet activation/ aggregation activity.
Method: Using flow cytometry fifty-six recombinant secreted or surface-localized staphylococcal proteins were screened for their capacity to activate platelets. Experiments were performed by measurement of the activation markers P-selectin and $\alpha_{IIb}\beta_3$ conformation, using whole blood, platelet-rich-plasma (PRP), and washed platelets in buffer from a constant set of donors. Furthermore, micropattern protein array (MiPA) chips were functionalized with His_6-tagged staphylococcal proteins to assess the interactions on a single cell level. Real-time calcium mobilization assay (Fluo-4AM), single platelet imaging, parallel plate microfluidics, quantitative image processing and immunofluorescence (P-selectin expression) were used to detect platelet activation kinetics and markers.
Result: This study verified the potential of Eap, a member of the SERAM (secretable expanded repertoire adhesive molecules) family, to trigger platelet activation and aggregation. Additionally, this investigation further revealed the chemotaxis inhibitory protein CHIPS, the formyl peptide receptor-like 1 inhibitory protein FLIPr, both inhibiting neutrophil chemotaxis, as well as the major autolysin Atl as potent platelet activators. The domains of Atl and Eap required for platelet activation were narrowed down. MiPa chips enabled to follow platelet activation by the candidates at single platelet level. Likewise, platelet aggregation activity of these candidates in whole blood was determined.
Conclusion: Taken together, we present here two members of the SERAM family (Eap and Atl) and two additionally secreted proteins of *S. aureus* with characteristic to activate and aggregate human platelet. These results emphasize the importance and complexity of *S. aureus*-platelet interactions. Interestingly, Atl and Eap were also shown to interact with the platelet-derived protein thrombospondin-1 (Kohler *et al*., 2014, Hussain *et al*., 2008).

Presentation: Monday, 12 September 2016 from 8:30 – 8:45 in room Donausaal 4-5.

008/MPV

***In vivo* proteomic analysis of a pathogen isolated from the cerebrospinal fluid during acute meningitis**

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Introduction: The adaptation of pathogens to various host milieus requires a variety of virulence factors implicated in immune evasion mechanisms and transmigration of barriers. However, the success of encountering a host niche depends mainly on the ability of the microorganism to adapt its physiology to changing host niche conditions ensuring thereby maintenance of bacterial fitness and multiplication. In this regard proteins are the functional key-players and the dynamics of the proteome reflects the adaptation to a certain host niche. *Streptococcus pneumoniae* (pneumococci) is a commensal of the human upper respiratory tract but also one of the leading pathogen causing bacterial meningitis. Pneumococci produce a versatile repertoire of virulence factors to evade the immune defence mechanism and to enable them crossing of the blood-brain barrier.
Objective: Isolating pneumococci from the cerebrospinal fluid (CSF) of infected mice and assessment of bacterial protein profiles by employing an *in vivo* proteomics approach.
Materials and Methods: A comprehensive and qualitative mass spectrometry (MS) spectra library was first established by analyzing the proteome of pneumococci cultured under different conditions such as stress or heat. The spectra library and the SpectraST search tool created enables bacterial proteome analysis even in the presence of eukaryotic proteins. Mice were intracisternally infected with 10^5 cfu of *Streptococcus pneumoniae* D39 or its isogenic mutants generated by allelic replacement. Bacteria were isolated by a dual filter extraction step and high sensitive MS including a spectra to spectra comparison search were conducted.
Result: The combined MS measurements of pneumococcal *in vitro* samples for the spectra library resulted in the identification of 7,597 unique peptides corresponding to 1,165 proteins. Combining pneumococci recovered by the filter-based method from the CSF yielded approximately 200,000 bacteria. Nevertheless, more than 685 proteins were identified by SpectraST database searches in the infection dose (control sample) and 249 proteins from the *in vivo* on filter digest. Some of the identified proteins were exclusively detected in the *in vivo* on filter digest, namely ComDE, a two-component signal transduction system, and AliB, a substrate binding protein of an ABC transporter system involved in nutrient/peptide uptake. In the murine meningitis model, intracisternal infection with mutant strains deficient in AliB,

ComDE, or a combination of AliB-ComDE resulted in an attenuated meningeal inflammation and disease course compared to that induced by the wild-type strain D39.
Conclusion: The *in vivo* proteomics approach is a powerful tool to characterize the protein dynamics of pathogens during dissemination in the host and identifies crucial players involved in virulence, fitness and gene regulation.

Presentation: Monday, 12 September 2016 from 8:45 – 9:00 in room Donausaal 4-5.

009/MPV

Systematic site-directed mutagenesis of CagL, an outer protein of the *Helicobacter pylori cag* type IV secretion system, reveals novel functional domains

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The genome of virulent *Helicobacter pylori* strains contains the *cag* pathogenicity island (*cag*PAI) that plays a crucial role in the outcome of *H. pylori* infection and encodes the *cag* type IV secretion system (CagT4SS). During the infection, *H. pylori* use the CagT4SS to translocate effectors such as CagA and peptidoglycan into epithelial cells, leading to a proinflammatory response. Here, we focused on the T4SS tip protein CagL, which is essential for CagT4SS functions. CagL interacts with host cells and integrin. Based on the CagL secondary structure, analysis of global interstrain variation and CagL-specific amino acid motifs, we aimed to identify novel motifs which are involved in host cell contact and the interaction with other Cag proteins.
In order to characterize CagL protein function in detail, we constructed a set of isogenic chromosomal non-marked mutants in *H. pylori* containing site-directed deletions or amino acid substitutions in protein motifs located in predicted loops and CagL unique sequences. These mutants were tested for CagL expression, localization and for gastric epithelial cell activation. Purified recombinant CagL variants were tested for integrin and cell interaction.
Based on the above characterization, we were able to dissect CagL into two different protein domain categories: domains that influenced the stability and subcellular localization of CagL and other *cag*PAI proteins, and motifs that influenced CagL interactions with integrin and gastric epithelial cells. In particular, in addition to the known CagL RGD motif, we identified at least one novel motif which contributed to integrin binding and influenced CagL interaction with cells. The characterization of the mutants in different contexts will be discussed.

Presentation: Monday, 12 September 2016 from 9:00 – 9:15 in room Donausaal 4-5.

010/MPV

Hsp70 is required for the uptake of the ADP-ribosylating *Clostridium perfringens* iota toxin in mammalian cells

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Bacterial ADP-ribosylating toxins cause severe diseases such as diphtheria, cholera and other life-threatening enteric diseases in humans and animals. As an AB-type toxin, the *Clostridium (C.) perfringens* iota toxin employs an efficient uptake mechanism involving binding to a specific cell-surface receptor via its B-component, receptor-mediated endocytosis and pH-dependent translocation across endosomal membranes into the target cell cytosol. Here, the enzymatic active A-component ADP-ribosylates G-actin resulting in F-actin depolymerization and cell rounding. After internalization of the toxin into endosomes, a translocation pore is formed by the B-component and the A-component has to be unfolded to translocate through this narrow pore [1]. In our latest study, we reported that Hsp70 is required for the membrane translocation of the A-component from acidic endosomes into the cytosol (Fig.1) [2]. The pharmacological Hsp/Hsc70-inhibitor VER-155008 (VER) binds to the ATP-binding site of Hsp/Hsc70 with an inhibitory effect. We showed that VER protects mammalian cells from intoxication with the iota toxin and prevents membrane translocation of the A-component into the cytosol (Fig.2). Other steps of toxin mode of action, i.e. receptor binding and enzyme activity, are not affected by VER. Moreover, a novel developed Hsp70-specific inhibitor that targets the substrate binding domain also inhibits intoxication of cells with iota toxin. Thereby, we demonstrated that both the ATP-binding and the substrate binding domain of Hsp70 are required for the efficient translocation of the iota toxin across membranes [2]. Both isoforms, Hsp70 and Hsc70, directly interacted *in vitro* with the A-component of the iota toxin. Interestingly, the A-component displayed a significantly enhanced binding to Hsp/Hsc70 in its denatured i.e. unfolded confirmation [2]. This finding is plausible considering that the A-component also unfolds during membrane translocation in the cell. For the iota toxin, and also other pathogenic ADP-ribosylating toxins, an involvement of other host cell chaperones, namely Hsp90 and peptidyl-prolyl *cis/trans* isomerases of the cyclophilin and FK506-binding protein families, during membrane translocation was discovered [1]. These newly identified interaction partners of bacterial ADP-ribosylating toxins might serve as attractive novel drug targets to inhibit membrane translocation of the A-component and therefore prevent intoxication of target cells and clinical symptoms. A toxin-based therapeutic strategy to treat patients suffering from infection with toxin-producing bacteria constitutes an attractive addition to the existing antibacterial therapy with antibiotics, in particular in the case of (multi-)resistant bacteria.

References

[1] Barth, H. (2011) Naunyn Schmiedebergs Arch. Pharmacol. *383*, 237-245
[2] Ernst, K., Liebscher, M. et al. (2016) Sci. Rep. *6*, 20301

Figure 1

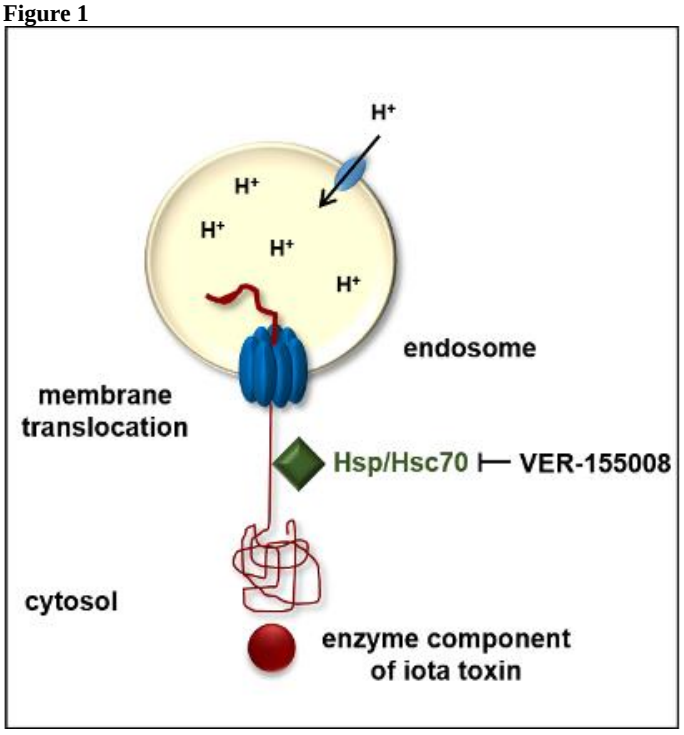
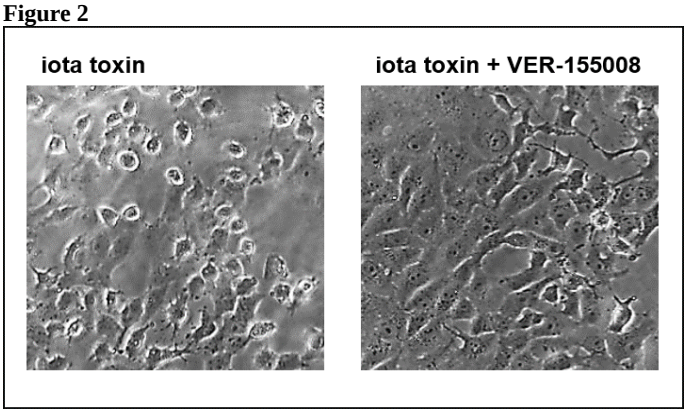


Figure 2



Presentation: Monday, 12 September 2016 from 9:15 – 9:30 in room Donausaal 4-5.

011/MPV

Pro-inflammatory Cytokine and Chemokine Release of Brain Microvascular and Peripheral Endothelial Cells upon Infection with *Neisseria meningitidis*

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Background: *Neisseria meningitidis* (meningococci) is a major cause of epidemic meningitis and septicemia. To establish systemic infection, *N. meningitidis* attaches to and invades into host endothelial cells, thus triggering an inflammatory response with subsequent release of cytokines. Previous studies showed an LPS-independent increase of interleukin-8 (IL-8) in different cell lines challenged with meningococci *in vitro*. In this study, we investigated if peripheral and brain microvascular endothelial cells differ in terms of IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1) release. Additionally, we tested clinical *N. meningitidis* isolates and isogenic mutant strains to determine bacterial factors that contribute to endothelial cytokine release.

Method: We used human microvascular endothelial cells (HBMEC) as a model for brain endothelium and EA.hy926 cells as a model for peripheral endothelium. Both cells lines were infected with meningococci and tested for release of the respective chemokines, and[j1] adherence and invasiveness of meningococcal strains was determined. A panel of meningococcal isolates from patients and healthy carriers were employed to investigate if the clinical presentation correlates with our model. Furthermore, we used lipooligosaccharide (LOS)- and capsule-deficient mutants, heat-killed (HK) bacteria and bacterial supernatants to study the effects of meningococcal factors to IL-8 release.

Result: Clinical isolates did not differ significantly in terms of adherence and invasiveness; however, the LPS-deficient mutant barely invaded into endothelial cells while the non-encapsulated mutant presented a hyper-invasive phenotype. Moreover, our data show that there is a differential pattern of chemokine and cytokine release comparing peripheral and brain endothelial cells with minor correlations between clinical isolates and IL-8 release. Remarkably, capsule- and LOS-deficient mutant strains induced the highest IL-8 release. Treatment of cells with HK bacteria or meningococcal culture supernatant also resulted in IL-8 release, indicating that invasion into host cells is not essential for cytokine release.

Conclusion: Our data indicate that brain endothelial cells show a differential cytokine and chemokine release compared to peripheral endothelial cells after meningococcal infection. While clinical *Neisseria meningitidis*isolates differ slightly in their IL-8 response, LOS- and capsule-deficient mutants induced enormous IL-8 release. According to our findings, cytokine release does not essentially require previous invasion into host endothelial cells, which may contribute to a better comprehension of meningitis pathology.

Presentation: Monday, 12 September 2016 from 9:30 – 9:45 in room Donausaal 4-5.

012/MPV

Comparison of different variants of aggregative adherence fimbriae (AAF) of *Escherichia coli*

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Introduction: The pAA encoded "aggregative adherence fimbriae" (AAF) are regarded as one of the most important virulence factors of enteroaggregative *Escherichia coli* (EAEC), as these adhesins convey adherence to the intestinal epithelium as well as biofilm formation, which are key functions in EAEC pathogenesis thereby promoting the intestinal colonization. Also the Shiga toxin-producing *E. coli* (STEC) O104:H4 outbreak strain expresses AAF/I fimbriae. Other examples of hybrid *E. coli* strains have been described as well, which combine biomarkers of various *E. coli* pathotypes and also express AAF fimbriae or which at least display an aggregative adherence phenotype. Meanwhile, five AAF major structural subunit variants are known (AAF/I - AAF/V). Against this background, we raised the question whether the structural differences of the five AAF fimbrial variants may correlate with different functions.

Materials and Methods: In order to compare the five AAF variants with regard to their contribution to biofilm formation, autoaggregation and aggregative adherence, we subcloned the AAF determinants in the expression vector pBAD24. Heterologous expression of AAF/I to AAF/V fimbriae was induced in an identical strain background (*E. coli* strain DH5a), and the contribution of the different AAF fimbrial variants to bacterial autoaggregation, adherence to human epithelial cells as well as to biofilm formation was compared under identical growth conditions.

Results and Discussions: The five different *aaf* gene clusters were successfully expressed in an isogenic *E. coli* K-12 strain background. Our results demonstrate that expression of AAF/I,

AAF/III and AAF/V fimbriae significantly contributed to the bacterial phenotypes tested, whereas expression of AAF/II and AAF/IV fimbriae had only a weaker impact on the aggregative and adhesive phenotype. Our data suggest that the expression of different AAF-fimbrial variants is not necessarily associated with different adhesive phenotypes. However, if individual AAF fimbrial types can be exchanged without an alteration of bacterial virulence traits will have to be investigated in the future.

Presentation: Monday, 12 September 2016 from 9:45 – 10.00 in room Donausaal 4-5.

WORKSHOP 03
Neues zu Verfahren der mikrobiologischen Diagnostik (StAG DV)
12 Sept. 2016 • 08.30–10.00

013/DVV

Novel Multiplex PCR for detection of the most prevalent carbapenemase genes in Gram-negative bacteria within Germany

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Introduction: The increasing incidence of carbapenem resistant Gram-negative bacteria elicit major concern worldwide. The activity report from the NRZ Bochum for 2014 (DOI 10.17886/EpiBull-2016-002) revealed both an increase in carbapenemase detection since 2009 and the prevalence of different carbapenemases in major Gram-negative pathogens (*Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) from Germany. Current methods for the identification of carbapenemases often rely on phenotypical methods before a targeted PCR but this is time-consuming and there are problems with false positive results.
Objective: The aim of this study was to develop a simple multiplex PCR for the identification of the most prevalent carbapenemase-groups in Gram-negative bacteria in Germany.
Method: The prevalence of different carbapenemases in Germany was identified from the Epidemiologisches Bulletin publication: out of 1830 carbapenemase-containing isolates (excluding copy strains, two carbapenemases per strain in 20 isolates) the **c**arbapenemase-groups identified were VIM (429 isolates), OXA-48 (389), OXA-23 (350), KPC (334), NDM (190), OXA-40 (63), OXA-58 (39), IMP (23), GIM (15), GES (11), ISAbaI-OXA-51 (5), IMI (2), FIM (2) and DIM (1). The most frequent carbapenemase-groups were listed as potential targets and the DNA sequences for all known carbapenemase genes within each group were downloaded from NCBI database, aligned, and conserved regions identified. PCR primers were designed to anneal to these conserved regions. Two multiplex PCRs were proposed and tested under single and multiplex conditions with genomic DNA and crude lysates from strains containing the carbapenemases. Strains of unknown carbapenemase status were tested and results were verified by singleplex PCR.
Result: In the first multiplex PCR, the carbapenemase groups VIM, KPC, OXA-40, NDM, OXA-48 and OXA-23 were included with product sizes of 202, 312, 413, 517, 611 and 718 base-pairs respectively. The attempt to include IMP carbapenemases in the PCRs failed because of the diversity in the IMP-genes and no conserved regions were found. Alignment of subgroups of the IMPs allowed primer design but led to multiple PCR-products in the multiplex PCR and IMP was therefore excluded. The second multiplex included IMI, GES, GIM and OXA-58 with product sizes of 206, 416, 508 and 616 base-pairs respectively. PCRs showed positive bands as expected. No unexpected PCR products were observed. PCR results of strains with unknown carbapenemase status were confirmed in all cases.
Conclusion: A fast and easy method was developed for detection and differentiation of 10 of the most prevalent carbapenemase groups found in Germany, i.e VIM, OXA-48, KPC, OXA-23, NDM, OXA-40, OXA-58, GIM, GES and IMI. Due to the diversity

of the IMP-carbapenemases a multiplex PCR does not seem to be a suitable method for their identification.

Presentation: Monday, 12 September 2016 from 8:30 – 8:45 in room Konferenz 2-3.

014/DVV

Rapid phenotypic determination of carbapenem resistance in Gram-negative rods directly from positive blood cultures

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Introduction: Timely administration of appropriate antimicrobial treatment is essential for survival of patients with sepsis. Thus, the detection of important resistance phenotypes should be achieved as soon as possible. Particularly, the alarming increase of carbapenem resistance in Gram-negative bacteria necessitates the development of novel rapid diagnostic tests.
Objectives: In this proof-of-principle study, we aimed (a) to investigate accuracy and rapidity of the laser-scattering method (LSM) for resistance detection directly from positive blood cultures (BCs) and (b) to determine the optimal preparation procedure for positive BCs to be applied for this method.
Materials and Methods: Ten meropenem-susceptible (seven *Pseudomonas aeruginosa* and three *Klebsiella pneumoniae*) and nine meropenem-resistant (six *P. aeruginosa* and three *K. pneumoniae*) isolates were mixed with 10 ml human whole blood in a final concentration of 10 cfu/ml. Inoculated blood was introduced into BACTEC Plus Aerobic/F bottles and incubated in the BACTEC 9240 automated BC system. Upon positive signal, BC samples were processed by either filtration/dilution or lysis/centrifugation method. For filtration/dilution, positive BC broth was filtered through a 5 µm syringe filter, followed by 1:100 dilution of filtrate with cation-adjusted Mueller-Hinton broth (CA-MHB). For lysis/centrifugation (Sepsityper kit, Bruker, Bremen, Germany), 1 ml of positive BC was mixed with 200 µl lysis buffer, followed by centrifugation. After supernatant was discarded, 1 ml washing buffer was added and again centrifuged. The pellet was dissolved in CA-MHB, standardized to 0.5 McFarland suspension and diluted to produce final inoculum of $5x10^5$ cfu/ml. The samples with or without meropenem in breakpoint concentration 2 µg/ml were measured by LSM in BacterioScan^{TM}216R instrument (BacterioScan Inc., St Louis, US). For each sample pair, growth trend ratios were computed at each minute by the real-time working SCARM filter. Based on these ratios, receiver operating characteristic (ROC) curve analyses were done.
Result: Sensitivity and specificity for detection of carbapenem resistance achieved both 90% after two hours of incubation, and reached 100% after three hours. For *K. pneumoniae*, it was possible to discriminate between resistant or susceptible isolates after only approx. one hour incubation time. Blood components remaining after the filtration/dilution procedure caused disturbance of optical measurement, which considerably hampered evaluation. Further dilution may eliminate the problem but provides inoculum counts, which are lower than recommended for standard susceptibility testing.
Conclusion: LSM using BacterioScan^{TM}216R combined with lysis/centrifugation procedure appears very promising for rapid determination of carbapenem resistance in Gram-negative rods directly from positive BCs.

Presentation: Monday, 12 September 2016 from 8:45 – 9:00 in room Konferenz 2-3.

015/DVV

Benefit of Terminal Subcultures for Detection of Candidemia using the BD BACTEC FX™-System

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Question: The sensitivity of conventional blood culture analysis for detection of candidemia is poor. One possible explanation for this lack of sensitivity is that the detection threshold of commercial blood culture analysis systems is not reached due to insufficient growth of *Candida* spp. inside the blood culture bottles. If this is indeed the case, terminal subcultures might help to detect candidemia in these patients.
Method: The BD BACTEC FX blood culture analysis system together with the BACTEC PLUS-Aerobic/F and Anaerobic/F blood culture bottles (Becton Dickinson GmbH) were used. From July 2009 until April 2016 terminal subcultures on Sabouraud-agar were performed after 5 days of incubation in the BD BACTEC FX system from all negative blood culture bottles for which mycological analysis was requested.
Result: Altogether 55,384 blood cultures were analyzed during the study period and a total of 29.959 terminal subcultures were performed (54.1 %). *Candida* spp. were detected in 473 blood cultures (0.9 %) of 236 patients.
In 124 of the *Candida*-positive blood cultures (26.2 %) growth was detected in the aerobic and the anaerobic blood culture bottle. In 321 blood cultures (67.9 %) only the aerobic bottle and in 11 blood cultures (2.3 %) only the anaerobic bottle was positive.
Of the 332 blood culture pairs, in which only one bottle was positive, *Candida* spp. grew in the terminal subcultures of the corresponding negative bottles in 155 cases (46.7 %). More importantly, terminal subcultures revealed *Candida* spp. in 17 blood cultures of 16 patients (6.8 % of patients with candidemia) in which both the aerobic and the anaerobic blood culture bottle were reported negative after 5 days of incubation. The species causing these false-negative results were *C. glabrata* (n = 11; 64.7 %), *C. albicans* (n = 5; 29.4 %) and *C. guilliermondii* (n = 1; 5.9 %), respectively. Notably, of all *C. glabrata* (n = 79), *C. albicans* (n = 287) or *C. guilliermondii*-candidemias (n = 2), 13.9%, 1.7 % or 50%, respectively, were only detected by terminal subculture.
Conclusion: By performing a terminal subculture from negative blood culture bottles the rate of candidemia detection could be increased by 6.8 % of patients. Especially *C. glabrata* is insufficiently detected by the BACTEC FX blood culture analysis system. For every additionally detected candidemia patient an average of 1872 terminal subcultures corresponding to 3744 Sabouraud-agar plates have to be performed.

Presentation: Monday, 12 September 2016 from 9:00 – 9:15 in room Konferenz 2-3.

016/DVV

Biofilms in prosthetic valve endocarditis- composition, complications and consequences

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[2]*German Heart Institute Berlin, Studienzentrale, Berlin, Germany*
[3]*Charité-Universitätsmedizin Berlin, Institut für Mikrobiologie, Berlin, Germany*

Infective endocarditis (IE) is a life-threatening disease associated with high mortality. Diagnosis of IE is still challenging. Re-infection of the prosthetic valves, in particular in cases with unidentified pathogens, is a severe complication with poor post-operative survival. Thus, diagnosis of the causative microorganisms is crucial for the patient's outcome. Microbiological diagnosis is based mainly on culture techniques.

However, these often fail due to previous antibiotic therapy or fastidious microorganisms.
Our aim was to detect, visualize and identify the infectious agents in native and re-infected prosthetic valves and to study their biofilm formation using Fluorescence *in situ* Hybridization (FISH) combined with 16S rRNA-gene PCR and sequencing.
Patients with native and prosthetic valve endocarditis were included over a 10-year period. Our study comprised 386 valves, out of which were 88 prostheses (21%). We compared routine microbiological outcomes with FISH and PCR results.
We found comparable spectra of pathogens in native and prosthetic valve endocarditis. All cases showed mono-species infections. Using FISH we showed impressive biofilm structures within the prosthetic valves ranging from single cells to highly organized biofilms. We also found FISH positive bacteria in culture-negative cases and samples from patients under antibiotic therapy. Furthermore, bacteria were visualized not only on the valves but also in between the fibers of the suture strings, being difficult to access for immune cells.
FISH not only allows timely therapeutical decisions, but also analysis of pathogenesis of prosthetic valve endocarditis, i.e. re-colonization by the initial microorganisms or infection by a new species during or post-surgery.

Presentation: Monday, 12 September 2016 from 9:15 – 9:30 in room Konferenz 2-3.

017/DVV
Humoral Immune Responses to Selected Recombinant *Chlamydia pneumoniae* Antigens
J. Hagemann*[1], U. Simnacher[1], J. Maile[2], M. Brill[2], F. Struck[2]
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[2]*Mikrogen GmbH, Neuried, Germany*

Introduction: Reliable serodiagnosis and estimate of the clinical relevance of *Chlamydia* (*C.*) *pneumoniae*, which is thought to be a common cause of respiratory tract infections, is often challenging due to its high seroprevalence in healthy individuals, cross-reactivity with other chlamydial species, and substantial limitations in commercially available serodiagnostic tools.
Aim: We therefore analysed the humoral immune response to defined recombinant *C. pneumoniae* antigens in MIF-characterised sera of infected patients in order to establish new serologic markers.
Materials and Methods: Immunoreactive proteins were identified by use of immunoproteomic analysis, gene expression library screening and literature research. Out of these, ten promising surface, virulence-associated, as well as hypothetical proteins were selected for recombinant synthesis. Evaluation of the antigens was carried out on the basis of well-characterised human sera, in which antibodies to recombinant antigens were detected by use of a strip immunoassay. Qualitative and quantitative analysis was performed for both IgM- and IgG-response.
Result: We could show different antibody patterns in early and late infection. In late or past infection, MOMP, OMP2 and YwbM seem to be the most reactive antigens, all of them showing a correlation between MIF IgG titre and band reactivity as well as band intensity of reactive antigens. The virulence-associated SINC-orthologue YwbM was the most sensitive and specific antigen, showing no cross-reactivity with sera of *C. trachomatis* patients. In early infection, recombinant antigens still lack diagnostic sensitivity. However, IgM antibodies to MOMP and TARP were regularly observed, suggesting these antigens to be addressed early in humoral immune response.
Summary and Outlook: We suggest YwbM to be a promising marker for reliable species-specific diagnosis of *C. pneumoniae* especially in late or past infections. Further work on IgM reactive antigens is needed to establish a serological marker to reliably identify early and very early infections.

Presentation: Monday, 12 September 2016 from 9:30 – 9:45 in room Konferenz 2-3.

018/DVV
Total Lab Automation - Introduction into the classic workflow - a one year experience
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Introduction: Microbiology as we know it today is dominated by manual work. This includes labeling, inoculation and transport of media. For some years now solutions for partial automation exist. However, total lab automation using one machine for labeling, inoculation, transport, incubation, reading, work-up and disposal of plates is a new development.
Aim: In April 2015 a TLA (BD Kiestra) was assembled at the Department for Infectious Diseases, Medical Microbiology and Hygiene at the University of Heidelberg. It consists of a 24 slot BarCodA, an InoculA with a safety hood, 4 ReadA Compacts and 7 work places directly attached to the system. This is a description on how we validated the machine, adjusted our workflows and accelerated reporting. The description includes the limits we encountered during implementation.
Materials and Methods: After assembly the performance of the TLA was reviewed. This included performance with plates from 4 different manufacturers, continuous performance with 2500 plates, temperature consistency in the incubators during one week, potential spilling from sample to sample and growth curves for MRSA, VRE and MRGN using selected ATCC strains as well as patient isolates. According to the results from the growth curves incubation and imaging programs for different kinds of media were determined for the TLA. 525 screening samples (nose swabs, rectal swabs, wound swabs) were inoculated in parallel (classic (Previ Isola) and TLA (InoculA)) and results compared. After go-live results with patient samples were reviewed in order to determine the indispensable incubation times for MRSA, VRE and MRGN. Imaging time points were optimized to current workflows and working hours.
Result: The TLA is capable of working with plates from all manufacturers tested.
The TLA hardware is capable of running 2500 plates in a row.
Temperature is extremely consisted over a period of one week.
We have no evidence for spilling from one sample to the other.
Current selective media support the growth of MRSA within 20 hours, VRE within 36 hours and MRGN within 20-24 hours.
The comparison between classic inoculation and reading with inoculation and reading using the TLA revealed an overall higher positivity rate with the TLA (about 10% more positives).
Current data allow a final read for nose swabs at 20 hours after start of incubation and a final read for rectal and wound swabs at 36 hours.
Summary: Implementation of a TLA into a University microbiology lab is a demanding process using a lot of human and financial resources. It necessitates a review of current protocols and continuous adjustments of work-flows and protocols as well as training and continuous education of personnel. This effort is rewarded by a high quality of inoculation, a standardized incubation and speeding up of the reporting process.

Presentation: Monday, 12 September 2016 from 9:45 – 10.00 in room Konferenz 2-3.

WORKSHOP 04
Klinische Mikrobiologie & Qualitätssicherung (StAG KM & Komm. QS)
12 Sept. 2016 • 08.30–10.00

019/KMV

Poultry-associated multi-drug resistant *Salmonella* spp., *Campylobacter* spp. and *Arcobacter* spp. in urban Ghana

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Frequent overuse of antibiotics in poultry farming has contributed to the emergence of antibiotic resistance. Consumption of meat contaminated with *Salmonella*, *Campylobacter*, and *Arcobacter* may infect humans and lead to emergence of multidrug resistance (MDR). Although the general transmission mode for the enteric bacteria under investigation is known (faecal-oral), it remains speculative which pathogen vehicles are predominant in Africa. In Ghana, chicken meat is locally available, however the majority is imported from Brazil, the United States, or the Netherlands.
This study aimed to investigate the level of contamination of local and imported chicken meat with MDR *Salmonella*, *Campylobacter* and *Arcobacter* spp.
Material from frozen and fresh meat samples was cultured for *Salmonella*, *Campylobacter* and *Arcobacter* using enrichment broths and selective media. Bacterial isolates were identified by Gram staining, biochemical testing, and MALDI TOF MS. Antibiotic susceptibility testing was preformed according to CLSI guidelines.
In total, 200 samples were collected. The highest contamination was seen for *Arcobacter* (n=50; 25%), followed by *Salmonella* and *Campylobacter* (each n=17, 8.5%). Contamination for all bacteria was highest for local meat: *Salmonella*, *Campylobacter* and *Arcobacter* were isolated from 76.5% (n=13), 86% (n=43) and 82.4% (n=14) of local meat samples, respectively. Antibiotic susceptibility testing revealed that 23.5% (n=4) of *Salmonella* were MDR. Resistance to Fluorquinolones (FQ) for *Salmonella*, *Campylobacter* and *Arcobacter* was identified in 62.5%, 76.5% and 52% of isolates, respectively.
This study highlights that despite concerns of contamination risks due to imports of meat, contamination for all bacteria was highest for local meat. Of major concern is the overall high level of FQ resistance seen as this may lead to the increase of FQ resistance in the human population.

Presentation: Monday, 12 September 2016 from 8:30 – 8:45 in room Konferenz 1.

020/KMV

Multidrug-resistant *Achromobacter animicus* causing wound infection in a street child in Mwanza, Tanzania

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Introduction: The species *Achromobacter animicus* first described in 2013 has been isolated from sputum of humans suffering from respiratory infections. Literature regarding *A. animicus* is limited. Herein we document first case of *A. animicus* wound infections.
Objective: To characterize a multidrug-resistant *A. animicus* isolated from chronic posttraumatic wound infection in a street child from African continent.
Materials and Methods: Phenotypic identification of the organism was performed using gram staining, culture on Blood and MacConkey Agars and VITEK®-MS. Further taxonomic identification was done by sequencing of the complete 16S rRNA gene and multilocus sequence typing (MLST) of 7 housekeeping genes. Antimicrobial Susceptibility testing and extended spectrum beta lactamase production was confirmed using VITEK® 2 system and E-Test respectively.
Result: The isolate was phenotypically identified as a gram-negative bacterium with inconclusive genus. The complete 16S rRNA gene provided 99.6%, 99.6% and 99.7% match with *Achromobacter xylosoxidans* (CP014065), *Achromobacter denitrificans* (CP013923) *and Achromobacter animicus* (NR_117615) respectively. The MLST unveiled 4 new alleles, i.e. *gltB*_82, *lepA*_114, *nrdA*_99 and *nuoL*_92, that resulted into the new sequence type ST-320, which was closely related to other *A. animicus* genotypes. Single locus sequencing of nrdA_765 gene confirmed the isolate to be *A. animicus*. The isolate was found to be resistant to ampicillin, trimethoprim-sulfamethoxazole, piperacillin/tazobactam, cefotaxime, ceftazidime and tetracycline, but susceptible to ciprofloxacin, gentamicin, imipenem, meropenem, and ertapenem, and intermediate susceptible to moxifloxacin.
Conclusion: For the first time, this case report highlights the role of multidrug- resistant *A. animicus* in causing wound infections which is important information to clinicians and clinical microbiologist. There is a need to improve microbiological services in developing countries in order to identify uncommon human pathogens. This will assist in updating the database of automated identification systems; simplifying identification of these pathogens and track their epidemiology.

Presentation: Monday, 12 September 2016 from 8:45 – 9:00 in room Konferenz 1.

021/KMV

A microbiome based strategy for the prediction of infectious infertility

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Inflammatory processes triggered by infections are a major cause of tubal factor infertility in females. Major obstacles in early diagnosis of females at risk in acquiring infectious infertility are often asymptomatic infections as underlying cause. Early diagnosis of patients at risk could facilitate clinical strategies in preventing infectious infertility. Therefore, we aimed to establish a method to precisely predict infectious infertility on the basis of diagnostic tests and microbiome analysis using next-generation-sequencing.
To establish a prediction method for infectious infertility, we conducted a prospective study on females with infectious infertility (ININF) in comparison to females with non-infectious infertility (nININF), female sex workers (FSW) and fertile females. We performed diagnostic testing for sexually transmitted pathogens using PCR-tests for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and *M. genitalium*. Cultivation dependent diagnostics were performed for *Candida* sp., Group B Streptococcus, *Gardnerella vaginalis*, *E. coli*, and *Klebsiella* sp. Furthermore, we tested the serum of the females for presence of IgG and IgA antibodies targeting *C. trachomatis* antigens MOMP, OMP2, TARP, CPAF, and HSP60.

Microbiome sequencing was performed from cervical swabs. We isolated bacterial DNA using the MO BIO PowerSoil® Kit. The V3V4 region of the bacterial 16S rRNA gene was amplified and the amplicon library was sequenced on a MiSeq sequencing system from Illumina. Raw reads were processed using mothur and thereby all sequences with ambiguous bases, sequences with homopolymer length greater than 9, sequences conducted of being chimeric and sequences that were classified as non-bacterial were removed. A subset of 2500 reads/sample was used for statistical analysis of the microbial composition based on taxonomic assignment and operational taxonomic units (OTU).
We could not identify single markers for infectious infertility from diagnostic and microbiome sequencing data. Therefore, we conducted a logistic regression model for prediction of infectious infertility integrating a) PCR and culture dependent diagnostic testing, b) serology for antibodies targeting *C. trachomatis* and c) the first ten taxa from the microbiome sequencing. Our model was able to predict infectious infertility with an accuracy of 93.8 %, correctly predicting ININF in 17 of 18 cases. Importantly, all cases of nININF could be separated from ININF integrating the microbiome data. The same model excluding the microbiome data predicted only 13 of 18 cases correctly.
Our results show a new strategy for prediction of infectious infertility. However, the model has to be refined in future studies with enhanced patient numbers to improve and validate its performance. The results also highlight the potential of NGS-techniques in future diagnostics.

Presentation: Monday, 12 September 2016 from 9:00 – 9:15 in room Konferenz 1.

022/KMV

Tedizolid susceptibility in linezolid- and vancomycin-resistant *Enterococcus faecium* isolates

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Vancomycin-resistant enterococci (VRE) are of ever-increasing importance, most notably in high-risk patient populations. Therapy options are often limited for these isolates, and apart from tigecycline and daptomycin, oxazolidinon linezolid is frequently administered. The broad usage of linezolid, however, has driven the emergence of linezolid-resistant VRE strains (LR-VRE), further shortening therapeutic options. Second generation oxazolidinon tedizolid has the advantage of being active against a specific subset of LR-VRE, i.e. isolates expressing the plasmid-encoded chloramphenicol-florfenicol resistance- (*cfr*-) gene. Here we tested activity of tedizolid-activity in a collection of 30 LR *Enterococcus faecium* VRE (MIC range 32 - 256 mg/L) isolated between 2012 and 2015 from clinical and screening specimens. By pulsed field gel electrophoresis (PFGE) isolates were assigned to 16 clonal lineages. In three cases, linezolid-susceptible progenitor isolates of LR-VRE were isolated, thus demonstratingthe de nove emergence of the linezolid resistant phenotype. PCR did not detect *cfr*, *cfr*(B) or novel oxazolidinone resistance gene *optrA* in LR-VRE. All isolates, however, carried mutations within the 23S rDNA. Compared to linezolid, tedizolid MICs were lower in all isolates (MIC range 2 - 32 mg/L), but remained above the FDA tedizolid breakpoint for *E. faecalis* at 0.5 mg/L. Thus, related to the predominant resistance mechanism, tedizolid is of limited value for treatment of most LR-VRE and represents a therapeutic option only for a limited subset of isolates.

Presentation: Monday, 12 September 2016 from 9:15 – 9:30 in room Konferenz 1.

023/KMV

Local resistance profile of bacterial isolates in uncomplicated urinary tract infections (LORE study)

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Whereas microbiological tests are routinely performed in complicated urinary tract infections (UTI), only little is known on the antibiotic resistance in uncomplicated UTI in Germany since bacterial resistance testing is usually omitted. The German clinical guidelines for uncomplicated UTI recommend primarily the use of fosfomycin or nitrofurantoin and cotrimoxazole should rather be avoided.
The LORE study aimed in the determination of the local resistance profile to antibiotics in uncomplicated UTI. The main goal was to identify alternative drugs for the first-line treatment instead of fosfomycin, which should be rather limited to severe infections with multi-resistant bacteria, or instead of nitrofurantoin in order to avoid its side effects.
During the period of twenty-two months, twenty-nine practitioners and gynaecologists of the Kiel regions recruited female patients with uncomplicated UTI and the age of 16 to 65. Patients with gravidity, diabetes, or chronic diseases or after antibiotic therapy were excluded. The urine samples were tested by four local laboratories according to the EUCAST rules with regard to the bacterial load, the bacterial species, and the sensitivity to the common antibiotics ampicillin or amoxicillin without or with beta-lactamase inhibitors, piperacillin, ceftazidime, cefpodoxime, imipenem or meropenem, ciprofloxacin, cotrimoxazole, fosfomycin, and nitrofurantoin. The LORE study was performed in the Kiel region in close collaboration with general practitioners and gynaecologists from the local medical network Praxisnetz Kiel. This study was supported by the Robert Koch-Institute, Berlin.
Totally, 954 samples were collected and 896 patients were included into the study. A significant bacterial load of at least 100,000 colonies per ml were found in 491 samples (55%). Gram-negative bacteria were isolated in 521 cases. The most frequently isolated bacterium was Escherichia coli (N = 450; 70% of all samples with bacterial isolates). Other Gram-negative bacteria were Klebsiella, Proteus, Citrobacter, Enterobacter, and Morganella in descending order. Gram-positive bacteria were isolated in 216 cases. Fosfomycin reached a sensitivity of 98%, nitrofurantion 94%, and cotrimoxazole 85% if all samples were included. However, regarding just the most relevant Gram-negative bacteria, fosfomycin reached 98%, nitrofuantoin 95%, cefpodoxime 95%, ciprofloxacin 96%, and cotrimoxazole 85%. The sensitivity rates for ampicillin or amoxicillin without or with beta-lactamase inhibitors or for piperacillin ranged between 37 and 67%.
The LORE study described the local bacterial resistance profile in uncomplicated UTI. The sensitivity rates of 95% for cefpodoxime and 86% for cotrimoxazole in Gram-negative bacteria suggest their use instead of the presently recommended drugs fosfomycin and nitrofurantoin.

Presentation: Monday, 12 September 2016 from 9:30 – 9:45 in room Konferenz 1.

321/KMV

Frequency, Quality and Cost of Syphilis diagnostics in Germany: A retrospective health care utilization database analysis

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Syphilis (Sy) is a major public health problem in Europe since it´s re-emergence in the late 1990`s and early 2000`s. However, data on the economic impact of Syphilis on health care systems is scarce. Therefore, the aim of our study was to assess the associated annual diagnostic and therapeutic costs and to gain epidemiological information on Sy patients by retrospectively analysing socioeconomic data for the years 2009-2012. Data was provided by a statutory health insurance company (DAK-Gesundheit) covering more than 5.5 million insured persons. Results were compared to other sources of data such as nationwide notification data e.g. and quality of diagnostic testing for Sy in Germany studied by EQA was taken into consideration. In our cohort the incident diagnosis of Sy was coded on average for 2,300 outpatients and 850 inpatients annually which by extrapolating these findings to the German population level would result in more than 32,000 annual cases (incidence 40/100,000 inhabitans). These figures far exceed numbers from mandatory nationwide notification (incidence of 6.1/100,000 inhabitans in 2013 e.g.). In our cohort more than 70,000 diagnostic tests were ordered annually (accounting for direct medical costs in our cohort of more than 370,000 Euro/y and extrapolated to more than 1 million diagnostic tests nationwide) with two thirds of them being performed as part of maternity services. Considering the many tests performed as part of preblood donation testing it is obvious that the large majority of diagnostic testing is performed on a low risk population which together with proficiency testing data is raising concerns about test quality and standardization of diagnostic assays used. Findings from this study suggest ongoing issues related to care for Sy and may help to improve future disease management.

Presentation: Monday, 12 September 2016 from 9:45 – 10:00 in room Konferenz 1.

HAUPTSYMPOSIUM 01
Molecular Microbiology
12 Sept. 2016 • 12.30–13.30

024/INV

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Microbial communities have co-evolved with humans for millions of years. They inhabit all surfaces of the human body, including the respiratory tract. Specific sites harbor specialized bacterial communities and it is increasingly recognized that these different micro-ecosystems play a major role in maintaining human health. The respiratory tract is a complex organ system which primary role in human physiology is the exchange of oxygen and carbon dioxide. The human airways are colonized with niche-specific bacterial communities: it is increasingly recognized that one of their main functions might be to prevent respiratory pathogens from gaining a foothold on the mucosal surface and spreading to the lower respiratory tract. Our current research addresses how the healthy URT microbiome is established, and what ecological and environmental factors direct early and subsequent development of respiratory microbial communities. Moreover, we focus on studying the relationship between respiratory microbiota development and development of respiratory infections.

Presentation: Monday, 12 September 2016 from 12:30 – 13:00 in room Donausaal 3.

025/INV

ROLE of Zinc in group A streptococcal pathogenesis

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Streptococcus pyogenes (Group A Streptococcus; GAS) is a Gram-positive human pathogen responsible for a wide spectrum of diseases ranging from pharyngitis and impetigo, to severe invasive diseases including necrotizing fasciitis and streptococcal toxic shock-like syndrome. Zinc is recognized as an important metal ion in relation to nutritional immunity and zinc deficiency is linked to increased susceptibility to bacterial infection. Zinc stress impairs glucose metabolism through the inhibition of the glycolytic enzymes phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase. In the presence of zinc, a metabolic shift to the tagatose-6-phosphate pathway allows conversion of D-galactose to dihydroxyacetone phosphate and glyceraldehyde phosphate, partially bypassing impaired glycolytic enzymes to generate pyruvate. Additionally, zinc inhibition of phosphoglucomutase results in decreased capsule biosynthesis. To investigate the role of zinc in innate immune defense against GAS, we analyzed the clinically important GAS M1T1 wild-type strain, and the phenotypes of two isogenic mutants and corresponding complemented mutants. The targeted GAS czcD gene encodes for a putative zinc efflux pump and the adjacent gczA gene encodes a putative Zn-dependent activator of czcD expression. Compared to wild-type and complemented cells, both mutants exhibited reduced ability to grow in the presence of zinc. Transcriptional analyses indicate that gczA up-regulates czcD in response to zinc. The gczA regulator also induces galactose metabolism, circumventing zinc-induced blockage of glucose uptake, and the zinc susceptible CTP synthase. Both czcD and gczA are up-regulated in contact with human neutrophils. Zinc efflux plays a critical role in GAS pathogenesis, as both czcD and gczA mutants displayed increased susceptibility to killing by human neutrophils and reduced virulence in a murine infection model. Taken together, these results demonstrate that zinc homeostasis is an important contributor to GAS pathogenesis and innate immune defense against infection. Strategies to manipulate zinc homeostasis in order to reduce GAS infection are discussed.

Presentation: Monday, 12 September 2016 from 13:00 – 13:30 in room Donausaal 3.

HAUPTSYMPOSIUM 02
Vaccinology
12 Sept. 2016 • 15.00–16.30

026/INV

How to Control the Most Successful Pathogen on Earth

S. H. E. Kaufmann*[1]
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Introduction: Tuberculosis (TB) is a global health threat with 2 billion individuals infected with *Mycobacterium tuberculosis* (Mtb). Although only 5% to 10% of these individuals develop active disease, TB ranks high in morbidity and mortality with approximately 9.6 million individuals developing active disease and 1.5 million dying annually. General agreement exists that better intervention measures are urgently needed with a vaccine that prevents pulmonary TB in all age groups being decisive.
Objective: Create a genetically modified BCG (VPM1002) with improved safety and protective efficacy.
Patients, Materials and Methods: A recombinant BCG was created which expresses listeriolysin and is devoid of urease C. Vaccine efficacy and safety were tested preclinically in appropriate animal modes. Healthy individuals were enrolled in two phase I, one phase IIa and one phase II trial.
Result: Preclinical animal models revealed that the vaccine VPM1002 is more efficacious and safer than BCG. Phase I and IIa trials demonstrated its safety and immunogenicity in adults and neonates. An ongoing phase IIa trial assesses its safety and immunogenicity in HIV-exposed neonates.
Conclusion: VPM1002 has been licensed to Vakzine Projekt Management, Hanover, Germany and sub-licensed to Serum Institute India, Pune, India. Based on excellent results in preclinical models and clinical trials encourage further development of VPM1002. Currently a phase IIb trial in neonates and a phase III trial in adults are being prepared for submission to regulatory agencies.

Presentation: Monday, 12 September 2016 from 15:00 – 15:30 in room Donausaal 3.

027/INV

From the genome to the development and future perspectives of a broadly protective vaccine against MenB

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Introduction: Most of the vaccines available today, although very effective, have been developed using conventional technologies. The vaccinology field is evolving very rapidly and the new available technologies have opened alternative ways in designing improved vaccines or novel vaccines against infections for which preventive measures do not exist. In this context, a new approach named “Reverse Vaccinology”, based on the identification of novel antigens through bioinformatics analysis of a bacterial genome, has been instrumental to the identification of a vaccine against *Neisseria meningitidis* serogroup B, a bacterium causing a devastating disease characterized by meningitis and sepsis.
Result: The most promising antigens identified by this novel technology, NHBA (Neisserial Heparin Binding Protein) fHbp (factor H binding protein), and NadA (Neisseria Adhesin A), combined with Outer Membrane Vesicles derived from the New Zealand outbreak strain NZ98/254 are the basis for a broadly protective meningococcal serogroup B vaccine, 4CMenB. This vaccine has been approved for use from two months of age in 38 countries worldwide (Europe, Australia, Canada and South America) and in US for use in individuals 10 through 25 years of age.

The development of a novel vaccine based on previously unknown antigens opened many scientific questions. Their function, immunogenicity, and ability to be effective targets for antibody recognition in different strains representing the meningococcal genetic diversity will be described. The results of these studies have provided many insights in the mechanism of virulence and pathogenesis of meningococcus and have been the basis of a new epidemiological tool (MATS, Meningococcal Antigen Typing System) to evaluate vaccine coverage.
Conclusion: 4CMenB vaccine is expected to reduce the incidence of meningococcal disease, providing added public health benefits.

Presentation: Monday, 12 September 2016 from 15:30 – 16:00 in room Donausaal 3.

028/INV
Effects of pneumococcal conjugate vaccination on serotype distribution and burden of pneumococcal disease in children and adults in Germany
M. van der Linden*[1]
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Backgrounds and Aims: *Streptococcus pneumoniae* remains a leading cause of pneumonia, sepsis and meningitis and disproportionately affects young children and the elderly. In July 2006, vaccination with pneumococcal conjugate vaccine was generally recommended in Germany for all children ≤ 24 months. PCV13 was licenced for adults in 2011 and uptake in adults has increased since. In this study, we present the burden of disease and serotype distribution among children and adults with invasive pneumococcal disease (IPD) before and after the start of childhood and adult vaccination.
Method: Pneumococcal isolates recovered from children and adults with IPD were serotyped at the GNRCS using the Neufeld-Quellung-reaction.
Result: In 2014-2015, 84 IPD isolates recovered from children <2 years were sent in, of which only 15 had PCV13 serotypes. This represents a reduction of 45% compared to 2005/2006 (before vaccination introduction) and a reduction of 33% since the introduction of higher-valent vaccination. Among the PCV13-non-PCV7 serotypes, reductions were observed for serotypes 1 (-78%), 3 (-63%), 6A (-100%), 7F (-94%) and 19A (-75%).
Among adults PCV7 serotypes have decreased from 40-45% before childhood vaccination to 3% in 2014-2015. PCV13-non-PCV7 serotypes have reduced from 47.1% in 2010-2011 to 28.0% in 2014-2015.
Non-PCV13 serotypes have gained in importance, with serotypes 10A, 15A/B/C and 24F being most prevalent among children, and 10A, 12F, 15A and 23B among adults. Of these, 15A and 23B are often penicillin non-susceptible.
Conclusion: Childhood pneumococcal conjugate vaccination has strongly affected the serotype distribution among both children and adults.

Presentation: Monday, 12 September 2016 from 16:00 – 16:30 in room Donausaal 3.

WORKSHOP 05
Psychologische Aspekte zur Implementierung von Präventionsmaßnahmen (StAG HY)
12 Sept. 2016 • 18.00–19.30

029/HYV
Why is hand hygiene compliance often suboptimal? - A psychological approach to explain the "hand hygiene paradox"
S. Diefenbacher*[1], J. Keller[1]
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Introduction: Hand hygiene plays an important role in infection prevention (Allegranzi & Pittet, 2009). Nevertheless, hand hygiene compliance is often insufficient (Huis et al., 2012). (Social) Psychology provides promising approaches to explain this paradox. We refer to the distinction between consciously planned and automatic types of behavior as proposed in prominent dual-process models (Ajzen & Fishbein, 2000). Considering that hand hygiene is a relatively frequent but short behavior that is typically only complementary to a focal activity, the underlying processing mode can be assumed to be automatic in nature.
Aim: We examined whether hand hygiene can be conceptualized as an automatic type of behavior. For this purpose, we tested the explanatory power of (a) habit as an element of automatic processing as well as (b) knowledge and explicit attitude towards hand hygiene as elements of conscious processing with respect to hand hygiene behavior.
Method: We assessed habit (SRBAI; Gardner et al., 2012), explicit attitude and subjective knowledge about hand hygiene with self-reports and hand hygiene using the Day Reconstruction Method (Kahneman et al. 2004), direct observation (cf. Sassenrath et al., 2016) or video-based observation.
Result: All studies consistently showed significant positive correlations between habit and hand hygiene. Additionally, we found a significant positive relationship between explicit attitude and hand hygiene in one (of two) studies. However, after including habit in a stepwise regression analysis this relationship was no longer significant. In an additional regression model habit was a better predictor of hand hygiene than knowledge.
Conclusion: The findings suggest that hand hygiene should be conceptualized as an automatic (not conscious) type of behavior. The findings have far-reaching implications for hand hygiene trainings in hospitals, where hand hygiene is typically considered to be a conscious type of behavior.

References
[1] Allegranzi, B., & Pittet, D. (2009). Role of hand hygiene in healthcare-associated infection prevention. *J Hosp Infect, 73*, 305-315.
[2] Gardner, B., Abraham, C., Lally, P., & de Bruijn, G. J. (2012). Towards parsimony in habit measurement: Testing the convergent and predictive validity of an automaticity subscale of the Self-Report Habit Index. *Int J Beh Nutr Phy, 9*, 102.
[3] Huis, A., van Achterberg, T., de Bruin, M., Grol, R., Schoonhoven, L., & Hulscher, M. (2012). A systematic review of hand hygiene improvement strategies: a behavioural approach. *Implement Sci, 7*, 92.
[4] Kahneman, D., Krueger, A. B., Schkade, D. A., Schwarz, N., & Stone, A. A. (2004). A survey method for characterizing daily life experience: The day reconstruction method. *Science, 306*, 1776-1780.
[5] Sassenrath, C., Diefenbacher, S., Siegel, A., & Keller, J. (2015). A person-oriented approach to hand hygiene behaviour: Emotional empathy fosters hand hygiene practice. *Psychol Health, 31*, 1-23.

Presentation: Monday, 12 September 2016 from 18:00 – 18:15 in room Donausaal 3.

030/HYV

How important is hygiene in the hospital? A short survey of participant's knowledge, attitudes and expectations about hygienic standards in hospitals

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Introduction: The media frequently discusses on the subject of healthcare associated infection (HAI), which corresponds to the importance of the problem. The growing media presence of the HAI´s issue could result in uncertainties in general public. There are numerous scientific papers about patient-related data (morbidity, mortality, lethality et cetera), while there is very limited data available about the influence of the threatening
Presentation: attitudes and expectations of patients, who are to be treated preventively, diagnostically or therapeutically in future.
Objective: The purpose of this survey is not only to find out which attitudes and expectations people had towards hygienic standards in hospitals but also how they estimate the risk to acquire an infection in hospitals generally. Moreover, the study was undertaken to estimate the general public´s knowledge of HAI´s and their potential transmission of pathogens.
Method: In the context of a public event in Leipzig (called *"Medizin Erlebnisparcour"* University Hospital Leipzig) a survey was conducted, by interviewing passers-by. Participants were asked a series of questions on their attitudes about hygienic standards in hospitals: "What is most important to you when you think about your stay in the hospital?" (scale 0=not important to 4= very important) and "What are you increasingly concerned about (scale 0= no concerns to 4=deep concerns). Furthermore, they were asked, what they know about HAI´s (open question) and the most common potential transmission path. Finally, the participants were asked, how they estimate the risk to acquire an infection in hospitals (in percentage terms).
Result: In total, 246 participants answered the questionnaire. Mean age of the participants was 42.2 ± 18.7 years. Most of the participants were women (75.6 %). The participants considered the compliance of hygienic standards to be the most important issue (♀M=3.8 vs.♂M=3.9; p=.539) between the physicians specialist knowledge (♀M=3.4 vs.♂M=3.7; p<.001). Most commonly, they report that they were increasingly concerned about acquiring an infection in hospitals (♀M=3.3 vs.♂M=3.2; p=.617). The majority of participants are well-informed about HAI´s and furthermore they know that pathogens are mostly transferred via hands (62 % of all respondents). It is remarkable, that they overestimate their own risk to acquire an infection in hospitals (♀=37% vs.♂ =32%; p=.088).
Conclusion: The results show that the majority of participants have a profound knowledge of HAI´s. Surprisingly, the own risk to acquire an infection in hospitals is overestimated. These results could possibly be affected by the growing media presence of the HAI´s issue. Attention should be paid to improve the risk communication on the burden of HAI´s.

Presentation: Monday, 12 September 2016 from 18:15 – 18:30 in room Donausaal 3.

031/HYV

Sustainability takes time: Effects of the psychologically tailored interventions on hand hygiene compliance in the PSYGIENE cluster-randomized controlled trial after two years of follow-up

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Introduction: First-year follow-up results of the PSYGIENE cluster-randomized controlled trial, a project funded by the German Federal Ministry of Health (grant no.: INFEKT-019) and conducted on the intensive care and hematopoietic stem cell transplantation units at Hanover Medical School, had shown increased alcoholic-based hand hygiene compliance both in the study arm using interventions psychologically tailored to wards based on the Health Action Process Approach (HAPA), and the study arm using the standard German Clean Care is Safer Care-campaign (Aktion Saubere Hände [ASH]) [1].
Objective: To test whether the psychologically tailored PSYGIENE-interventions lead to sustainable increases in hand hygiene compliance compared to the ASH after two years of follow-up.
Method: Tailored interventions targeted wards and were informed by problem-focused interviews with physicians and staff nurses (response rates: 100%) and a written survey on HAPA-factors (physicians: 71%; nurses: 63%). In educational sessions for physicians and nurses, and feedback discussions with staff nurses, 29 behaviour change techniques (BCTs) [2] were used in the "tailoring"-arm, and 15 in the ASH-arm. Compliance observations adhered to WHO-/ASH-standards.
Result: Given similar baseline compliance (tailoring: 54%, ASH: 55%, p=.581), tailoring was associated with increases in both follow-up years (2014: 64%, 2015: 70%, p=.001), while compliance in the ASH-arm decreased from 68% in 2014 to 64% in 2015 (p=.007). Comparisons of increases from 2013 to 2015 and compliance in 2015 were also in favour of the "tailoring"-arm (p=.005 and p=.001). While trends among nurses were similar, among physicians tailoring vs. ASH did not differ in increases from 2013 to 2015 (+15% and +12%, p=.658) and rates in 2015 (63% vs. 61%, p=.632). However, the increase of +6% in the "tailoring"-arm from 2014 to 2015 differed from the respective decrease of -9% in the ASH-arm (p=.016).
Conclusion: After two years, psychological tailoring based on the HAPA-model was associated with a more sustainable increase in hand hygiene compliance, despite limited didactic methods (educational training sessions and feedback discussions) and the restriction of the tailored interventions to one year. However, regarding physicians still more research on interventions geared to this target group is needed.

References

[1] von Lengerke T, Lutze B, Krauth C, et al. Promoting hygienic hand disinfection as an ongoing task: Results of the PSYGIENE cluster-randomized controlled trial to (re-)raise compliance of physicians and nurses based on psychological tailoring in a tertiary intensive care setting [Abstract]. Int J Med Microbiol 2015; 305(Suppl 1): 27
[2] Michie S, Richardson M, Johnston M, et al. The behavior change technique taxonomy (v1) of 93 hierarchically clustered techniques. Ann Behav Med 2013; 46(1): 81-95

Presentation: Monday, 12 September 2016 from 18:30 – 18:45 in room Donausaal 3.

032/HYV

How to improve being an infection control role model?

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Introduction: In 2003 70% of respondents to a survey reported that attending physicians would rarely comply with infection control guidelines (1). In 2012 attending physicians were aware of their function as role models when interviewed during a conference, however there was a discrepancy between their willingness to perform as role models, their self-perception and the assessment by others which indicated feedback and communication deficits (2).

Attending physicians were also asked what would help them to optimize their hygiene performance and whished for direct feedback by infection control practitioners, feedback of outcome data like infection rates and observational data like hand hygiene compliance and that they would be influenced by reminders like placards, leaflets or screen savers while social media reminders, apps and smartphones were less likely to be accepted.

Methods and Results: As a potential intervention we developed artistic motivational placards (figure 1) and a „speak-up"-campaign (figure 2). However a comprehensive research study to analyze the effectiveness and efficacy of each individual strategy is difficult to perform due to multiple confounding variables, Hawthorne effect, and lack of reliable end points. In an informal survey of physicians during 40 hours infection control training courses (Hygienebeauftragte Ärzte) 101 (85%) of 118 participants would use the motivational placards in their respective institutions but only 23 (19%) would initiate a speak up-campaign.

Conclusion: While motivational placards with the goal of improving role modelling are accepted by a large majority of physicians, speak up campaigns are met with skepticism. It seems that potential side effects like irritating patients, reducing trust and interfering with the doctor-patient relationship are more important reasons against such campaigns than fear of criticism.

Reference

[1] Hyg Med 2003; 28: 13-16 2,) Hyg Med 2012; 37: 408-412

Figure 1

Figure 2

Presentation: Monday, 12 September 2016 from 18:45 – 19:00 in room Donausaal 3.

033/HYV

Which duties for infection control link nurses (ICLN) have proven to be viable and successful in supporting infection prevention and control (ICP) activities in acute care hospitals? - A literature review.

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Background: ICLN are seen as important backup for the ICP-Teams in hospitals. The German Robert-Koch-Institute (RKI) recommends the introduction of ICLN in every ward and functional unit in German hospitals. This literature review aims at finding duties that were shown to be viable and successful.

Method: A systematic literature review was performed on PubMed and Cochrane databank, using predefined search terms. Studies that evaluated the implementation of / existing ICLN systems by means of staff- or patient-based outcome parameters were included and screened for duties of ICLN. References of all included publications were checked and selected according to the same criteria.

Result: Of 353 hits 10 studies met the inclusion criteria. In two studies ICLN were tasked to collect data of health care associated infections (HAI). One study demonstrated that this task was viable and produced valuable data that had not been available before. It was shown in an audit conducted by the NHS in England, that the ICLN system was seen as successful (50%) or very successful (20%) by wards on which ICLN had been implemented.

In three studies the effectiveness of ICLN giving tutorials or teaching ward nurses was explored. An increases rate in correct urine catheter practice was achieved in two studies. Focus interviews in the third study yielded, that the presence of ICLN as

resource for infection prevention and control (ICP) issues was much appreciated.
The duty to monitor ICP practices such as hand hygiene or compliance with contact isolation was described in two studies. Both parameters were found to increase significantly after the implementation of ICLN.
ICLN were also successfully tasked with early outbreak detection and the assessment of patients to ensure appropriate placement. HAI rates were found to be lower after the implementation of ICLN.
To identify and report ICP matters back to the ICP-Team was also described as another duty. The presence of ICLN was appreciated. Rates of hospital acquired (HA) MRSA and HA-MRSA bacteraemia decreased.
In three studies ICLN were invited to participate in the development of guidelines and contribute to solutions for hygiene related problems. Rates of correct urine catheter practice were found to increase, HAI rates decreased. In one study duties were not clearly defined. ICLN were tasked to participate in ICP rounds and then help implement recommendations deriving from these rounds. They were challenged to develop their individual plan of action with the support of the ICP team. HAI rates were found to be lower after the intervention.
Conclusion: The importance of defining duties for ICLN has been demonstrated. The RKI-recommendations of duties for ICLN however are broad and imprecise. This analysis might help to define tasks for ICLN that have shown to be viable and successful by supporting ICP activities, producing valuable data and reducing HAI rates.

Presentation: Monday, 12 September 2016 from 19:00 – 19:15 in room Donausaal 3.

034/HYV

Electronic hospital monitoring modules as a standarized instrument for in-fection hygiene monitoring at hospitals and medical facilities – a model project for North Rhine-Westphalia

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Background: Infection hygiene monitoring at hospitals and other medical institutions is done in accordance with §17 of the Public Health Service Act (ÖGdG, 2013) and the Pro-tection from Infection Act (IfSG) (IfSG, 2013) by the lower health agencies. For further developing the recent pattern, inspection sheets were developed using electronic modules for hospital monitoring, which should guarantee a flexible and modular monitoring instru-ment for the lower health agencies in North Rhine-Westphalia (NRW).
Goal: The modules for hospital monitoring should be tested for (non-)implementation, ap-plicability and practicability in everyday practice at the health departments. The key objec-tive of the evaluation was to get the widest possible range of information about non-use and valuation of the electronic modules for hospital monitoring.
Methods: A combination of different qualitative and quantitative methods from the social sciences was used, including target group-specific questionnaires, participatory observa-tions and non-responder-analysis.
Results: The non-responder-analysis showed that the electronic modules for hospital monitoring were not rejected as such, but due to reasons like insufficient personal re-sources or missing technical conditions.
Positive findings included a good structure, a comprehensive content of modules and a wide acceptance and support by hospital epidemiologists. Negative aspects identified by the health departments are long storage and loading times, slow handling, long data pro-cessing times, and uncertainties regarding data privacy
Conclusions: It was found that modules for hospital monitoring contribute substantially to standardising infection hygiene monitoring at hospitals. In accordance with the needs we developed optimizations and appropriate treatment recommendations.

Presentation: Monday, 12 September 2016 from 19:15 – 19:30 in room Donausaal 3.

WORKSHOP 06
Microbial pathogenesis and pathogens within the gastrointestinal tract (FG MP & FG GI)
12 Sept. 2016 • 18.00–19.30

035/GIV

Infection, innate immune signalling and cancer in the stomach - stem cell-derived organoids as a new model

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Question: Gastric cancer is the third leading cause of cancer deaths worldwide. Gastric cancer originates from stem cells and the development is causally related to chronic infection with the bacterium Helicobacter pylori. The infection drives inflammation via innate immune signaling. Here we describe human gastric organoids grown from adult stem cells as new model for H. pylori research.
Method: Gastric stem cells were identified using mouse models of Lgr5-DT-EGFP and Troy-Lacz-IRES-EGFP. Human gastric organoids were grown from whole glands or single cells isolated from resection material from gastric cancer patients. Organoids were described using immunohistochemistry and mRNA analysis. H. pylori was microinjected into the lumen of the organoids. Microarray and RT PCR was used to anaylse the host response.
Result: Troy and Lgr5 are Wnt target genes specifically expressed at the bottom of gastric glands. While Lgr5 marks stem cells in the antrum, in the corpus Troy and Lgr5 together mark a subpopulation of differentiated chief cells that can regain stem cell capacity upon damage. Mouse and human gastric stem cells can initiate ever-expanding 3 dimensional organoids in vitro. The organoids harbor stem cells, progenitor cells as well as four lineages of the stomach. The self-organize into domains harboring either lineages of the pit or the gland. Infection of organoids with Helicobacter pylori reveals cell-type specific inflammatory responses. Organoids grown from tumors as well matched healthy tissue allow evaluation of drug responses.
Conclusion: Human gastric organoids are a very useful source of differentiated primary cells as well as stem cells. Their 3-dimensional organization mimics the in vivo situation. Organoids can be infected by H. pylori and mount a strong inflammatory response. Organoids can be generated from virtually every patient, allowing the establishment of living biobanks. Organoids may in the future help to bridge the gap between mouse models and patient trials and allow patient specific treatments.

Presentation: Monday, 12 September 2016 from 18:00 – 18:15 in room Donausaal 4-5.

036/GIV

Multiplexed polymerase chain reaction (PCR) for the diagnosis of intestinal pathogens in patients with persistent digestive disorders: a case-control study from Mali

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Background: Persistent abdominal pain and persistent diarrhoea (≥2 weeks) are common and challenging symptoms in endemic settings that are characterised by poor sanitation and hygiene. Molecular diagnostic techniques such as polymerase chain reaction (PCR) are more sensitive than conventional methods and may improve the diagnosis of aetiologic agents of infectious intestinal disorders.
Method: From August 2014 to May 2015, stool samples from patients with persistent diarrhoea (≥2 weeks) and/or persistent abdominal pain (≥2 weeks) and matched asymptomatic controls were recruited during a prospective case-control study by the NIDIAG consortium in Niono, central Mali (http://www.nidiag.org). Stool samples were fixed in ethanol and transferred to Homburg, Germany, where multiplex real-time PCR assays (R-Biopharm; Darmstadt, Germany) were carried out for the detection of six pathogenic bacteria (*Campylobacter* spp., *Escherichia coli* pathovars, *Salmonella* spp. and *Yersinia enterocolitica*) and four intestinal protozoa (*Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba histolytica* and *Giardia intestinalis*).
Result: The study cohort comprised 553 patients and 547 asymptomatic controls. 79.8% of symptomatic patients and 75.0% of asymptomatic controls were diagnosed with at least one intestinal pathogen. Of note, ≥3 co-infections were found in 22.8% of the cases and 13.9% of the controls. The most frequently detected bacteria were enteroaggregative *E. coli* (EAEC) (39.9%) and *Campylobacter* spp. (35.3%), followed by enteroinvasive *E.coli* (EIEC; 15.5%) and enterotoxigenic *E. coli* (ETEC; 13.9%). *G. intestinalis* (20.5%) and *D. fragilis* (16.2%) were the most common parasites, while *Cryptosporidium* spp. and *E. histolytica* were rarely encountered. With the exception of *G. intestinalis* (27.3% *vs.* 13.5%, $p<0.001$), most pathogens were not significantly more frequent in cases than controls.
Conclusion: Stool-based multiplex PCR is a sensitive tool for the rapid detection of multiple pathogens but poorly discriminates symptomatic patients from matched controls. The high number of co-infections suggests that persistent digestive disorders in endemic settings are complex 'multi-pathogen events' rather than being caused by a single infectious agent.

Presentation: Monday, 12 September 2016 from 18:15 – 18:30 in room Donausaal 4-5.

037/GIV

Development and Application of a Novel Cag type IV Secretion Reporter Assay in *Helicobacter pylori*

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The chronic gastric pathogen *Helicobacter pylori* infects approximately half of the world population. It colonizes the gastric mucosa persistently, causing chronic gastritis, gastric or duodenal ulcers as well as gastric cancer and MALT lymphoma. The development of these malignant disorders is mostly due to the *H. pylori* Cag type IV secretion system. This molecular machine specifically transfers the bacterial protein CagA into gastric cells. Inside the host cell, CagA becomes phosphorylated and finally leads to changes in eukaryotic cell morphology and gene expression. CagA translocation is routinely analyzed by Western blotting to determine the level of phosphorylated CagA protein. Although this method has been used for years, it is time-consuming, labor-intensive and unsuitable for high-throughput screenings. We have established a novel reporter system which is based on a fusion of a TEM-1 β-lactamase to full-length CagA. TEM-1-CagA translocation into target cells is monitored by TEM-1-mediated conversion of the fluorescent β-lactam derivative CCF4. This fast, sensitive and highly specific method enables CagA translocation analysis by fluorescence microscopy, flow cytometry and fluorescence-assisted plate reading for high-throughput screening approaches. We applied this assay to study CagA translocation dynamics as well as the type IV signal recognition and secretion process. Moreover, we have performed medium-throughput screenings of different compound libraries to identify specific Cag type IV secretion inhibitors. This screening procedure resulted in preliminary identification of various compounds that were able to inhibit CagA translocation.

Presentation: Monday, 12 September 2016 from 18:30 – 18:45 in room Donausaal 4-5.

038/MPV

Hypoxia-induced dormancy in *Coxiella burnetii* is regulated by the activation status of Stat3

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The intracellular bacterium *Coxiella burnetii* is the causative agent of Q-fever, a zoonosis that can clinically manifest as acute Q fever or chronic Q fever. While acute Q fever is a mild flu-like illness, which can also develop into a severe pneumonia or hepatitis, chronic Q fever is characterized by bacterial persistence and mainly manifests as endocarditis. Although, axenic *C. burnetii* growth requires hypoxic conditions and tissue oxygen levels are low at sites of infection, information about the influence of hypoxia on intracellular fate and persistence of *C. burnetii* is lacking.
Therefore, we investigate the influence of hypoxia on *C. burnetii* replication in macrophages, their primary target cells in the body. Whereas bacteria grow readily in host cells under normoxic conditions, we demonstrate that *C. burnetii* cannot replicate in macrophages exposed to a hypoxic atmosphere. This replication defect is not caused by altered intracellular trafficking or by bacterial killing. Our results rather indicate that *C. burnetii* enters a dormancy period under hypoxic conditions. Importantly, our results further indicate that dormancy in *C. burnetii* occurs in response to altered activation status of the host cell transcription factor signal transducer and activator of transcription 3 (Stat3) under hypoxic conditions. Dormant bacteria might be less sensible to immune defense mechanisms or antibiotic treatment. Therefore, hypoxic areas could provide a niche for *C. burnetii* persistence. Thus, modulating the activation status of Stat3 might support the treatment of chronic Q fever.

Presentation: Monday, 12 September 2016 from 18:45 – 19:00 in room Donausaal 4-5.

039/MPV

Role of proteins belonging to the OMP biogenesis machinery for virulence of *Yersinia enterocolitica*

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The outer membrane (OM) of Gram negative bacteria acts as a physical barrier protecting Gram negative species from harmful environmental influences. At the same time it facilitates the import of nutrients, the export of proteins, the passage of signaling molecules and also harbors proteins associated with virulence. Transmembrane traffic particularly is facilitated by membrane-integral proteins. In order to insert these into the OM, an essential oligomeric membrane-associated protein complex, the beta-barrel assembly machinery (BAM) is required. Being essential for the biogenesis of outer membrane proteins (OMPs) the BAM machinery and also periplasmic chaperones (termed the OMP biogenesis machinery as an entity herein) may serve as attractive targets to develop novel antimicrobial or antiinfective agents. We aimed to elucidate which proteins belonging to the OMP biogenesis machinery have the most important function in granting bacterial fitness, facilitating biogenesis of dedicated virulence factors and determination of overall virulence. To this end we used the enteropathogen *Yersinia enterocolitica* (*Ye*) as a model system. We individually knocked out all non-essential components of the Bam machinery (BamB, C and E) as well as the periplasmic chaperones DegP, SurA and Skp. In summary, we found that the most profound phenotypes were produced by the loss of BamB or SurA with both knockouts resulting in significant attenuation or even avirulence of *Ye* in a mouse infection model. Thus, both BamB and SurA shall be addressed as targets for the development of new antimicrobials in the future.

Presentation: Monday, 12 September 2016 from 19:00 – 19:15 in room Donausaal 4-5.

040/MPV

A neonatal CNS infection model following mucosal challenge with *Listeria monocytogenes*

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Introduction: Bacterial infections with a manifestation in the central nervous system (CNS) represent an important cause of morbidity and mortality in neonates. However, mechanisms of host susceptibility, route of infection and underlying mechanisms of inflammation in the CNS remain ill-defined.

Objective: The aim of this study was to establish a model of neonatal CNS infection with *Listeria monocytogenes* following mucosal challenge in order to investigate the cellular and molecular mechanisms of bacterial tissue tropism and innate immune responses.

Materials and Methods: Neonatal C57BL/6 mice were infected intranasally with *L. monocytogenes*. To determine bacterial dissemination, pups were sacrificed at various time points and organs were obtained for replica plating. Tissue tropism and immune responses were analyzed by immunohistochemistry, electron microscopy, flow cytometry and qRT-PCR.

Result: In contrast to the established gastrointestinal tropism of *L. monocytogenes*, bacteria were mainly reisolated from the brain, particularly from the olfactory bulb and the cerebrum. Only very few *Listeria* were found in the cerebellum and the brain stem as well as in the blood, suggesting a non-hematogenous dissemination from the nasal cavity to the CNS. Once inside the cranium, *Listeria* induced a multifocal meningo-encephalitis as evaluated by histo-pathological examination. Interestingly, mucosal invasion was restricted to the olfactory epithelium and completely independent of the two major listerial invasins InlA and InlB. Nevertheless, electron microscopic examination clearly showed that, during early time points, *Listeria* resided in non-myeloid supporting cells. Later, wild-type bacteria were found to be associated with axon bundles projecting from the olfactory cavity to the CNS. In contrast, an isogenic *Listeria* mutant lacking ActA, which facilitates intracellular motility and cell-to-cell spread, was still able to induce internalization into the olfactory epithelium but was entirely restricted to the olfactory mucosa and could not overcome the cribriform plate. Once inside the brain, wild-type *Listeria* were targeted by various immune cells. Flow cytometric and immune-histochemical analyses showed an accumulation of bacteria with a concomitant recruitment of $CD45^+CD11b^+$ microglia/macrophages and a significant increase of infiltrating $Ly6C^+$ monocytes/macrophages. As expected, mRNA of key cytokines mediating intracranial inflammation and monocyte attraction, such as Tnfα, Cxcl2, Ccl2, and Ccl7, was highly upregulated.

Conclusion: The possibility to study neonatal CNS infection by *L. monocytogenes* following the natural route is essential for understanding onset and progression of disease. This robust and standardized *in vivo* model reflecting the pathogenesis of neonatal *Listeria* infections may, thus, be crucial for discovering new therapeutic approaches.

Presentation: Monday, 12 September 2016 from 19:15 – 19:30 in room Donausaal 4-5.

WORKSHOP 08
Virulence and epidemiology of zoonotic pathogens (FG ZO)
12 Sept. 2016 • 18.00–19.30

041/ZOV

Development of a virulence gene scoring system for ESBL-producing *E. coli* isolates from human and animal sources

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Extended-spectrum β-lactamase (ESBL)-producing multidrug-resistant *Escherichia (E.) coli* have become a significant problem in human and veterinary medicine. While many studies address the antimicrobial resistance characteristics of these bacteria, only few studies have investigated their virulence properties so far. This pilot study evaluates combining whole genome sequencing, a phenotypic virulence assay and statistical modelling for assessment the pathogenic potential of ESBL-producing *E. coli* strains from various sources.

All investigated *E. coli* isolates were submitted to next generation sequencing of whole genomes. Assembled contigs were queried using *in-silico* methods. This included screening for selected virulence and resistance genes as well as multi-locus sequence typing. Concatenated multi locus sequence typing (ConMLST) was used to uncover phylogenetic relationships between the isolates. Subsequently, 40 isolates (20 human and 20 animal isolates) were randomly chosen and tested for virulence in the *Galleria (G.) mellonella* model. To identify differences in larvicidal effects, Cox regression analysis was conducted and a stepwise multinominal logistic regression was used to identify over- or underabundant virulence factors. To evaluate the observed larvicidal effect in *G. mellonella*, growth in human serum was tested subsequently.

The majority of the tested 101 *E. coli* isolates carried genes or operons, respectively, encoding products that had been associated with bacterial iron metabolism, serum resistance and adhesion. More than half of the 40 more closely investigated isolates (56 %) were found to be clearly larvicidal as defined by killing larvae of *G. mellonella* within 24 hours after inoculation of 7.4×10^6 cfu of the respective *E. coli* isolate. Cox regression identified ConMLST phylogenetic group C as most larvicidal, exhibiting on average a 2.24-times higher risk of larvicidal effects ($p < 0.001$) than the least larvicidal phylogenetic group A. The sequence type cluster (STC) 23 was the most larvicidal STC being 2.24-times ($p < 0.001$) more larvicidal than STC 131. Occurrence of *iss* (HR = 2.52, $p < 0.001$) and *ompT* (HR = 2.29, $p < 0.001$) increased hazard, while *usp*, *sitA* (HR = -8.84; HR = -3.18, $p < 0.001$ for both) and *kpsMT II* (HR = -2.79, $p < 0.001$) gene cluster decreased the hazard for larvicidal effects in *G. mellonella*. Strains encoding only aerobactin (HR = 1.89, $p < 0.001$) show an increase in larvicidal effects compared to strains without any siderophore.

The identified genes and gene combinations are promising candidates to establish a genome based scoring system for predicting virulence of ESBL-producing *E. coli* strains in extraintestinal infection. Future studies will expand on these findings and serve to test and refine the preliminary scoring system.

Presentation: Monday, 12 September 2016 from 18:00 – 18:15 in room Konferenz 2-3.

042/ZOV

ESBL-plasmids influence chromosomally-encoded genes important for biofilm formation and motility in *E. coli*

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Backgrounds and Objectives: ESBL-producing *E. coli* have become abundant all over the world and especially clonal lineages of ESBL-associated STs 131 and 648 seem to play an important role. This study aimed to investigate the influence of ESBL-plasmid-encoded non-resistance genes on chromosomally-encoded features of ST131 and ST648 *E. coli*.

Materials and Methods: Seven triplets of ESBL-carrying wild-type (WT) strains, their corresponding ESBL-plasmid-"cured" variant (PCV) and a complementary ESBL-carrying transformant (T) where analysed in long-term colony, swimming motility and Omnilog® Phenotypic Microarray assays, whole-genome sequence and RNA sequence analysis.

Result: For some of the triplets we detected enhanced curli and/or cellulose production and a reduced swimming capacity of the WT and T strain compared to their PCV. RNA sequencing revealed the chromosomally-encoded *csgD*-pathway as a key factor involved. Omnilog® results pointed towards a similar metabolic behavior of WT, PCV and T.

Conclusion: Our phenotypic and RNA sequencing results clearly indicate an influence of ESBL-plasmids on the chromosomally encoded central hub (*csgD*) in the subtle interactions between a sessile and planktonic way of life in multi-resistant *E. coli*, presumably contributing to their pandemic success.

Presentation: Monday, 12 September 2016 from 18:15 – 18:30 in room Konferenz 2-3.

043/ZOV

Circulation of clonal *bla*$_{\text{CTX-M-15}}$-encoding fluoroquinolone-resistant *Escherichia coli* ST410 isolates in humans, animals, food and the environment in Germany

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Background: Multidrug-resistant *Escherichia coli* frequently harbour extended-spectrum beta-lactamase (ESBL) genes, thereby impairing the treatment options in case of an infection with bacteria carrying these genes. In particular, isolates coding for CTX-M-type ESBLs are often isolated from humans as well as from companion animals, livestock, wild animals and the environment, raising concern regarding exchange and spread of clonal isolates and/or plasmids between and among these host populations and habitats, respectively. To address this question, we performed a detailed molecular epidemiological analysis of CTX-M-15-producing *E. coli* isolated from various sources in Germany.
Materials and Methods: Whole genome sequencing using Illumina technology was performed for 121 CTX-M-15-producing *E. coli* isolates from humans (n=47), companion animals (dogs, cats, horses, n=25), livestock (pigs, cattle, poultry, n=26), food (n=16) and the farm environment (n=7). Based on the whole genome sequences, the multi-locus sequence type (ST) of these isolates and phylogeny based on single nucleotide polymorphisms (SNPs) were assessed. Additionally, genomes were scanned for recognized virulence genes.
Result: 26 STs were detected among the CTX-M-15-producing *E. coli* isolates. ST410 was the most frequent ST (31/121) and was detected in isolates from humans (n=9), companion animals (n=4), livestock (n=8), food (n=4) and farm environment (n=6). Based on SNP analysis, five clades (A-E) were identified within the ST410 isolates. Isolates of clade B were present in all four populations and their core genomes differed by less than 75 SNPs from each other. In addition, isolates of clade B and C were clonally marked by chromosomal insertion of the *bla*$_{\text{CTX-M-15}}$ gene either in the *rhs*E locus (clade B) or in a defective lambdoid prophage (clade C). Virulence genes found in these isolates were mostly related to iron acquisition and adhesion to eukaryotic cells. All ST410 isolates displayed a fluoroquinolone resistance.
Conclusion: Our data provides strong evidence for circulation of fluoroquinolone-resistant CTX-M-15-producing *E. coli* ST410 clones that are closely related to each other and display a broad-host-range including humans. As the isolates originated from diseased humans and animals, virulence of these isolates is assumed. Further studies will address the presence of these clones in other European countries by investigating a larger set of isolates.

Presentation: Monday, 12 September 2016 from 18:30 – 18:45 in room Konferenz 2-3.

044/ZOV
Cytotoxic action of Shiga toxin on human erythroid progenitor cells
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Introduction: Hemolytic anemia is associated with the onset of hemolytic-uremic syndrome (HUS), which patients develop upon infection with Shiga toxin (Stx)-producing enterohemorrhagic *Escherichia coli* (EHEC). The mechanism of hemolytic anemia is poorly understood and partially occluded blood vessels are believed as the major reason for mechanical erythrocyte disruption leading to intravascular hemolysis.
Objective: Here we questioned whether Stx2a is capable of targeting CD34$^+$ human stem/progenitor cells (HSPCs) and their erythroid-committed descendants.
Materials and Methods: We employed an *ex vivo* erythropoiesis model using cytokine-mobilized CD34$^+$ peripheral blood stem cells. Stx2a-mediated cellular injury and expression of Stx-receptors globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer) of *in vitro* propagated erythroid progenitor cells were monitored.
Result: CD34$^+$ HSPCs were negative for Stx-receptors and resistant toward Stx2a. Toxin sensitivity and receptor expression increased immediately after initiation of erythropoietic differentiation and peaked for basophilic and polychromatic erythroblast development accompanied by toxin-caused apoptosis and necrosis. Susceptibility to Stx2a declined during differentiation to orthochromatic erythroblasts and reticulocytes, which became less sensitive and resistant, respectively.
Conclusion: We conclude that Stx2a-caused hemolytic anemia might be the result of both intravascular hemolysis (mechanical damage) and extravascular hemolysis (in the bone marrow) underlying HUS-associated premature death of erythrocytes.

Presentation: Monday, 12 September 2016 from 18:45 – 19:00 in room Konferenz 2-3.

045/ZOV
The immunopathogenic potential of *Arcobacter butzleri* - more than a commensal?
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Background: Only limited information is available about the immunopathogenic properties of *Arcobacter* infection *in vivo*. Therefore, we compared the pathogenic potential of *Arcobacter butzleri* with *Campylobacter jejuni* and commensal *E. coli* as pathogenic and harmless reference bacteria, respectively.
Methodologies and Principal Findings: Gnotobiotic IL-10$^{-/-}$ mice generated by broad-spectrum antibiotic compounds were perorally infected with *A. butzleri* (strains CCUG 30485 or C1), *C. jejuni* (strain 81-176) or a commensal intestinal *E. coli* strain. Either strain stably colonized the murine intestines upon infection. At day 6 postinfection (p.i.), *C. jejuni* infected mice only displayed severe clinical sequelae such as wasting bloody diarrhea. Gross disease was accompanied by increased numbers of colonic apoptotic cells and distinct immune cell populations including macrophages and monocytes, T and B cells as well as regulatory T cells upon pathogenic infection. Whereas *A. butzleri* and *E. coli* infected mice were clinically unaffected, respective colonic immune cell numbers increased in the former, but not in the latter, and more distinctly upon *A. butzleri* strain CCUG 30485 as compared to C1 strain infection. Both, *A. butzleri* and *C. jejuni* induced increased secretion of pro-inflammatory cytokines such as IFN-γ, TNF, IL-6 and MCP-1 in large, but also small intestines. Remarkably, even though viable bacteria did not translocate from the intestines to extra-intestinal compartments, systemic immune responses were induced in *C. jejuni*, but also *A. butzleri* infected mice as indicated by increased respective pro-inflammatory cytokine concentrations in serum samples at day 6 p.i.
Conclusions and Significances: *A. butzleri* induce less distinct pro-inflammatory sequelae as compared to *C. jejuni*, but more pronounced local and systemic immune responses than commensal *E. coli* in a strain-dependent manner. Hence, data point towards that *A. butzleri* is more than a commensal in vertebrate hosts.

Presentation: Monday, 12 September 2016 from 19:00 – 19:15 in room Konferenz 2-3.

046/ZOV

Mass-spectrometry-based-Phyloproteomics (MSPP) of *Campylobacter fetus*

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Introduction: The bacterial species *Campylobacter fetus* divides up into three different subspecies. *C. fetus ssp. fetus* (*Cff*) is commonly associated with enzootic abortion especially in sheep and bacteremia in humans, whereas *C. fetus* ssp. v*eneralis* (*Cfv*) typically causes bovine genital campylobacteriosis causing fertility problems with considerable economic losses. Recently, a third subspecies - *C. fetus* ssp. t*estudinum* (*Cft*)- has been isolated from various reptile species, which is obviously a mere colonizer in these hosts.
Objective: Confirmation of the isolate and discrimination between the subspecies of *C. fetus* can be performed by laborious biochemical or molecular methods. In this study we aimed to establish avery fast and highly reliablemass-spectrometric technique calledMass-spectrometry-based-Phyloproteomics (MSPP) to replace these biochemical and molecular subtyping methods.
Method: A total number of 33 *C. fetus* isolates were analyzed performing MALDI-TOF-based intact cell mass spectrometry (ICMS) and evaluated to establish a *C. fetus*-MSPP scheme. Twenty isolates originated from colonized cattle, five from aborted calf fetuses, two from aborted sheep fetuses, one from a colonized pig and five were isolated from human blood. At all 21 *Cff*, 11 *Cfv* and 1 *Cft*have been analyzed.MLST was used as reference method.
Result: Based on the known genome sequences of three *C. fetus*isolates representing all three subspecies, 15 biomarker masses vis-à-vis 14 ribosomal proteins and a non-ribosomal protein were assigned to a particular gene locus.
Different isoforms in the detectable biomarkers resulting in biomarker mass shifts were associated with their amino acid sequences and included into the *C. fetus*-MSPP evaluation scheme.
Based on this isoform list we were able to differentiate all three subspecies and additionally the most significant MLST clonal complexes out of the nine clonal complexes represented in the examined isolate collection. Based on the combined amino acid sequences a MSPP-based UPGMA-tree was constructed.
Conclusion: In this study the principle of MSPP-typing, previously demonstrated on a *C. jejuni* ssp. *jejuni* and a *C. jejuni* ssp. *doylei* isolate collection, has been successfully adapted on *C. fetus*.
The relevant subspecies/MLST CC can be discriminated using MSPP. This confirms the hypothesis that the MSPP-method bears a high potential of an easy-to-perform typing method. An extension of the isoform database should be aspired in the future.

Presentation: Monday, 12 September 2016 from 19:15 – 19:30 in room Konferenz 2-3.

WORKSHOP 09
Multiresistant bacteria crossing borders: Migration, tourism, and more (FG PR)
13 Sept. 2016 • 08.30–10.00

047/PRV

Multidrug-resistant gram-negative bacteria in patients from abroad and refugee patients admitted to a German University Hospital: same but different?

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Question: Patients arriving from high-prevalence countries (HPC) for multidrug-resistant gram negative bacteria (MDRGN), assigned as ABROAD, and refugees (REF) recently (<1yr) arrived from HPC are predestined to carry MDRGN. Better knowledge of the epidemiology of MDRGN among ABROAD and REF is essential to define demands on specified and cost-effective measurements for infection control of these cohorts in hospital settings. Objective of this is to investigate the epidemiology of MDRGN in REF and ABROAD admitted to University Hospital Frankfurt (UHF), Germany, between December 2015 and May 2016.
Method: Totally, n=108 REF and n=73 ABROAD were screened for MDRGN by rectal swab on day of admittance. Rectal swabs were streaked onto selective CHROMagarTM ESBL plates (Mast Diagnostica, Paris, France). Identification of MDRGN species, antibiotic susceptibility testing and detection of carbapenemase encoding genes in case of carbapenem resistance (CR) were conducted as previously described [1]. MDRGN was defined as previously described [1].
Result: The demographic characteristics of REF and ABROAD are different. In REF 52.8% samples (CI_{95}=42.9-62.5) were positive for at least one MDRGN, thereof 1.6% (0.0-8.7) with CR. In ABROAD 39.7% samples (28.5-51.9) were positive for at least one MDRGN, whereof 41.4% (23.5-61.1) were positive for at least one MDRGN with CR. In total, n=17 MDRGN with CR were detected in ABROAD, thereof the most frequently detected MDRGN with CR was *A. baumannii* (47.1%; 22.9-72.2).
Conclusion: MDRGN prevalence in both cohorts is higher than in patients living in the Rhine-Main-region and admitted to an intensive/intermediate care unit at UHF (16.7%). Prevalence of MDRGN with CR in REF is significantly lower than in ABROAD. At UHF, REF and ABROAD are screened and pre-emptively isolated on day of admittance. For both groups, diligent hygiene management is needed.

Reference

[1] Reinheimer C, Kempf V, Göttig S, Hogardt M, Wichelhaus T, O'Rourke F et al. Multidrug-resistant organisms detected in refugee patients admitted to a University Hospital, Germany June–December 2015. Euro Surveill. 2016;21(2):pii=30110.

Presentation: Tuesday, 13 September 2016 from 8:30 – 8:45 in room Donausaal 3.

048/PRV

Detection of extended spectrum betalactamases and carbapenemases in isolates from patients and returning soldiers from international war and crisis zones

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Introduction: The Medical Service of the German Armed Forces is in regular contact with patients and returnees from war and crisis zones that are colonized or infected with multidrug resistant pathogens. The predominant causative organisms are Gram-negative rod-shaped bacteria. This contribution gives an overview of the detected extended spectrum betalactamases (ESBL) and carbapenemases in respective isolates from the years 2007 till 2015.

Method: Beta-lactamase producing Gram-negative isolates from patients from Libya, Syria and Ukraine who were treated at German Armed Forces Hospitals, from German soldiers returning from mostly (sub)tropical sites of deployment and from European soldiers deployed in Western African Mali were included in the assessment. Copy strains were excluded by rep-PCR typing. The isolates were subjected to in-house multiplex PCRs targeting frequent ESBL enzymes (bla_{TEM} and bla_{SHV} with subsequent Sanger sequencing to exclude non-ESBL variants, bla_{CTX-M} divided in the groups I-IV [Bonnet 2004; Pitout et al. 2004] by different PCRs) and carbapenemases (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{AIM}, bla_{DIM}, bla_{GIM}, bla_{SIM}, bla_{KPC}, bla_{BIC}, and bla_{OXA-48}).

Result: Although there were region-specific variations, bla_{CTX-M} group I and bla_{SHV} enzymes predominated among the ESBL-positive strains; bla_{CTX-M} group IV was considerably less frequent. Detected bla_{TEM} enzymes were not associated with ESBL phenotypes. In ESBL-positive strains directly isolated in Mali from European soldiers, bla_{CTX-M} group I remained the only detectable mechanism. Identified carbapenemases comprised bla_{NDM}, bla_{OXA-48}, bla_{BIC}, and bla_{VIM}.

Discussion: The Medical Service of the German Armed Forces has to deal with an influx of multidrug resistant pathogens due to the treatment of patients and returnees from high endemicity sites. Military medical surveillance can help to provide insights in the distribution of resistance mechanisms in areas that are otherwise difficult to assess due to ongoing conflicts.

References

[1] Bonnet R (2004) Growing group of extended-spectrum betalactamases: the CTX-M enzymes. Antimicrob Agents Chemother 48, 1-14.
[2] Pitout JD, Hossain A, Hanson ND (2014) Phenotypic and molecular detection of CTX-M-beta-lactamases produced by *Escherichia coli* and *Klebsiella* spp. J Clin Microbiol 42, 5715-5721.

Presentation: Tuesday, 13 September 2016 from 8:45 – 9:00 in room Donausaal 3.

049/PRV

Current situation of antibiotic resistance in refugees in the Saarland

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The global dynamics of colonization and infection with bacteria resistant to antibiotics are still quite unclear. Especially the MRSA situation and the increase of antibiotic resistance in gram-negative bacteria is of high interest in the health system. A number of factors play a role, mostly not well studied and/or documented. Around the globe, the rate of antibiotic-resistant bacteria is quite variable. Different local circumstances induce persistent country- or region-specific resistance clusters.

By evaluating the microbiological results of refugees shortly after their arrival, the hypothesis of the presence of indigenous resistance clusters in their homelands is to be investigated.

For 2015, the official statistics for Germany show a total of 1.091.894 asylum seekers. In the Saarland, the smallest non-city federal state, 25.596 asylum seekers were recorded (2.3%), 87% from Syria, 3% from Afghanistan, 3% from Iraq, and 7% from other countries. In the refugee reception center in the Saarland a medical check is performed upon arrival with hospital admissions as medically indicated. We evaluated the available microbiological data of refugees for inpatient treatment from January 2015 to May 2016. Our laboratory serves several hospitals in the area, representing approx. 40% of all inpatients in our state.

Of 92 anal/rectal swabs for screening of MRGN*, 16 were 3MRGN positive (17.4%), and an additional 22 were positive for 2MRGN (24%). In the same period, the ICU patients of a tertiary care hospital in the Saarland served by our laboratory had a prevalence for 3MRGN of 3.7%.

Of 79 nasal/throat swabs for screening of MRSA, 13 were positive (16.5%). In the same period, the ICU patients of the above mentioned hospital had a prevalence for MRSA of 2.1%. Former studies on MRSA prevalence in the Saarland showed positivity rates of 2.2% on hospital admission (2010), and 4.8% in long term care facilities (2013/14).

All results show a burden with antibiotic resistant bacteria significantly higher in refugees than in the native high risk population.

Examinations of refugees from non-European regions regularly show colonization with antibiotic-resistant bacteria in a higher proportion than the native population in Germany. It is unclear if acquisition occurs in the home countries or on the run. Many of these resistance clusters appear to have formed independently of nosocomial selection pressure. The *Acinetobacter baumannii* strain, which became known as Iraqibacter in 2003, and the USA300 strain of community acquired MRSA are examples of the emergence and persistence of certain types of resistance in geographically circumscribed areas. We must bear in mind that an epidemiologically relevant spreading of resistance can arise by migration.

*3MRGN = gram-neg. rods with resistance against penicillins, 3rd gen. cephalosporines and quinolones, 2MRGN = scheme for pediatrics without the attention to quinolones.

Presentation: Tuesday, 13 September 2016 from 9:00 – 9:15 in room Donausaal 3.

050/PRV

Prevalence of multidrug-resistant bacteria in refugees: a retrospective 12-month surveillance study in a university pediatric hospital in Germany

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Introduction: The global prevalence of colonization with multidrug-resistant bacterial organisms differs considerably. High rates of colonization with ESBL-producing *Enterobacteriaceae*

have been reported from the Middle East. As newly arrived refugees originate mainly from this area it can be hypothesized that the prevalence of colonization with certain kinds of multidrug-resistant organisms might be higher in the refugee population.
Aim: To determine the prevalence of colonization with multidrug-resistant bacteria in refugees that arrived in Germany within the previous 6 months.
Materials and Methods: Between 01-01-2015 and 31-12-2015 148 pediatric patients from refugee accommodations were admitted to our hospital. A policy of screening for multidrug-resistant organisms at admission in this patient population was instituted. Screening for the presence of MRSA, *Enterobacteriaceae* with resistance to oxyimino-cephalosporins or carbapenemase-producing *Enterobacteriaceae* was performed with selective media.
Result: The age range of the patients fell between 1 day and 16 years with a median age of 3 years. 60.8% of the patients were male. A previous hospitalization was not known for any of the patients. Screening for MRSA colonization at admission was performed in 64 (43.2%) patients, with positive results in 10 patients (15.6%). 126 patients (85.1%) were screened for oral or rectal colonization with multidrug-resistant Gram-negative bacteria, 78 (61.9%) of whom had any positive result. *Escherichia coli* with resistance to cefotaxime and/or ceftazidime accounted for the 74 (94.9%) of these results and concomitant resistance to fluoroquinolones was present in 32.4%. The remaining patients were colonized with *Klebsiella pneumoniae* resistant to cefotaxime and/or ceftazidime. 8 patients were colonized by both *E. coli* and *K. pneumoniae* with resistance to oxyimino-cephalosporins. In one of these patients the *K. pneumoniae* isolate produced an OXA-48 carbapenemase.
Summary: The prevalence of colonization with MRSA as well as with *E. coli* and *K. pneumoniae* displaying resistance to cefotaxime and/or ceftazidime in pediatric refugee patients is considerably higher than in patients resident in Germany. Worryingly, even one carbapenemase-producing *K. pneumoniae* was detected in the rectal swab of one patient. Screening for colonization with multidrug-resistant bacteria might be important not only for infection control purposes but also to guide empiric treatment and will be continued at our institution.

Presentation: Tuesday, 13 September 2016 from 9:15 – 9:30 in room Donausaal 3.

051/PRV

Prevalence of active tuberculosis in migrants: a prospective 12 month surveillance study in Germany

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Introduction: In the European Union tuberculosis case notification rates are among the lowest in the world. The prevalence of active tuberculosis in migrant populations has to be analysed to get valid data for the decision of screening activities.
Aim: Therefore, we prospectively quantified the risk of active tuberculosis among migrants at time of arrival screening. Results may help assessing the impact of active screening for tuberculosis, defining more precisely the need for infection control strategies and allowing timely tuberculosis therapy.
Method: Investigation was performed as regular part of the admission screenings for people arriving at Friedland, Germany, a primary major receiving center during one year.
Result: In 11.773 refugees 16 x-ray investigations gave the suspicion of active tuberculosis, thereof 11 cases could be verified, thereof 9 cases were classified as microscopically positive. This data translate into rates of 136 per 100.000 suspected cases, 93 per 100.000 verified cases, and finally 76 per 100.000 infectious cases, respectively. Prevalence was higher in asylum seekers coming from Russia and Eritrea compared to the main origins of current migration Libya, Afghanistan and Iraq. One case of MDR-tuberculosis could be detected in a migrant from Russia. The incidence did not vary with the season.
Conclusion: Prevalence rates of tuberculosis in asylum seekers are higher than in the many native European populations. Rates seem to reflect the prevalence in the home countries. X-ray investigation during first examination seems to be a valuable component in detecting infectious tuberculosis cases and therefore may prevent transmission and allow timely tuberculosis therapy.

Presentation: Tuesday, 13 September 2016 from 9:30 – 9:45 in room Donausaal 3.

052/PRV

Antimicrobial resistance markers in *Klebsiella pneumoniae* clinical isolates

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Introduction: The number of hospital-acquired infections caused by antimicrobial-resistant pathogens is increasing world-wide. Management of nosocomial infections, in particular those caused by multidrug-resistant Gram negative bacteria, requires a strategy that includes not only the development of new anti-microbial compounds and a rational use of anti-microbial agents, but also early diagnosis for targeted treatment and the implementation of effective infection control principles.
Aim: Here, we analyzed the Resistome of clinical *K. pneumoniae* isolates retrieved from the "National Reference Laboratory for multidrug-resistant Gram-negative bacteria for Germany" by whole genome sequencing (WGS) to deliver a broad overview over the most prevalent mechanisms of antimicrobial resistance (AMR) and the phylogeny of these multi-drug resistant isolates.
Materials and Methods: DNA libraries of 89 clinical *K. pneumoniae* isolates were prepared using the NEBNext Ultra DNA Library Kit and sequenced on an Illumina MiSeq with an average coverage of 40-fold. Genomes were assembled using the A5 assembly pipeline and AMR markers where identified i) by BLASTing all genomes against the Comprehensive Antibiotic Resistance Database (CARD) and ii) by mapping the reads against the Arg-ANNOT database via srst2. srst2 was also used to perform *in silico* Multilocus Sequence Typing (MLST). Antibiotic susceptibility testing was performed by agar dilution.
Result: WGS revealed the presence of several fluoroquinolone resistance markers, predominantly chromosomal mutations in *gyrA* and *parC, b*ut also several plasmid mediated quinolone resistance determinants like qnrA, qnrB, qnrS and the aminoglycoside modifying enzyme AAC(6')-Ib-cr, which also confers reduced susceptibility to quinolones. Identified carbapenemases were of types KPC (KPC-2 and KPC3), NDM-1, OXA-48 and VIM (VIM-1 and VIM-2). Furthermore, we identified extended-spectrum beta-lactamases like CTX-M-14 and CTX-M-15, CMY-4, DHA-1 and several types of OXA-, SHV-, and TEM-(extended-spectrum) beta-

lactamases. Several aminoglycoside modifying enzymes were also identified like AAC(3')-IIa/c, AAC(6')-Ib/-Ib-cr, ANT(2")-Ia and APH(3')-Ia and -VIa, respectively.
In-silico sequence typing revealed the presence of high risk sequence types (STs) 101 (n=14), 14 (n=7), 15 (n=6), 258 (n=6), 395 (n=5) and 512 (n=4) with 31 different STs in total and four additional, yet undescribed STs.
Discussion: Here, we were able to study the phylogeny and the presence of AMR conferring mutations and horizontally acquired genes of a broad collection of clinical *K. pneumoniae* isolates and linked these information with the antibiotic susceptibility profiles. Therefore, we were able to generate data, which might be useful in the combat against the current crisis in antimicrobial resistance.

Presentation: Tuesday, 13 September 2016 from 9:45 – 10.00 in room Donausaal 3.

WORKSHOP 10
Adhesins and pathogen-induced host signaling (FG MP)
13 Sept. 2016 • 08.30–10.00

053/MPV

The role of factor H polymorphism in complement evasion by malaria parasites

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Background: The alternative pathway of human complement (alternative complement pathway; ACP) is the first line of defense against microbial invaders, but might be harmful for self-cells. In order to prevent any damage by the ACP, human cells use a variety of membrane-anchored and fluid-phase regulators, the most prominent of which is factor H (FH), which is present in human body fluids. During host-pathogen co-evolution, many microbes developed the ability to bind regulators such as FH to imitate human cells and to thus avoid complement-mediated lysis. Recent studies have shown that the blood stages of the human malaria parasite *Plasmodium falciparum* hijack FH to inactivate C3b and to block the further ACP cascade. Noteworthy, FH is prone to single nucleotide polymorphisms (SNPs), including the replacement of tyrosine (Y) by histidine (H) at amino acid 402. This FH-SNP has been related to autoimmune defects like age-related macular degeneration (AMD). To date, nothing is known about the role of FH-SNPs in *P. falciparum* ACP evasion and the potential link between AMD and *falciparum* malaria in Africa.
Objective: We aim to evaluate whether the FH-SNP Y(402)H impairs ACP evasion by *P. falciparum*, using recombinant FH variants as well as sera from Ghanaian donors that exhibit different FH-SNP variants. In follow-up work we intend to unveil potential links between the incidences of FH-SNPs and malaria infections in four different malaria-endemic regions of Ghana.
Method: Genotyping, Western blotting and ELISA were performed to determine the presence of FH-SNP Y(402)H variants in the sera of the Ghanaian donors. The effect of FH-SNP Y(402)H on plasmodial complement evasion was investigated by quantitative Western blotting and ELISA as well as cofactor and decay-accelerating activity assays. To observe parasite viability, *in vitro* replication assays were conducted using cell culture medium supplemented with the recombinant FH variants and the different sera samples.
Result: Our current data indicate that recombinant FH-H(402) binds more efficiently to *P. falciparum*-infected red blood cells than FH-Y(402). In consequence, C3b inactivation appears to be more effective, when the infected red blood cells were incubated with recombinant FH-H(402) compared to incubation with FH-Y(402), suggesting that the FH-SNP H(402) promotes plasmodial complement evasion more efficiently. Furthermore, ELISA demonstrated the presence of 35% fh-yy and 65% fh-yh alleles, but the absence of the fh-hh allele, in the sera of the Ghanaian blood donors (n = 27). This is in accord with reports by others that AMD is more prevalent in Caucasians than Africans.
Conclusion: Our current data indicate that the FH-SNP H(402) mediates ACP evasion by the *P. falciparum* blood stages more efficiently that Y(402). Noteworthy the presence of the FH-SNP H(402) variant is uncommon in Africans and it remains to be elucidated, if FH-SNP Y(402) might have an immune-protective

effect by preventing severe malaria infections in people of malaria-endemic Africa.

Presentation: Tuesday, 13 September 2016 from 8:30 – 8:45 in room Donausaal 4-5.

054/MPV

Supra-cytotoxic-concentrations of α-toxin are required to activate ADAM10

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Introduction: Staphylococcus aureus α-toxin serves as an important virulence factor in lethal pneumonia in mice. The effect is partially attributed to ADAM10-dependent cleavage of E-cadherin, resulting in the disruption of epithelial barrier function (1). In vitro, the effect was recapitulated with a human cell line (1), and it was reported to occur at sublytic concentrations of α-toxin. Notably however, murine cells are generally rather tolerant to α-toxin, while human epithelial cells are susceptible.
Aim: Here, we wished to directly compare doses of α-toxin required for cytotoxicity and activation of ADAM10, respectively, in the same cell type.
Materials and Methods: We studied the response of human HaCaT cells to purified wild type α-toxin. Cellular ATP levels, a sensitive measure of cytotoxicity at sub-cytocidal toxin-concentrations, were measured using a luciferase assay. Cleavage of E-cadherin and activation of p38MAPK were detected by Western-blot. Changes in cellular Ca^{2+}-concentration were recorded fluorometrically.
Result: We show that α-toxin concentrations causing efficient cleavage of E-cadherin in HaCaT cells are far higher than concentrations needed to cause massive loss of ATP. Processing of E-cadherin and activation of p38MAPK at supra-cytotoxic concentrations of α-toxin were blocked by treatment with divalent cation chelator EGTA, although activation of p38MAPK by low concentrations of α-toxin remained unaffected (2). We conclude that supra-cytotoxic concentrations of α-toxin are required to trigger E-cadherin cleavage, presumably because only under these conditions a portal of entry is opened which allows rapid increase of intracellular Ca^{2+} concentration.
Summary: At ~10 nM, α-toxin exerts significant cytotoxicity to human epithelial cells; but efficient cleavage of E-cadherin requires µM concentrations.

References

[1] Inoshima I, Inoshima N, Wilke GA, Powers ME, Frank KM, Wang Y, Bubeck Wardenburg J. A Staphylococcus aureus pore forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. Nat Med. 2011;17(10):1310-4.
[2] von Hoven G, Rivas AJ, Neukirch C, Klein S, Hamm C, Qin Q, Meyenburg M, Füser S, Saftig P, Hellmann N, Postina R, Husmann M. Dissecting the role of ADAM10 as a mediator of S. aureus alpha-toxin action. Biochem J. 2016.

Presentation: Tuesday, 13 September 2016 from 8:45 – 9:00 in room Donausaal 4-5.

055/MPV

A common pattern of molecular interactions: Pneumococcal adhesins interacting with fibronectin type III repeats

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Introduction: *Streptococcus pneumoniae* (the pneumococcus) is a Gram positive commensal of the human upper respiratory epithelia. Pneumococci employ versatile strategies to successfully reside as a commensal in the nasopharyngeal cavity. However they are in particular feared for their high capacity to cause severe infections. Major strategies include the expression and exposure of various MSCRAMMS interacting specifically with host ECM components to promote the interaction of pneumococci with host eukaryotic cells. Pneumococcal adherence and virulence factor A (PavA) and B (PavB) represent two major MSCRAMM. Present study unravels the molecular interaction of PavA and PavB with the type III repeats of the human adhesive glycoprotein fibronectin (Fn).
Materials and Methods: Recruitment of fibronectin by pneumococci was assessed by immunoblots and flow cytometry. Direct protein-protein interaction approaches such as Far western ligand overlay and Surface Plasmon Resonance (SPR, BiacoreT100) were employed to delineate the interaction of pneumococcal FnBPs PavA and PavB with heterologously expressed FnIII repeats. To narrow down the interaction domains in all Fn type IIII repeats, synthetic peptide arrays consisting of immobilized 15mer Fn-peptides spanning FnIII1-15 were employed in a ligand overlay assay with PavA and PavB. Potential interaction domains were synthesized and binding to PavA and PavB respectively was investigated via SPR (BiacoreT100). In addition the topology of the recognized epitopes via peptide array analysis was identified by using specific softwares Fibcon and by Target-Template Alignment mode of Swiss-Model.
Result: Flow cytometric analysis confirmed pneumococcal sequestration of fibronectin from human plasma in a dose-dependent manner. Far western blots and SPR studies further indicated that pneumococcal FnBPs preferentially target FnIII repeats for their interaction with human Fn. Peptide array analysis and binding studies using short Fntype III derived peptides identified potential hot spots in FnIII repeats for pneumococcal PavA and PavB. The epitopes identified were topologically found to cluster on the inner strands of both b-sheets which form the fibronectin domains. Strikingly, both FnBPs exploited, despite that they are structurally completely diverse, similar domains in Fntype III repeats for their interaction with the fibronectin molecule.
Conclusion: Pneumococci interact with the human adhesive glycoprotein fibronectin by preferentially targeting the Fntype III repeats via its FnBPs PavA and PavB. Present study and the experimental approaches revealed a common pattern of molecular interactions between pneumococcal FnBPs and human FN. Specific epitopes recognized in this study can potentially be used as antimicrobial targets in further scientific endeavors.

Presentation: Tuesday, 13 September 2016 from 9:00 – 9:15 in room Donausaal 4-5.

056/MPV

Molecular determinants involved in extracellular matrix binding protein mediated *Staphylococcus epidermidis* adherence to fibronectin

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The great majority of implant-associated, nosocomial infections are caused by usually harmless skin commensal *Staphylococcus epidermidis*. The opportunistic pathogenicity of *S. epidermidis* is essentially linked with biofilm formation on artificial surfaces. During biofilm formation, attachment of *S. epidermidis* to surface organized fibronectin (Fn) is of crucial importance. The Extracellular Matrix Binding Protein (Embp) is known to be a fibronectin binding protein. We addressed the question to which extent the modular organization of Embp contribution to FN binding. Indeed, in wild type *S. epidermidis* 1585 Embp-expression was essential for adherence to immobilized Fn. Bioinformatic analysis of the Embp primary amino acid sequence predicted the presence of 21 **F**ound in **V**arious **A**rchitectures (FIVAR) modules while additional experimental evidence suggested 170 amino acid (aa) repetitive units in this area. Moreover, in the central part 38 alternating FIVAR and G-related albumin binding sites (GAFIV) where expected comprised of 125 aa repeating units. Recombinant subfragments taken from FIVAR or GAFIV region provided biochemical evidence for a direct interaction between Embp and Fn. *In trans* expression of FIVAR modules in surrogate host *S. carnosus* TM300 proved that this interaction is also sufficient for bacterial adherence to surface organized Fn. Surprisingly, expression of Embp was not sufficient for recruitment of soluble Fn to the bacterial cell surface, a key feature of well characterized Fn-binding protein A (FnBPA) from *S. aureus*. Thus, we speculated that Embp uses a Fn-interaction mode different from FnBPA, itself binding to N-terminal Fn type I domains. Far western blotting experiments in which recombinant overlapping Fn subdomains were immobilized and recombinant Embp was used as a ligand provided clear evidence that Embp does not interact with Fn type I_{1-5} but closely to the Hep-2-binding site containing Fn type III subdomains 12-14. Since Embp expressing *S. epidermidis* 1585 and *S. carnosus* TM300 bound to immobilized Fn III_{12-14}, this interaction is clearly also relevant for Embp-mediated adherence of *S. epidermidis* to Fn. However, since Hep 2-domain specific mAB FNH3-8 did not inhibit *S. epidermidis* adherence to Fn, Embp and heparin binding sites are obviously independent. To further confirm this interaction binding of recombinant Embp fragment to a Fn type III_{12}-peptide library could be demonstrated. Embp is, thus, a staphylococcal Fn binding protein using a novel mode of interaction involving Fn type III_{12} domain.
Since Fn type III_{12} is cryptic in the soluble Fn state but becomes accessible during Fn fibrillogenesis, our data suggest that the Fn conformation state has a profound influence on *S. epidermidis* - Fn interactions. This could have important implications for the pathogenesis of implant associated *S. epidermidis* infections.

Presentation: Tuesday, 13 September 2016 from 9:15 – 9:30 in room Donausaal 4-5.

057/MPV

Infection of epithelial cells with Chlamydia trachomatis inhibits TNF-induced apoptosis at the level of receptor internalisation while leaving non-apoptotic TNF-signalling intact.

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Chlamydia trachomatis is an obligate intracellular bacterial pathogen of medical importance. C. trachomatis develops inside a membranous vacuole in the cytosol of epithelial cells but manipulates the host cell in numerous ways. One prominent effect of chlamydial infection is the inhibition of apoptosis in the host cell, but molecular aspects of this inhibition are unclear. Tumour necrosis factor (TNF) is a cytokine with important roles in immunity, which is produced by immune cells in chlamydial infection and which can have pro-apoptotic and non-apoptotic signalling activity. We here analysed the signalling through TNF in cells infected with C. trachomatis. The pro-apoptotic signal of TNF involves the activation of caspase-8 and is controlled by inhibitor of apoptosis proteins (IAPs). We found that in C. trachomatis-infected cells, TNF-induced apoptosis was blocked upstream of caspase-8 activation even when IAPs were inhibited or the inhibitor of caspase-8-activation, cFLIP, was targeted by RNAi. However, when caspase-8 was directly activated by experimental over-expression of its upstream adapter FADD, C. trachomatis was unable to inhibit apoptosis. Non-apoptotic TNF-signalling, in particular the activation of NF-κB, initiates at the plasma membrane while the activation of caspase-8 and pro-apoptotic signalling occur subsequently to internalisation of TNF receptor and the formation of a cytosolic signalling complex. In C. trachomatis infected cells, NF-κB-activation through TNF was unaffected while the internalisation of the TNF-TNF-receptor complex was blocked, explaining the lack of caspase-8-activation. These results identify a dichotomy of TNF signalling in C. trachomatis-infected cells: apoptosis is blocked at the internalisation of the TNF receptor but non-apoptotic signalling through this receptor remains intact, permitting a response to this cytokine at sites of infection.

Presentation: Tuesday, 13 September 2016 from 9:30 – 9:45 in room Donausaal 4-5.

058/MPV

Role of HIF-1α in Infections with *Streptococcus pneumoniae* at the Blood-Brain Barrier

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The homeostasis of the central nervous system is maintained by the blood-brain barrier (BBB), which is made up of the microvascular endothelial cells and supported by pericytes and astroglia. The BBB functions to protect the brain from circulating toxins and delivers nutrients. In bacterial meningitis with *Streptococcus pneumoniae* (SPN), the BBB breaks down leading to the infection of the brain parenchyma and severe neurological sequelae.

Untreated meningitis is almost always fatal which highlights the need to better understand this deadly disease. Mechanisms underlying the transmission of meningeal pathogens across the BBB are still poorly understood. Based on our previous reports showing a general activation of *hypoxia inducible factor* (HIF) -1α in bacterial infections, we hypothesized that the activation of HIF-1α leading to secretion of vascular endothelial growth factor (VEGF) is involved in the invasion process of pathogens across the BBB. To test our hypothesis, we performed pneumococcal infections of brain ECs *in vitro* and permeability assays of EC monolayers using dextrans of various molecular sizes. Localization of the *S. pneumoniae* on ECs was visualized using confocal immunofluorescence microscopy. Expression of several components of EC cellular junctions (claudins, occludins) was analyzed by quantitative (q) RT-PCR and Western blotting. Our results demonstrate an increase in paracellular permeability of EC monolayers to dextrans of various sizes. Immunofluorescence analysis showed localization of pneumococci predominantly at the cell-cell junctions, qRT-PCR analysis revealed an upregulation of HIF-1α and VEGF. Western blot demonstrated downregualtion of VE-cadherin, adherens junctions member and upregulation of HIF-1α upon infection *in vitro*. These results suggest a paracellular approach for pneumococcal transmigration, potentially involving HIF-1 activation in ECs. Furthermore, *in vivo* analysis of human and murine brain tissue samples demonstrated upregulation of HIF-1α in the brain vessels in meningeal infections indicating HIF-1α as a potential mediator of vessel permeability. To determine the functional role of HIF-1α in the translocation of pneumococcal pathogens, we performed *loss-of-function* experiments by using the HIF-1α inhibitor echinomycin and HIF-1a *si*RNA. Our results indicate a protective effect of HIF-1 inhibition on vessel permeability upon infection in human and murine brain ECs. Expression for cell-junction-associated proteins and permeability studies *in vivo* upon infection in EC-specific HIF-1 knock-out mice are currently underway.

This work is funded by DFG-SFB 815 (Redox-Regulation) program.

Presentation: Tuesday, 13 September 2016 from 9:45 – 10.00 in room Donausaal 4-5.

HAUPTSYMPOSIUM 03
Cellular Microbiology
13 Sept. 2016 • 11.30–11.45

059/INV

Sensing the enemy within: Innate Immune recognition of intracellular bacteria

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The AIM2 inflammasome detects double-stranded DNA in the cytosol and induces caspase-1-dependent pyroptosis as well as release of the inflammatory cytokines interleukin 1β (IL-1β) and IL-18. AIM2 is critical for host defense against DNA viruses and bacteria that replicate in the cytosol, such as Francisella tularensis subspecies novicida (F. novicida). The activation of AIM2 by F. novicida requires bacteriolysis, yet whether this process is accidental or is a host-driven immunological mechanism has remained unclear. By screening nearly 500 interferon-stimulated genes (ISGs) through the use of small interfering RNA (siRNA), we identified guanylate-binding proteins GBP2 and GBP5 as key activators of AIM2 during infection with F. novicida. We confirmed their prominent role in vitro and in a mouse model of tularemia. Mechanistically, these two GBPs targeted cytosolic F. novicida and promoted bacteriolysis. Thus, in addition to their role in host defense against vacuolar pathogens, GBPs also facilitate the presentation of ligands by directly attacking cytosolic bacteria.

Presentation: Tuesday, 13 September 2016 from 11:45 – 12:15 in room Donausaal 3.

060/INV

Cytosolic immune responses of host hepatocytes against *Plasmodium* infection

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Xenophagy is a selective autophagy process that targets intracellular pathogens. To investigate possible xenophagy processes during the non-pathogenic liver stage of *Plasmodium* parasites, we infected human cell lines expressing the autophagy marker protein LC3 coupled to GFP with *Plasmodium berghei* sporozoites. Invasion was accompanied by the attempt of the host cell to isolate the parasite in an LC3-positive compartment. To confirm that this is a physiological event, we infected transgenic mice expressing LC3-GFP in hepatocytes and again observed a strong accumulation of LC3 in a membrane around the parasite. Interestingly, this membrane is not a newly generated autophagosome but instead LC3 is incorporated into the parasitophorous vacuole membrane (PVM). To decipher the molecular events resulting in LC3 incorporation into the PVM, we used the CRISPR-Cas9 editing system to knockout genes crucially involved in different autophagy processes. We generated gene knockouts for FIP200 and Atg5 in human HeLa cells. Infection of Atg5- and FIP200-deficient cells with *Plasmodium* sporozoites showed that whereas Atg5 was essential for LC3-labelling of the PVM, the process was independent of a functional FIP200. This suggests that LC3-labelling of the *Plasmodium* PVM follows a non-canonical autophagy pathway. Additional experiments indicate that in *Plasmodium*-infected hepatocytes, selective autophagy and starvation-induced canonical autophagy compete for the same autophagy-related molecules. Activation of canonical autophagy led to higher parasite survival during the liver stage suggesting the host cell gives preference to starvation-induced autophagy over xenophagy. Importantly, even solidly attenuated parasites can survive in starved cells and proceed to the pathogenic blood stage.

This observation has far reaching consequences for the generation of attenuated *Plasmodium* parasites as live vaccines.

Presentation: Tuesday, 13 September 2016 from 12:15 – 12:45 in room Donausaal 3.

061/INV

Bug Extermination: New Immune Strategies for Poking Holes in Pathogens

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Microbial infection triggers potent innate and adaptive immune responses. Bacterial products, such as LPS or flagellin, in the host cytosol signal pathogen invasion, which activates potent immune alarms in macrophages, dendritic cells and mucosal epithelial cells. These alarms, called inflammasomes, activate inflammatory caspases (caspases 1/4/5/11) to trigger an inflammatory death (pyroptosis) and release of inflammatory cytokines. An important substrate of the inflammatory caspases is Gasdermin D. Mice genetically deficient in gasdermin are resistant to sepsis. Gasdermin D cleavage causes pyroptosis thorough an unknown mechanism. We found that the N-terminal fragment of gasdermin D (GSDMD-NT) forms membrane pores that destroy the integrity of the plasma membrane, leading to the release of cytosolic proteins, including GSDMD-NT itself. GSDMD-NT binds to phospholipids on the inner leaflet of the cell membrane and to cardiolipin on bacterial membranes. Because of its lipid-binding preferences, GSDMD-NT kills from within the cell, but does not harm neighboring mammalian cells. GSDMD-NT also directly kills intracellular and cell-free bacteria in vitro. Thus GSDMD-NT not only kills infected or injured mammalian cells, but also kills the bacteria that activate pyroptosis.
Innate and adaptive killer lymphocytes (NK cells and cytotoxic T cells) recognize infected cells and kill them by releasing cytotoxic granule death-inducing proteases, called granzymes, and pore-forming proteins that deliver the granzymes into target cells. What happens to intracellular microbes during this process is unclear. We found that the antimicrobial granule pore-forming protein, granulysin (absent in mice) delivers granzymes into extracellular and intracellular bacteria, parasites and fungi, where they generate superoxide, inactivate oxidative defense enzymes and kill microbes oxidatively. Superoxide scavengers and overexpression of microbe antioxidant proteins inhibits microbial death. Microbe death occurs within minutes before the host cell is killed, limiting the spread of infection. Anaerobes, which don't generate superoxide, are still killed, but more slowly. Proteomics analysis of granzyme substrates in 3 bacteria suggests that cleavage of essential proteins disrupts critical metabolic and biosynthetic pathways. We term this microbe programmed cell death 'microptosis'. In 3 intracellular infection models (*L. monocytogenes, T. cruzi, T. gondii*), granulysin-transgenic mice clear infection better and survive infections that are lethal to wild-type mice.

Presentation: Tuesday, 13 September 2016 from 12:45 – 13:15 in room Donausaal 3.

HYGIENESYMPOSIUM
MRGN Isolierung: Pro und Contra (FG PR)
13 Sept. 2016 • 11.45–13.15

062/PRV

Nosocomial infection after colonization with multidrug resistant Gram-negative organisms (MDRGNO) - What are relevant clinical factors that determine whether a colonization leads to an infection?

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Background: The number of patients colonized with multidrug resistant Gram-negative organisms (MDRGNO) is growing world-wide. Subsequent infections with these organisms are associated with limited treatment options and elevated mortality and morbidity. With additional and costly prevention measures hospitals are trying to prevent these infections. But how often do these patients develop an infection with MDRGNOs compared to infections with other bacteria and what are the risk factors. Within a hospital setting we aimed to determine the incidence of these MDRGNO infections as well as the risk factors associated with these infections after rectal MDRGNO colonization.
Materials and Methods: The setting of this study was a German university hospital with over 3.200 beds. The study period was two years (2014 and 2015). We included all patients that were found rectally colonized with multidrug resistant *Escherichia* (*E.*) coli or *Klebsiella* spp. and subsequently stayed at least 3 days in our hospital. The patients were then prospectively tracked, looking for microbiological examination indicating a possible infection. Cases were manually reviewed by infection control professionals to identify a new infection with either the colonization organism or any other organism. In order to analyze risk factors for a bacterial infection we conducted a nested case-case-control study. Cases were either MDRGNO infections or infections with any other bacteria, controls were patients without bacterial infection with an onset between MDRGNO colonization and hospital discharge. Data were analyzed using univariate and multivariate regression models.
Result: Within the study period 1,982 patients fulfilled the inclusion criteria. The patients were found to be colonized with the following MDRGNOs: *E.coli* 78.2% (n=1,549), 19.0% (n=377), K. pneumoniae and 2.8% (n=56) with both. Of these patients 4.8% (n=95) developed a bacterial infection with the colonization bacteria, 8.1% (n=161) with any other bacteria and 0.8% (n=16) with both. Patients, colonized with MDRGNO K. pneumoniae developed significantly more often an infection in comparison to MDRGNO *E.coli* colonized patients (7.7% vs. 3.9%, $p<0.01$). Independent risk factors for a nosocomial infection after MDRGNO colonization overall were surgical operations (OR = 2.4; CI95% 1.1 - 5.1), treatment with corticosteroids (OR = 3.8; CI95% 1.9 - 7.5) and devices such as urinary catheter (OR = 2.3; CI95% 1.2 - 4.3).
Conclusion: A colonization with MDRGNO does not necessarily lead to an infection with the same pathogen. Some MDRGNO, like K. pneumoniae seem to be associated with an increased risk of nosocomial infection. These results should be taken in consideration in infection prevention guidelines suggesting species-specific hygiene measures.

Presentation: Tuesday, 13 September 2016 from 11:55 – 12:05 in room Donausaal 4-5.

063/PRV

Prevalence of MDR *E. coli* and *K. pneumoniae* recovered from Urine Specimens from patients in the Cologne metropolitan area

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Introduction: Multidrug-resistant (MDR) Gram-negative bacteria are an increasing problem. While data from single institutions are available, little is known about the regional prevalence in the in- and outpatient setting.
Aim: This study was conducted to assess the regional prevalence of MDR *E. coli* and *K. pneumoniae* in the Cologne metropolitan area.
Materials and Methods: Species identification and susceptibility data from all positive urine specimens were prospectively analyzed over a one year period (1.1.2015-31.12.2015) as part of a regional surveillance project (mre-netz regio rhein-ahr). Secondary isolates were excluded. Data from participating hospitals and laboratories were anonymously pooled. Data from all tertiary care centers, and approximately 90% of hospitals and private practitioners in the Cologne metropolitan area were available and could be included in the study. MDR *E. coli* and *K. pneumoniae* were defined according to current MRGN criteria of the Robert-Koch Institute.
Result: A total of 103.074 patients were included in the analysis. Among these, 65% were hospitalized when the sample was obtained. Patents had a mean age of 62 years (range, 0-105 years), 33% of patients were male. Overall, *E. coli* and *K. pneumoniae* accounted for 53% of pathogens.
Prevalence of 3MRGN was 6.7% in *E. coli* and 5.9% in *K. pneumoniae* and differed between peripheral hospitals (8.4% and 6.1%, respectively), tertiary care centers (7.8%; 7.5%; p=0.082), and outpatients (4.9%; 4.5%; p<0.001). Similarly, overall prevalence of 4MRGN was 0.1% in *E. coli* and 0.6% in *K. pneumoniae* also differing between peripheral hospitals (0.1% and 0.8%, respectively), tertiary care centers (<0.1%; p=0.036 and 1.2%; p=0.19), and outpatients (0.1%; p=0.41 and <0.1%; p<0.001). In addition, 3MRGN were significantly more prevalent in male patients (*E. coli*, 9.3% vs. 5.3%; p<0.001; *K. pneumoniae* 9.7% vs. 4.1%; p<0.001), while in 4MRGN this differences could only be observed in *K. pneumoniae* (0.9% vs. 0.4%; p=0.03).
Conclusion: Prevalence of 4MRGN *E. coli* and *K. pneumoniae* in the Cologne metropolitan area was relatively low in 2015. While 4MRGN were most frequently isolated from patients hospitalized in tertiary care centers, prevalence of 3MRGN was highest in patients from peripheral hospitals and significantly more frequent in hospitalized patients.

Presentation: Tuesday, 13 September 2016 from 12:05 – 12:15 in room Donausaal 4-5.

HAUPTSYMPOSIUM 04
Emerging Pathogens
13 Sept. 2016 • 15.00–16.30

064/INV

Emerging *Chlamydia* infections in animals and their zoonotic potential

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Introduction: *Chlamydia psittaci* and *Chlamydia abortus* are well recognized for their zoonotic potential. *C. psittaci* is an obligate intracellular bacterium that causes respiratory disease in birds. In humans, this organism may cause psittacosis, a respiratory disease that can spread to involve multiple organs, and in rare untreated cases may be fatal. *Chlamydia abortus* is mainly responsible for ovine enzootic abortion, but it is also a dangerous pathogen for pregnant women. A few years ago, *C. abortus* was associated with pneumonia in humans. Interestingly, recent data underline the zoonotic potential of additional *Chlamydia* species such as *Chlamydia suis* and *Chlamydia caviae*.
Objective: Presenting an overview on recent zoonotic *Chlamydia* infections in Belgium and in The Netherlands. The following will be presented: 1) "atypical" *C. abortus* infections in humans, 2) managing a cluster outbreak of psittacosis in Belgium linked to a pet shop visit in The Netherlands and 3) asymptomatic *Chlamydia suis* and symptomatic *Chlamydia caviae* infections in humans.
Patients, Materials and Methods: Newly developed molecular diagnostic tests and culture were used to identify *C. psittaci*, *C. abortus*, tetracycline resistant *C. suis* and *C. caviae*.
Result: *C. psittaci*, *C. abortus*, *C. suis* and *C. caviae* were found in humans. *C. abortus* and especially *C. psittaci* and *C. caviae* caused severe pneumonia in humans. *C. suis* was demonstrated in slaughterhouse personnel and pig farmers, apparently without causing clinical symptoms.[HM1]
Conclusion: 'Veterinary' chlamydial agents have received less attention by physicians. Human medicine should be aware of the zoonotic potential of *Chlamydia* as there is accumulating evidence that these species are more abundant in animals than previously assumed. Also, recent data stress the need for a close collaboration between physicians, medical microbiologists, veterinarians and public health officials, as crucial information such as potential animal reservoirs with their associated *Chlamydia* can be missed.
[HM1]Ook hier nog C. abortus toevoegen?

Presentation: Tuesday, 13 September 2016 from 15:00 – 15:30 in room Donausaal 3.

065/INV

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The abstract has not been submitted.

066/INV

Frequent and unusual infectious diseases in refugees in Germany in terms of public health

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Since 2014 the number of asylum seekers and refugees in Germany has considerably increased. In 2015 according to unconfirmed estimates the number was around a million people. In many countries of origin there are infectious diseases that are not originary occuring in Germany or if they occur their are considerably infrequent. There are resulting different constellations concerning possible infectious diseases in refugees: untreated chronic infectious diseases acquired in the country of origin, infectious diseases or parasitic diseases acquired during migration, latent infections exacerbating during or after the stressful migration, or infectious diseases acquired in Germany that can potentially break out under ill-housed circumstances. There are different factors which might bedevil the situation: lack of language knowledge, mass accommodation, insufficient health screening after entering the country, limited on-site medical care, missing immunization records, lack of knowledge about the German health care system etc. The lecture highlights the subject in terms of public health against the background of individual medical cases.

Presentation: Tuesday, 13 September 2016 from 16:00 – 16:30 in room Donausaal 3.

WORKSHOP 12
Nosokomiale Ausbrüche
(StAG HY)
13 Sept. 2016 • 18.00–19.30

067/HYV

Weekly screening helps terminating nosocomial transmissions of Vancomycin resistant enterococci on an oncologic ward

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Background: Vancomycin resistant enterococci (VRE) are important causes of healthcare associated infections and often affect vulnerable patient groups such as patients on hematology/oncology wards. Halting the spread of VRE requires infection control bundle strategies such as patient isolation, enhanced hand hygiene, surface disinfection, and increased active surveillance measures. Distinguishing imported VRE-cases from nosocomially transmitted cases is often difficult. Appropriate screening strategies can help to differentiate in these cases and to detect clusters of VRE, resulting in increased awareness among healthcare workers.
Aim: To investigate the impact of weekly screening within the bundle of infection control measures in ending VRE transmissions on an oncologic ward.
Settings and Methods: In a 1500-bed tertiary care centre, a cluster of VRE colonisations and infections was detected on an oncologic ward between January and April 2015. Transmissions were classified as nosocomial colonisations or infections if they occurred >48 hours after hospitalisation and the initial screening was negative or not performed. Screening of all remaining patients on the ward was performed and whole genome sequencing-based typing was applied to determine the clonal relationship of isolated strains. Intensified hand hygiene, surface cleaning, patient isolation, and close surveillance were implemented. Moreover, the screening of all patients upon admission was initiated in May 2015. After an additional nosocomial VRE infection (detected in a blood culture sample), an additional weekly screening of all oncology patients was established in August 2015.
Result: Between January and April 2015 five VRE infections were detected in patients on the oncology ward. Screening of all remaining patients on the ward revealed 12/29 colonized patients, of which 10 were *per definition* nosocomial. After establishing infection control bundle strategies and VRE screening on admission, 7/40 VRE colonised patients on average were detected per week, showing no significant decline compared to the initial situation. Genomic typing revealed three different parallelly occurring clusters consisting of two v*anB*- and one *vanA*-cluster. After additional weekly screening, the number of colonised patients declined to 1/53 (p
Conclusion
Weekly screening is a basic condition for the successful management of outbreak situations and should be part of infection control strategies in case of spread of VRE and other multiresistant bacteria.

Presentation: Tuesday, 13 September 2016 from 18:00 – 18:15 in room Donausaal 3.

068/HYV

Vancomycin-resistant Enterococci as nosocial pathogens: invasive infections versus outbreak scenarios

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The presentation is a summary of the following two abstracts:

Vancomycin-resistant Enterococcus faecium (VREfm) has emerged as an important nosocomial pathogen worldwide because of its rapid spread, limited treatment options and high-level morbidity and mortality especially in intensive care and neutropenic patients. The aim of this study was to characterize the molecular epidemiology and virulence factors of VREfm from bacteremia cases from 2006 to 2015 in a single institution. A total of 25 VREfm from our institution and 5 VREfm from regional hospitals were available for further analysis to determine the genetic relatedness using PFGE, MLST, MLVA, rep-PCR (DiversiLab) and WGS; the presence of both vancomycin resistance genes vanA and vanB as well as virulence factors such as enterococcal surface protein (esp), hyaluronidase (hyl), and collagen adhesion (acm). MLST revealed 13 different sequences types (mainly ST17, ST18 and ST78), all belonging to clonal complex 17. In addition, 8 different MLVA types (mainly 159 and 12) were identified. WGS analysis defined at least 5 different main clusters. Analysis with PFGE demonstrated 7 subgroups (only two strains with identical patterns). The results with rep-PCR (DiversiLab) do only partially fit to the previous results. The vanA gene was present in 7/30 and the vanB gene in 23/30 isolates. The distribution of the esp, hyl, and acm genes showed 25 esp-positive (15 with 4 esp repeats), 22 hyl-positive and 30 acm-positive isolates.
In sum our broad analysis of the genetic relatedness by different typing methods revealed that our VREfm bacteremia isolates belong to different epidemiological MLSTypes with different virulence subsets. Interestingly, MLST and WGS showed the highest level of concordance.

The last years witnessed an enormous spread of vancomycin-resistant E. faecium (VREfm) strains worldwide. In our institution VREfm is now the predominant nosocomial gram-positive multiresistant
pathogen.
To assess the hyperendemic situation in more detail we have analyzed retrospectively the distribution of VREfm on an intensive care (mainly gastroenterology) unit over a one-year period (2014). We routinely screened new patients on admission for VRE according to our MRSA schedule and each new VREfm strain was kept for further MLST analysis. To trace possible VRE-transmission events we analyzed more than 22.000 patient days to determine the location of each individual patient (patient box and all neighbor patients) for the whole ICU stay.
In sum, we identified 86 cases representing 76 VRE-patients. On admission, 31 patients were known to be VRE-positive and 39 patients with no former record of VRE colonization were newly identified as VRE-positive by a rectal VRE-screening procedure. At least 7 different MLSTypes were identified thus far (22 VRE strains are not finished yet), predominantly ST 117, ST 192, ST 78 and ST 80. During the one-year study period 16 additional patients were newly identified as VRE-positive by additional screening or diagnostic microbiology and were therefore defined as nosocomial cases (positive at least 72 hours after admission). However, only one classical VRE-transmission scenario could be determined in which three patients within the same box became VRE-positive with ST 203. In another case, VRE transmission seems possible because ST 80 was found in a patient after a ST 80 VRE-positive patient was discharged from the same box. Much to our surprise, in all other nosocomial cases no index patients could be documented in the same box with the same MLSTyp.
These results put forward two questions which should be addressed in future work: 1.) Is the sensitivity of the screening method a problem (PCR from an enrichment selective bouillon)? and 2.) because only patients with known risk factor for MRSA were screened in our study, do we really know the risk factors for VRE-positive patients?

Presentation: Tuesday, 13 September 2016 from 18:15 – 18:30 in room Donausaal 3.

069/HYV

Emergence of linezolid- and vancomycin-resistant *Enterococcus faecium* in a department for hematologic stem cell transplantation

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Objective: Prevalence of vancomycin-resistant enterococci has increased in Germany. Here, we report the spread of linezolid- and vancomycin-resistant *Enterococcus faecium* (LVRE) in a German department for hematologic stem cell transplantation (HSCT).
Patients and Methods: In this retrospective analysis we included all patients with LVRE in a university-based department for HSCT in 2014 and 2015. Patients chart reviews were used to investigate the epidemiology and clinical outcome. Available LVRE isolates underwent detailed microbiological characterization and genotyping by pulsed-field gel electrophoresis (PFGE).
Result: In total, 20 patients with LVRE were identified within the observed time period. All except two patients underwent allogeneic HSCT. Surveillance culture results from incoming patients and chart review revealed that 10 of 20 patients were colonized at hospital admission. Eight of 10 patients with in-hospital acquired LVRE had previous linezolid treatment. Analysis of spatio-temporal patterns showed no evidence for LVRE patient-to-patient or environment-to-patient transmission within the HSCT department. In five cases (25%) LVRE bloodstream infection occurred. Nine LVRE isolates could be saved for characterization. Eight isolates carried *vanA*, one isolate *vanB*. All were susceptible to daptomycin. PFGE analysis showed that four different LVRE clones were responsible for the spread. One single genotype was present in six LVRE isolates whereupon the corresponding patients were all referred from the same hospital to the HSCT department.
Conclusion: This is the first report demonstrating the emergence of LVRE in a German HSCT department. (L)VRE screening at patients' admission and appropriate infection control strategies were sufficient to prevent any transmission. Further studies to identify risk factors and to determine optimal treatment in this predisposed patient collective are warranted.

Presentation: Tuesday, 13 September 2016 from 18:30 – 18:45 in room Donausaal 3.

070/HYV

Prolonged outbreak of clonal MDR/XDR *P. aeruginosa* on an intensive care unit: Ultra-filtrate bags as possible route of trasmission?

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Introduction: The outbreak occurred on a surgical, interdisciplinary intensive care unit of a tertiary care hospital. We detected a cluster of ICU patients colonized with of extensive drug resistant (XDR) *P. aeruginosa*. We initiated an outbreak investigation team, performed an exploring epidemiological analysis and initiated an epidemiology-based intervention.
Method: Within the outbreak investigation, we performed microbiological examinations of the sinks in the patient rooms and a retrospective case-control study. All patients admitted to the outbreak ICU between January 2012 and February 2014 were included in the case-control-study. Cases were patients colonized with the outbreak strain. Controls were patients with a different *P. aeruginosa* strain. Data on risk factors like devices, procedures, renal replacement therapy, antimicrobial chemotherapy, comorbidities and disease severity were collected. Risk factors were evaluated using multivariable conditional logistic regression analysis. Strain typing was performed using the repetitive element-based polymerase chain reaction (rep-PCR) DiversiLab system.
Result: The outbreak clone was found in the sinks of five patient rooms (31%). Altogether 21 cases and 21 (randomly chosen) controls were included. In the univariate analysis, there was no significant difference in age, comorbidities or other baseline data of the patients. Case patients had significantly more often a hemofiltration therapy than controls (33% vs. 5%, p=0,045). In the multivariate analysis, placement in a room with a colonized sink (OR 11.2, p=0.007) and hemofiltration (OR 21.9, p=020) were associated with elevated risk for colonization with the outbreak clone. In a subsequent evaluation of the work procedures associated with hemofiltration, we found that the ultra-filtrate bags were emptied in the sinks of the patient rooms. We exchanged the traps of the contaminated sinks and changed the working procedures concerning the hemofiltration machines to single-use ultra-filtrate bags. We then analyzed the incidence of *P. aeruginosa* in clinical cultures before the intervention and in the following 18 months. In this follow up period, the outbreak strain was detected only once, which proved the discontinuation of the outbreak (incidence rate before vs. after, 0.75% vs. 0.04%, pP. aeruginosaoverall was significantly decreased in comparison to the period during the outbreak (2.5% vs. 1.5%, p
Conclusion: In this outbreak, we demonstrated that inadequate handling of hemofiltration bags in contaminated sinks have the potential to act as drivers of a outbreak. However, the further distribution of the outbreak clone was most likely multifactorial (including contact transmission). The outbreak ended after the traps of the contaminated sinks were exchanged and working procedures associated to these sinks were reduced.

Presentation: Tuesday, 13 September 2016 from 18:45 – 19:00 in room Donausaal 3.

071/HYV

Dialysis drains: Origin of an outbreak of KPC (*Klebsiella pneumoniae* Carbapenemase) producing *Enterobacteriaceae*

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Sinks and drains are known sources of gramnegative bacteria, and outbreaks often relate to contaminated sewage systems. We describe a multispecies outbreak of KPC (*Klebsiella pneumoniae* Carbapenemase) producing *Enterobacteriaceae* in an ICU of a tertiary referral hospital related to dialysis drains.
In March 2016, we discovered 4 ICU patients to be colonized with KPC producing *Enterobacteriaceae (C. freundii, K. pneumoniae, E. aerogenes)*. All of the patients had a long, complicated medical history and a long hospital / ICU stay (> 1 month). All patients had already received contact isolation due to colonization with other multidrug resistant organisms or influenza viruses. One additional patient colonized with KPC producing *Klebsiella oxytoca* was identified retrospectively to be colonized in December 2015. Since KPC producing bacteria are infrequent in our hospital investigations were performed immediately.
Initial observations revealed no major hygiene related problems. The staff was familiar with isolation precautions and practices of hand hygiene. In fact, this particular ICU was the one with the highest usage of alcoholic hand rub in our hospital for many years. Extensive environmental examinations were implemented next taking samples from all parts of the ICU focusing on moist areas in the patients surroundings. Eventually, KPC producing *Enterobacteriaceae* were detected in drains for used dialysis fluids in different ICU rooms and even in rooms not hosting KPC colonized patients.
Continuous hemodialysis and hemofiltration techniques are often used in critically ill patients as cared for in an ICU. Installation of dialysis connection units at the patient's bedside is well established to provide supply of dialysis fluids. Often, the used dialysis fluid is collected in special bags with a volume of approx. 10 l which can easily be weighed and then drained into a wall mounted drainage system. In many ICUs, such drains are near the patients head and directly besides the infusion systems. Some manufacturers of those units recommend to use the outflows with a flexible tube, as to empty such waste bags and to reuse them. This very technique was performed in our ICU.
In our opinion, the risk of transmission of pathogens from the drains to patient is high. Due to our findings, we decided to change the dialysis waste management: All drains were closed and each filtrate drain bag was discarded into a closed container and transported to incineration. No further KPC producing *Enterobacteriaceae* have been detected in this ICU by screening all patients weekly.

Presentation: Tuesday, 13 September 2016 from 19:00 – 19:15 in room Donausaal 3.

072/HYV

Particularities of outbreaks on neonatal and paediatric intensive care units - results from surveillance of healthcare associated outbreaks in Germany, 2012-2015

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Background: Germany has established a national surveillance of healthcare associated (HAI) outbreaks according to the German Protection against Infection Act mandating the reporting by all healthcare workers in Germany to the local public health authorities and from there via the federal state authority to the Robert Koch Institute (RKI). Outbreaks on neonatal and paediatric intensive care units (NICUs/PICUs) may have severe consequences for the patients. Increased understanding of particularities of these outbreaks may allow development of more effective recommendations for outbreak detection and control.

Obejctive: To analyse epidemiological data from outbreaks on NICUs and PICUs compared to outbreaks on non-paediatric intensive care units (ICUs) to better understand their particularities.

Method: HAI-Outbreaks were reported from federal state authorities to RKI on standard paper forms. We analysed all paper-based reports of outbreaks between 1.1.2012 - 31.12.2015. We analysed information on: (1) aggregated data: date of onset, number of cases colonised, infected and deceased, infectious agent, multidrug resistance, type of ward; (2) individual data (linelist): information on diagnosis, time of diagnosis and microbiological findings.

Result: In total we retrieved information on 1,569 HAI outbreaks. 1,037 (66.1%) were due to viral pathogens, 417 (26.6%) due to bacteria, 5 due to parasites and 4 due to fungi. In total 19,926 individuals were affected by HAI outbreaks, 2,738 (13.7%) due to outbreaks caused by bacteria. 175 fatalities were reported, 148 (84.6%) of these in context of bacterial outbreaks. From NICUs and PICUs 53 (35 bacterial, 18 viral) outbreaks were reported. 437 children were affected by these outbreaks (341 by bacterial, 96 by viral outbreaks), ten of them died (9 within bacterial, 1 within viral outbreaks). Pathogen spectrum on NICUs differed from ICU outbreaks (figure 1). Antimicrobial resistance of bacterial pathogens increased on NICUs and PICUs (23,1% to 50%) as well as on ICUs (59,1% to 92,0%) between 2012 and 2015 (figure 2).

Conclusion: Outbreaks of antimicrobial resistant pathogens have increased up to 30% on NICUs and ICUs within the last four years. On NICUs we found a pathogen composition differing from ICUs. Special focus should be put on the prevention of multiresistant Klebsiellae and Staphylococci outbreaks. In comparison to ICUs viral outbreaks are more frequent in NICU outbreaks.

Figure 1

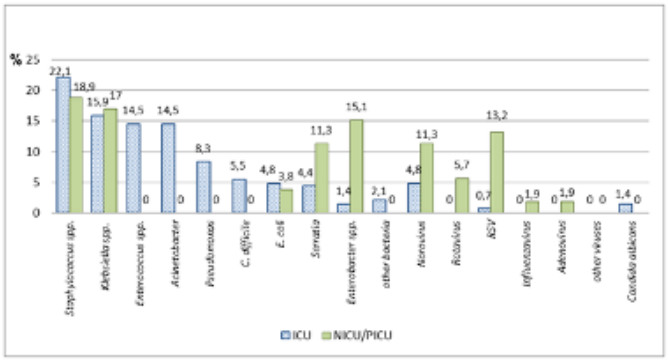

Figure 1: HAI outbreaks on neonatal and paediatric intensive care units (NICU and PICU outbreaks n=53) in comparison to non-paediatric intensive care units (ICU outbreaks n=145), 1.1.2012-31.12.2015

Figure 2

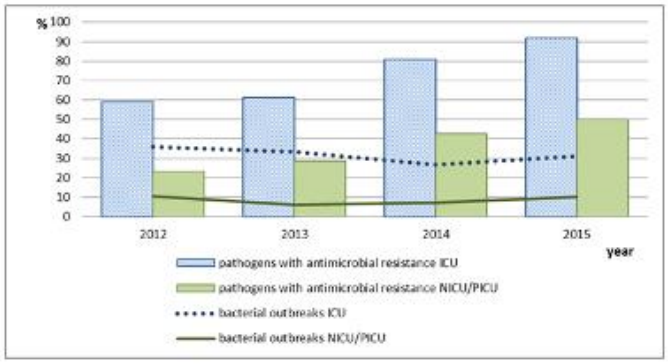

Figure 2: Pathogens with antimicrobial resistance in HAI outbreaks on neonatal and paediatric intensive care units (NICU and PICU outbreaks n=53) in comparison to non-paediatric intensive care units (ICU outbreaks n=145), 1.1.2012-31.12.2015

Presentation: Tuesday, 13 September 2016 from 19:15 – 19:30 in room Donausaal 3.

WORKSHOP 13
Symposium of the DFG Research Group 2251 „Acinetobacter“ (FG MP)
13 Sept. 2016 • 18.00–19.30

073/MPV

The phospholipases of *A. baumannii* - role in interbacterial competition and pathogenicity

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Introduction: *Acinetobacter baumannii,* which is an emerging threat in hospital environments due to its increasing resistance to antibiotics, is subject of the DFG research unit 2251. Despite its increasing role as hospital pathogen, knowledge with respect virulence mechanisms and survival of *A. baumannii* in the human host is scarce. Phospholipases are known to contribute to interbacterial competition and have been identified as virulence factors in different pathogenic bacteria.
Objective: Phospholipases are involved in the specific destruction of endosomal or phagosomal membranes, which releases the bacterium into the nutrient rich host cell cytoplasm. This together with the identification of potential phospholipase C and D genes in the genome *of A. baumannii* led to the suggestion that phospholipases might contribute to the survival and metabolic adaptation of *A. baumannii* to the human host.
Materials and Methods: To analyze the role of phospholipases in metabolic adaptation and virulence of *A. baumannii* ATCC19606^T we developed a marker-less mutagenesis system. A set of different phospholipase C and D mutants was generated and analyzed in *E. coli* killing assays, in a *Galleria mellonella* infection model as well as in invasion of A549 lung epithelial cells.
Result: Here we present the development of a highly efficient markerless mutagenesis system for *A. baumannii* using a suicide plasmid encoding for 1500 bp of the up- and down-stream region of the target gene as well as for a kanamycin resistance for positive selection and the levansucrase form *Bacillus subtilis* for negative selection. With this technique we generated a whole set of phospholipase single, double and triple mutants. Bacterial competition assays and *Galleria mellonella* infection studies revealed that the *A. baumannii* phospholipases are not crucial for interbacterial competition but play an important role in infection of *G. mellonella.* Furthermore, we tested the effect of phospholipases on invasion of human lung epithelial cells, showing that all of the tested phospholipases are necessary for full invasion efficiency.
Conclusion: The phospholipases C and D of *A. baumannii* ATCC19606^T are not essential for interbacterial competition, but function as specific tools to target eukaryotic cells.

Presentation: Tuesday, 13 September 2016 from 18:05 – 18:17 in room Donausaal 4-5.

074/MPV

Insertion sequences in the RND-type efflux pump regulatory genes *adeS* and *adeN* are associated with tigecycline resistance in *Acinetobacter baumannii*

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Introduction: Tigecycline (TGC) is one of the last-resort antibiotics for the treatment of infections caused by multidrug resistant *Acinetobacter baumannii*. However, resistance to TGC is now common. The mechanism of resistance is poorly understood but is thought to be mainly through efflux of the drug.
Objective: This study aimed to investigate the mechanism of TGC resistance in *A. baumannii* strains isolated from patients at hospitals in Greece and Spain between 2012 and 2014 as part of the MagicBullet clinical trial.
Materials and Methods: In total, 41 isolates from Spain and 100 isolates from Greece were investigated. The MICs were determined by Etest. TGC non-susceptibility was interpreted as MIC ≥ 2 mg/L using EUCAST resistance breakpoints for *Enterobacteriaceae*. The isolates were screened for insertion sequences in the RND-type efflux pump regulators *adeN* (regulator of AdeIJK) or *adeS* (regulator of AdeABC) by PCR. Insertion sequences were identified by Sanger sequencing. Gene expression was analysed by qRT-PCR.
Result: In Spain, 87.8% of *A. baumannii* strains were TGC non-susceptible, of which 8.3% displayed a disruption of *adeS* by IS*Aba1* and in 11.1% the gene was disrupted by an insertion sequence yet to be identified. A*deN* was disrupted in 38.7% of isolates (30.5% IS*Aba1*, 5.5% IS*Aba27*, 2.7% IS*Aba125*) and a one nucleotide deletion was observed in 5.5% of isolates. *AdeN* could not be detected by PCR in 8.3% of isolates (Table 1).
In Greece, 99% of isolates were TGC non-susceptible. *AdeS* could not be detected by PCR in 27.3% of isolates. In 9.1% of strains, *adeS* was disrupted by IS*Aba1*. In contrast, IS*Aba1* was found in *adeN* in 27.3% of isolates. In addition, IS*Aba125* was found in *adeN* in one isolate as well as a 87 nucleotide deletion in another strain (Table 1).
In all TGC susceptible strains from Spain *adeS* and *adeN* were undisrupted, while *adeS* was missing in the susceptible isolate from Greece. The mechanism of TGC non-susceptibility was still unknown for 12 strains from Spain and 35 strains from Greece.
The disruption of *adeS* or *adeN* was associated with an increased expression of the pumps *adeB* and *adeJ*, respectively. This was analysed in four different strains. Two displayed an *adeS* disruption by IS*Aba1* leading to a 9-fold to 13-fold increase of *adeB* expression. IS*Aba1* and IS*Aba125* were inserted into *adeN* in the other two isolates, which was associated with a 6-fold and 2-fold increase in the expression of *adeJ*.
Conclusion: Tigecycline resistance was associated with insertion sequences in the regulatory genes *adeS* and *adeN* leading to an increased expression of *adeB and adeJ*. Gene disruption by IS*Aba1* was most common and the disruption of *adeN* was more prevalent. Nevertheless, in about one-third of isolates the mechanism of TGC non-susceptibility remains to be elucidated.
Table 1: Overview of insertion sequences in efflux pump regulators *adeN* and *adeS* in TGC non-susceptible isolates. IS = insertion sequence.

Figure 1

	adeN		*adeS*	
Country (number of isolates)	**Spain (36)**	**Greece (99)**	**Spain (36)**	**Greece (99)**
gene not detected by PCR	3 (8.3%)	0 (0%)	0%	27 (27.3%)
IS*Aba1*	11 (30.5%)	27 (27.3%)	3 (8.3%)	9 (9.1%)
IS*Aba27*	2 (5.5%)	0 (0%)	0 (0%)	0 (0%)
IS*Aba125*	1 (2.7%)	1 (1%)	0 (0%)	0 (0%)
unknown IS	0 (0%)	0 (0%)	4 (11.1%)	1 (1%)
other	2 (5.5%)	1 (1%)	0 (0%)	0 (0%)
gene undisrupted	17 (47.5%)	70 (70.7%)	29 (80.6%)	62 (62.6%)

Presentation: Tuesday, 13 September 2016 from 18:17 – 18:29 in room Donausaal 4-5.

075/MPV

Probing natural competence of multidrug-resistant *Acinetobacter baumannii* via acquisition of the gentisate catabolic pathway

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Question: *Acinetobacter baumannii* is a gram-negative nosocomial pathogen notorious for its potential to develop multidrug-

resistance. Horizontal gene transfer (HGT) is critically shaping evolution of this pathogen, however the relevant pathways of HGT are not well-defined. Recently, natural competence was identified as a principle pathway of DNA uptake in *A. baumannii* but these studies were restricted to antibiotic-sensitive isolates due to the lack of suitable markers to tackle multidrug-resistant (MDR) isolates.
Method: Here, we introduce a simple transformation assay based on the acquisition of the gentisate catabolic pathway. The ability for growth on mineral medium with gentisate as the sole source of carbon is rare among members of the species *A. baumannii*.
Result: We identified strains capable of gentisate utilization which can serve as donors of DNA to transform gentisate non-utilizing strains. In this way we could demonstrate that natural competence is a typical feature of MDR strains belonging to international clone 1 (IC 1). Some MDR strains representing IC 2, IC 3, IC 4 and IC 5 could also be transformed. We found IC 7 to be not amenable to this novel assay due to its intrinsic capability to utilize gentisate.
Conclusion: This method offers a tool to study natural transformation in many multidrug-resistant isolates of *A. baumannii* without introducing an antibiotic resistance gene. Natural competence is a common trait of the IC 1 lineage and also detectable in strains belonging to other IC lineages suggesting a major impact of transformation competence on the evolution of this nosocomial pathogen.

Presentation: Tuesday, 13 September 2016 from 18:29 – 18:41 in room Donausaal 4-5.

076/MPV
Adaptation of *Acinetobacter baumannii* to desiccation
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Introduction: The opportunistic pathogenic bacterium *Acinetobacter baumannii*, which is subject of the DGF research unit 2251, is emerging in intensive care units in hospitals worldwide [1, 2] with recent outbreaks also in German hospitals. *A. baumannii* has the extraordinary capacity to adapt to and survive in dry environments which enables the bacterium to persist in the hospital environment [3], but the molecular basis for this trait is unknown.
Objective: A widespread strategy for the adaptation to low water activities is the uptake or synthesis of compatible solutes, small organic solutes that do not interfere with metabolism and other important cell functions [4]. We aim to elucidate the role of compatible solutes in *A. baumannii* for osmotic stress response as well as for desiccation resistance.
Materials and Methods: Growth of *A. baumannii* was analyzed in media with high osmolarities. The pool of compatible solutes in the bacteria grown under different conditions was investigated via NMR. In addition, we studied the survival of *A. baumannii* on dry surfaces.
Result: Our experiments revealed the unusual compatible solute mannitol in *Acinetobacter*. Mannitol is synthesized by an unusual bifunctional enzyme, the mannitol-1-phosphate dehydrogenase/phosphatase. The enzyme has been purified and its mode of activation is being analyzed.
Conclusion: Adaptation of *A. baumannii* to low water environments involves the synthesis of compatible solutes such as mannitol and glutamate.

References
[1] L. Dijkshoorn, A. Nemec, and H. Seifert. *Nat. Rev. Microbiol.* **5** (2007), 939-951.
[2] A. Howard, M. O'Donoghue, A. Feeney, and R. D. Sleator. *Virulence* **3** (2012), 243-250.
[3] A. Jawad, H. Seifert, A. M. Snelling, J. Heritage, and P. M. Hawkey. *J. Clin. Microbiol.* **36** (1998), 1938-1941.
[4] M. Roeßler, and V. Müller. *Environ. Microbiol.* **3** (2001), 743-754.

Presentation: Tuesday, 13 September 2016 from 18:41 – 18:53 in room Donausaal 4-5.

077/MPV
Insights into the biological function of the trimeric autotransporter adhesin (Ata) of *Acinetobacter baumannii*
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Crucial steps in bacterial infections are the adhesion to and invasion into human host cells. Among Gram-negative bacteria, trimeric autotransporter adhesins (TAAs) are wide spread modular constructed outer membrane proteins. TAAs mediate adhesion to different surfaces, but further biological functions can vary greatly between different bacterial species. The TAA of *Acinetobacter*, termed *Acinetobacter* trimeric autotransporter adhesin (Ata) was identified by bioinformatics; however, little is known about the biological functions of Ata in *A. baumannii*.
The objective of this study was to investigate to role of this protein in terms of adhesion, invasion and pathogenicity.
All experiments were done with *A. baumannii* ATCC 19606 (wt) and its isogenic *ata* deletion mutant (Δ*ata*). We performed static and dynamic adhesion assays onto abiotic surfaces (collagen, laminin, fibronectin, polystyrene) and biotic surfaces (HUVECs, A549), as well as in a new established dynamic organ infection model using human umbilical cord veins (HUCV). Binding rates were calculated by fluorescence microscopy and a qRT-PCR approach. Furthermore, HUVECs and A549 cells were used for investigating Ata-dependent invasion by counting intracellular bacteria after gentamicin protection assay and to determine cytotoxicity effects of the bacteria by quantifying the release of LDH. For *in vivo* pathogenicity, we used the *Galleria mellonella* infection model. Complement resistance was investigated by incubation of *A. baumannii* in normal human serum (NHS) and NHS-ethylene glycol tetraacetic acid (EGTA) for blocking of the alternative pathway with subsequent agar plating.
Adhesion experiments revealed that Ata mediates a distinctive binding to collagen, laminin and polystyrene. When human cells were infected, we could detect that Δ*ata* showed an up to 9-fold reduction of adhesion in static and dynamic infection conditions. When HUCVs were infected, an overall higher binding efficiency compared to the dynamic *in vitro* assay was detected. Gentamicin protection assays showed that invasion of *A. baumannii* into human cells is Ata-independent. In contrast, employing the LDH cytotoxicity *in vitro* assay and the *Galleria mellonella in vivo* infection model, deletion of *ata* significantly impaired virulence. After incubation of *A. baumannii* in NHS, we observed a better viability of the wild-type compared to Δ*ata*, but no survival when incubated with NHS-EGTA, indicating the essential role of an autoprotective function of the classical complement pathway.
Ata is a multifunctional TAA mediating adhesion to abiotic surfaces and human cells as well as pathogenicity. We observed an Ata-dependent survival of *A. baumannii* in NHS. Our findings might contribute in therapeutical options targeting Ata for inhibiting virulence of this pathogen.

Presentation: Tuesday, 13 September 2016 from 18:53 – 19:05 in room Donausaal 4-5.

078/MPV

Tracing DNA uptake machineries across the bacterial tree of life using a feature-aware phylogenetic profiling

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Introduction: Mining environmental genetic diversity via the direct uptake of free DNA allows naturally competent bacteria to rapidly adapt to changing environments. While natural competence is a highly versatile mechanism for accomplishing genetic innovation, its prevalence in contemporary bacteria is largely unknown. Phylogenetic profiles for individual building blocks of known DNA uptake machineries - i.e. the presence-absence patterns of orthologs to the corresponding genes across the bacterial domain - provide the means for rapidly identifying novel candidates for naturally competent bacteria. Yet, orthology of two sequences is a poor proxy for their functional equivalence, posing the risk of unspecific predictions.
Objective: Orthology - or more general - homology describes the evolutionary relationships of two sequences. It is only loosely connected to the similarity of two homologs' functions, which diverges as a function of evolutionary time since two sequences last shared a common ancestor. An automated and reliable *in silico* screen for the presence of a particular functionality, such as represented by a DNA uptake machinery, has to tie the inference of sequence homology and of protein function.
Materials and Methods: To establish the link between evolutionary relationships and functional equivalence of sequences we integrated a targeted ortholog search tool, *HaMStR*, with an assessment of the feature architecture similarities (FAS) between a protein of interest and its orthologs. The versatility of the new tool was demonstrated in a screen for the presence of 5 different types of DNA uptake machineries in more than 1,000 bacterial species.
Result: We developed software facilitating the establishment of feature-aware phylogenetic profiles across large and phylogenetically diverse species collections. Tracing of the individual components of 5 different DNA uptake machineries in the bacterial domain established a hitherto unprecedented *in silico* characterization of bacteria with respect to their potential of natural competence. Depending on the level of stringency we classify between 32 and more than 200 bacteria as capable of direct DNA uptake. The approach can be generalized to screening any gene interaction network of interest.
Conclusion: Screens for DNA uptake machineries via FAS-supported ortholog assignments classify at least 32 hitherto uncharacterized bacterial species as potentially naturally competent.

- ***This is a contribution of the DFG research group 2251 "Acinetobacter –***

Presentation: Tuesday, 13 September 2016 from 19:05 – 19:17 in room Donausaal 4-5.

079/MPV

CipA of *Acinetobacter baumannii* inhibits the alternative pathway of complement by interaction with complement C3.

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Introduction: Multidrug resistant *Acinetobacter baumannii* can cause severe infections in critically ill patients, mainly in intensive care settings. Infections caused by *A. baumannii* include pneumonia and bacteremia. To establish an infection, pathogens must first contend with the innate immune response. Complement represents a major barrier to invading pathogens, and only fragmentary information is available about factors contributing to immune evasion of *A. baumannii*. Here we show that CipA, a novel pathogenicity factor of *A. baumannii*, overcome complement-mediating killing by direct interaction of CipA with the central complement component C3.
Method: Following cloning and production in *Escherichia coli*, CipA was tested for its ability to bind diverse components of the alternative pathway including C3, C3b, Factor B as well as C5. To assess the impact of CipA on the alternative pathway, hemolytic and ELISA-based complement activation assays (Wielisa) were investigated. In addition,
Result: The inhibitory effect of CipA on the classical (CP) and alternative (AP) pathway was assessed through hemolytic assays. Increasing amounts of CipA were preincubated with NHS, before erythrocytes were added. Following incubation, erythrocyte lysis was determined by measuring the release of hemoglobin. In addition, the effect of CipA on the terminal sequwence (TS) was studied using purified complement components. While CipA neither inhibited the CP nor the TS, a strong inhibition of the AP was observed. Assuming that CipA might be able to interact specifically with the AP, we assessed binding of C3, C3b, C5, and Factor B to CipA. With the exception of Factor B, CipA bound all other complement components.
Discussion: To establish an infection in the host, little is known about how *A. baumannii* evades complement. By functional characterization of the protein interaction, here we show that CipA of *A. baumannii* specifically blocks the AP by direct interaction with complement components C3, C3b, and C5.

This is a contribution of the DFG research group 2251 "Acinetobacter –

Presentation: Tuesday, 13 September 2016 from 19:17 – 19:30 in room Donausaal 4-5.

WORKSHOP 14
Joint Workshop of StAG RK & FG MS
13 Sept. 2016 • 18.00–19.30

080/RKV

The genus *Fusarium* as causative agent of eye infections - epidemiology, antifungal susceptibility and diagnostics

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Eye infections by filamentous fungi such as the phytopathogenic genus *Fusarium* are prevalent in tropical and subtropical countries with agricultural economy. With the use of contact lenses *Fusarium* keratitis became also a problem in moderate climates. Between 01/2014 and 05/2016, the NRZMyk received 27 *Fusarium* isolates from patients with corneal infection in the age between 19 and 78 with a predominance of females (85%). A detailed study of five cases revealed that all five patients wore soft contact lenses and had regular contact to plant material.
With the dominating species *F. petroliphilum* and *F. keratoplasticum*, the *Fusarium solani* species complex (FSSC) represented two thirds of the pathogens followed by the *F. oxysporum* species complex (22%) and *F. proliferatum* (11%).
Antifungal susceptibility testing using the EUCAST microdilution reference method was performed for all isolates. *Fusarium* species are intrinsically resistant to echinocandins and to several azoles. The susceptibility profile against voriconazole, posaconazole and terbinafin differed depending on species complex or species highlighting the importance of species identification.
Although the elongation factor 1-α (ef-1α) is the marker of choice for a reliable species identification in *Fusarium* the resolution power of the ITS region is high enough to discriminate all relevant pathogens of eye infections. In order to allow a non-culture based diagnosis of *Fusarium* species from clinical material such as aspirates of the vitreous body or culture negative corneal abrasion we developed a *Fusarium* specific nested polymerase chain reaction (PCR) assay targeting the ITS region that is able to detect low amounts of *Fusarium*-DNA.
Incidence and outcome of *Fusarium* eye infections registered by the NRZMyk demonstrate the clinical importance of these infections and the need for their detailed documentation. Consequently, we set up a national register for mycotic keratitis in cooperation with the University Hospital for Eye Diseases in Düsseldorf.

Presentation: Tuesday, 13 September 2016 from 18:00 – 18:15 in room Konferenz 2-3.

081/RKV

Primary resistance of Helicobacter pylori to clarithromycin, levofloxacin and tetracycline in Germany

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Objective: Knowledge of antimicrobial resistance of *Helicobacter pylori* is needed to prescribe adequate treatments. The aim of this ongoing prospective study is to keep the development of primary antimicrobial resistance of *H. pylori* to key antibiotics such as clarithromycin under surveillance and continuously deliver data on resistance.
Method: From October 2014 until April 2016, a total of 746 adult patients from all across Germany who had not received any prior eradication treatment were enrolled. Clinical and epidemiological data were gathered; gastric tissue samples were taken and molecular genetically tested for mutations conferring resistance to clarithromycin, levofloxacin and tetracycline.
Result: Primary resistances were 10.1% for clarithromycin and 9.6% for levofloxacin; 1.5% revealed resistance and/or reduced susceptibility to tetracycline. We did not observe any correlation between resistances, the underlying gastric disease and the patients' country of origin. There was no significant difference in the resistance proportions among the different German federal states.
Conclusion: In our study population, the prevalence of clarithromycin and levofloxacin resistance is low as is resistance and/or reduced susceptibility to tetracycline. Patients without any prior eradication treatment may be treated empirically. Genotypic susceptibility testing is a reliable and quick strategy to update resistance data in regular intervals and greatly reduces logistic constraints.

Presentation: Tuesday, 13 September 2016 from 18:15 – 18:30 in room Konferenz 2-3.

082/RKV

Changing epidemiology of *Clostridium difficile* in Europe: experiences from an unexpected ribotype 018 outbreak in southern Germany

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Background: *Clostridium difficile* infection (CDI) is the most common cause of nosocomial diarrhoea. *C. difficile* ribotype 018 (RT018) has become the most prevalent strain in North Italy and has spread to several European countries as well as to East Asia (South Korea and Japan), where a growing number of RT018-related outbreaks have been reported. However, the RT018 strain has rarely been isolated in Central and Western Europe. In August 2015, *C. difficile* RT018 was detected in two hospitals in the German state of Baden-Wuerttemberg. An outbreak investigation was initiated in close cooperation between the local hospital hygiene team and the National Consultant Laboratory for *C. difficile*, followed by prospective ribotyping of all isolates for a period of two months. Here, we provide a preliminary report on this first RT018 outbreak in southern Germany.
Method: *C. difficile* strains were detected and characterized using antigen detection, stool culture and PCR. Subsequently, capillary electrophoresis ribotyping and multiple-locus variable-number tandem-repeat analysis (MLVA) were carried out on all *C. difficile* RT018 strains to investigate genetic relatedness and possible clonality.
Result: In total, nine *C. difficile* RT018 isolates could be identified. All RT018 strains were genetically related and clustered into two clonal complexes. When compared with historic isolates from Germany and with recent epidemic outbreak isolates from Milan, Italy, the identified isolates showed close relatedness with the epidemic Italian strains from Milan in 3 of 4 cases. In contrast, no direct relationship to the historic German isolates was found. Antibiotic susceptibility testing revealed resistance to moxifloxacin among the Italian and the German outbreak strains, while this

resistance pattern was absent in most historic German isolates. Follow-up investigations in the outbreak hospitals elucidated continued low-level spreading of RT018 isolates for a period of at least two months, despite strict application of hand hygiene and infection prevention strategies, including early diagnosis of patients with diarrhoea, single room isolation of patients with symptomatic CDI and contact precautions.

Discussion: The current investigation shows that the epidemic Italian *C. difficile* RT018 strains have reached southern Germany and might potentially spread further within Europe. Molecular subtyping using a combination of ribotyping and MLVA proved to be a powerful tool for rapid outbreak investigations.

Presentation: Tuesday, 13 September 2016 from 18:30 – 18:45 in room Konferenz 2-3.

083/MSV

WGS-based analysis of a cluster of CTX-M-15-producing *Klebsiella pneumoniae* in a neurorehabilitation centre

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Background: Extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* (*K. pneumoniae*) are important nosocomial pathogens. In particular, immunocompromised patients and patients suffering from underlying diseases are susceptible to severe infections. From April 2015 onwards, an increased incidence of patients colonized or infected with ESBL-producing *K. pneumoniae* was observed in a neurorehabilitation centre in Germany. Here, we report the whole-genome-sequencing (WGS)-based analysis of this setting including deduction of a diagnostic PCR for the major *K. pneumoniae* clone.

Materials and Methods: A total of 39 isolates were collected between April and December 2015. For WGS, DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) and the MagAttract (Qiagen). Sequencing libraries were prepared by applying the Nextera XT Library preparation kit and sequenced on a Miseq instrument (2 x 300 bp). Raw reads were trimmed and both mapping and *de novo*-assembly based approaches were applied for phylogenetic analysis of the isolates. Further, WGS data was used to determine the sequence types (multilocus sequence typing, MLST), resistance gene content and to set up a strain-specific multiplex-PCR.

Result: WGS analysis revealed the presence of not only one, but three different clusters (ST15, ST405 and ST414). The major cluster was formed by CTX-M-15-producing Klebsiella isolates belonging to ST15. Besides phylogenetic reconstruction of the various strains, whole-genome data allowed to elucidate the molecular basis of resistance phenotypes (alterations in the *mgrB* gene and the *ompk*35/36 genes) of two isolates exhibiting resistance to colistin and carbapenem antibiotics, respectively. In addition, characteristic regions in the major strain´s genome were identified and used to develop a specific multiplex-PCR based on three primer pairs for a rapid identification and classification of emerging isolates.

Conclusion: Our analysis provides a comprehensive insight into the phylogeny of the setting. Moreover, the use of whole-genome-sequencing allows rapid determination of resistance gene content, resistance mechanisms, MLST, and further issues, also in a retrospective manner, which represents an important benefit in comparison to traditional methods, such as pulsed-field-gel-electrophoresis (PFGE) typing.

Presentation: Tuesday, 13 September 2016 from 18:45 – 19:00 in room Konferenz 2-3.

084/MSV

Stationary or Mobile - NGS as practical tool for molecular diagnostics in emerging outbreaks

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Here, we report the first rapid and accurate next-generation-sequencing of Zika virus imported by a German tourist returning from Colombia.

This is an example for reconnaissance of unexpected outbreaks, for which NGS is a powerful technology aimed at identification and possible diagnostics. New technical advances now enable sequencing even during ongoing outbreaks and support *quasi-real-time* diagnostics as well as providing data for trace-back analysis when skillfully combined with bioinformatics.

In this study we sequenced, to the best of our knowledge, the first German Zika virus strain ZIKV-COL/TM7.16 isolated from a patient with acute febrile illness returning from the country of Columbia. After successful cultivation in African green monkey fibroblasts, we retrieved viral RNA and performed reverse transcription for subsequent Illumina Sequencing. The nearly complete genome sequence of strain ZIKV-COL/TM7.16 was assembled *de novo*. Sequence data of the terminal ends were obtained by 3' and 5' race PCR, respectively. The resulting complete high quality Zika virus genome may serve as reference data for scientists and physicians involved in Zika virus outbreak diagnostics.

In addition, we compared this Illumina-generated sequence with data obtained by MinION nano-pore-sequencing. This third-generation sequencing tool is the first field-able sequencer which, in combination with bioinformatics, provides the possibility of supporting on-site outbreak contact-tracing teams with rapid information amending classical diagnostic methods. As expected, raw-data of MinION showed higher sequencing error rates compared to Illumina technology. After assembly using software Canu and polishing by Nanopolish, almost 98% of the virus genome was covered in the resulting alignment and its sequence was correctly determined. Thus, this MinION sequencing approach can be used for reliable determination of sequence information and supporting outbreak reconnaissance in rapid deployable outbreak investigation teams.

Presentation: Tuesday, 13 September 2016 from 19:00 – 19:15 in room Konferenz 2-3.

085/MSV

Whole-genome sequence analysis of ESBL-producing *E. coli* of ST410 from different hosts and environments indicates interspecies transmission

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Backgrounds and Objectives: Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* were initially restricted to a human and veterinary clinical context. Recent findings however prove their occurrence in extra-clinical settings such as wildlife and the environment. For our study we comparatively analysed ESBL-producing *E. coli* using whole-genome sequence (WGS) data to get insights into their possible transmission between different habitats and hosts.
Materials and Methods: A total of 491 *E. coli* isolates from environmental (wild birds, dog faeces) and clinical origin (human, canine) were screened for the occurrence of ESBL-producing *E. coli*. A selection of isolates based on MLST and PFGE was whole-genome sequenced and WGS data of ten isolates of ESBL-producing *E. coli* of ST410 from different habitats was used for single nucleotide polymorphism (SNP) analysis of the maximum common genome (MCG) and PLACNET analysis of plasmids.
Result: Our analysis revealed, irrespective of their origin, a very high genetic similarity for the ten ST410 ESBL-producing *E. coli* isolates, differing by low numbers of SNPs only. Their clonal character was emphasized by similar resistance patterns, serotypes and plasmid profiles.
Conclusion: These results underline the mandatory nature of the "One Health" approach as we have initial evidence for a recent interspecies transmission of a successful and zoonotic clone of ST410 *E. coli* between wildlife, humans, companion animals, and the environment.

Presentation: Tuesday, 13 September 2016 from 19:15 – 19:30 in room Konferenz 2-3.

WORKSHOP 15
Infectious risks associated with food and the environment (FG PR)
14 Sept. 2016 • 08.30–10.00

086/PRV

Detection of colistin resistance gene *mcr-1* in *Escherichia coli* isolates obtained from piglets in the years 2008 and 2009

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Colistin (*syn.* polymyxin E), a cyclic polypeptide antibiotic, is regarded as a last-resort antibacterial agent to combat severe infections with multidrug-resistant Gram-negative bacteria in humans. Therefore, the use of colistin for treatment of animals, particularly food-producing animals, is critically reviewed. Reluctance is even increasing since 2015, when emergence of plasmid-encoded, horizontally transferable bacterial resistance against colistin was reported from China. Subsequent to the first report the responsible gene *mcr-1* was also detected in several bacterial isolates in Germany suggesting that this gene has been present in *E. coli* strains in pigs since 2010. In this study we tried to assess the first occurrence of *mcr-1* in Germany more precisely.
The PCR assay of Liu *et al*. (2016) was used to screen a collection of clinical *E. coli* isolates for the *mcr-1* gene. Isolates had been obtained from piglets in Germany from 1999 through 2009 and had been stored due to the possession of certain virulence genes (2,217 isolates from 1,934 piglets on 1,110 holdings). MIC data of *mcr-1*-positive isolates were determined using the VITEK2 system.
All isolates recovered in the years 1999 through 2007 (n = 1,705) tested *mcr-1* negative. However, *mcr-1* was detected in 4 (1.6 %) and 14 (5.3 %) of the tested isolates obtained in 2008 and 2009, respectively. These *mcr-1*-positive isolates originated from 1 of 118 (2008) and 6 of 124 (2009) tested pig holdings, respectively. The minimum inhibitory concentration (MIC) of colistin against these isolates was 4 to ≥ 16 µg/ml. These MICS were invariably graded resistant according to EUCAST definition (colistin *break point* for Enterobacteriaceae: 2 µg/ml). The *mcr-1*-positive isolates also encoded for the following combinations of recognized virulence factors: Stx2e/F18 fimbriae (12 isolates), Stx2e/ST-I/ST-II/F18 fimbriae (2), Stx2e/ST-I/ST-II/F18 fimbriae (2), and ST-I/ST-II (2).
Our results suggest that *mcr-1* circulates among pigs in Germany at least since 2008 and is increasingly prevalent in *E. coli* strains associated with this host species. In addition, *mcr-1* appears responsible for the colistin resistant phenotype of some strains of edema disease *E. coli* (EDEC) and enterotoxic *E. coli* (ETEC) which represent economically important pathogens of pigs worldwide. Future studies will unravel the genetic context of the *mcr-1* gene to allow for a comparative analysis of *mcr-1*-carrying plasmids from different animals species and human patients.
Liu *et al.* (2016). Lancet Infect Dis 16: 161-168.

Presentation: Wednesday, 14 September 2016 from 8:30 – 8:45 in room Donausaal 3.

087/PRV

Prevalence of *mcr-1* in *E. coli* from livestock and food in Germany, 2010-2015

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Question: In November 2015 the first plasmid-encoded colistin resistance gene *mcr-1* was detected in livestock and raw meat

samples as well as human beings in China [1]. Several further studies revealed its worldwide spread in Enterobacteriaceae. It could be isolated from different sources, like the environment, food, livestock, infected patients as well as asymptomatic human carriers. Colistin is a last choice antibiotic. So with the detection of carbapenemases-producing isolates which harbour the *mcr-1* gene as well, there is a concern regarding the spread of pan resistant Enterobacteriacea. Nevertheless, colistin is widely used for treating infections in food-producing animals. Although there are reports on *mcr-1* positive isolates, data on the prevalence of *mcr-1* are rare. Here we provide comprehensive data on the prevalence of *mcr-1* in *E. coli* from livestock and food samples in Germany in the years 2010-2015.

Method: Isolates analysed in this study originated from the German national monitoring program on zoonotic agents that includes resistance determination in commensal *E. coli* isolated from faecal and food samples of animal origin by broth microdilution method. Over 10,600 isolates of poultry, pig and cattle production were analysed for phenotypical colistin resistance based on the epidemiological cut-off value defined by EUCAST with MIC≥4mg/l. This resulted in 505 *E. coli* isolates designated as resistant to colistin, which were subsequently screened for the presence of the *mcr-1* gene by PCR/real-time PCR.

Result: The screening of phenotypically colistin resistant *E. coli* isolates resulted in 402 isolates (78.9%) harbouring the plasmid-encoded colistin resistance gene *mcr-1*. This corresponds to an overall prevalence of *mcr-1* of 3.8 %, but prevalence differs vastly between the productions chains. The highest prevalence was detected in the turkey food chain (10.7 %), followed by broilers (5.6 %). There is a decreasing trend in turkey caeca samples and turkey meat, whereas prevalence on turkey farm level is persistently high. A low prevalence was determined in pigs, veal calves and laying hens. The *mcr-1* was not detected in beef cattle, beef and dairy products in all years investigated.

Conclusion: So *mcr-1* detection occurred mainly in isolates which originated from poultry production chain. Since spreading of pan resistant isolates may occur, *mcr-1* prevalence should be carefully monitored and epidemiological data from other countries are urgently needed.

1. Liu Y, Wang Y, Walsh TR, Yi L, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. Lancet Infect Dis. 2015. doi: 10.1016/S1473-3099(15)00424-7.

Presentation: Wednesday, 14 September 2016 from 8:45 – 9:00 in room Donausaal 3.

088/PRV

Emergence of plasmid-mediated colistin resistance gene *mcr-1* in clinical *Escherichia coli* isolates from patients and poultry products in Germany

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Background: Colistin is one of the few remaining substances for treatment of infections with multidrug-resistant gram-negative pathogens. In November 2015 a high prevalence of the plasmid-mediated colistin resistance gene *mcr-1* in *Escherichia coli* from livestock and several human cases was reported from China. Thus, intensive screening of strain collections started worldwide to assess the extent of *mcr-1* distribution in human and livestock populations. In January 2016 laboratories throughout Germany were asked to send colistin-resistant isolates to reference laboratories for *mcr-1* screening. Here we report on the characterization of the colistin-resistant isolates that were sent to the Robert Koch Institute (RKI).

Materials and Methods: The *mcr-1*-screening by PCR included *E. coli* isolates from animals and animal products (n=211), clinical *E. coli* isolates from different studies (n=419), colistin-resistant clinical *Enterobacteriaceae* from the RKI strain collection (n=20) and colistin-resistant clinical isolates that were sent to the RKI since January 2016 (n=14). Antimicrobial susceptibility testing was performed by microbroth dilution and Etest. Presence of further resistance genes, e.g. beta-lactamase genes, and other mechanisms that are involved in colistin resistance were analysed by PCR and sequencing. Bacterial strain typing was performed by enzymatic macrorestriction and subsequent pulsed field gel electrophoresis (PFGE). Resistance gene transfer was tested in conjugation assays.

Result: Presence of gene *mcr-1* was confirmed in 16 colistin-resistant *E. coli* isolates from poultry products from 2011 and 2014. These isolates additionally were cephalosporin-resistant due to extended-spectrum beta-lactamases (ESBL) or AmpC-beta-lactamases (CMY-2). PFGE-typing revealed the presence of ten different *E. coli* clones with conjugative plasmids carrying *mcr-1*.
Among the clinical isolates collected in 2016 we confirmed *mcr-1* in five colistin-resistant *E. coli* isolated from urine (n=2), blood culture, wound swab and pharyngeal swab. Using PFGE-typing we differentiated four *E. coli* clones with ampicillin resistance due to production of TEM-1 beta-lactamase. All colistin-resistant *Klebsiella pneumoniae* (n=18), *Pseudomonas aeruginosa* (n=5) and *Acinetobacter baumannii* (n=1) did not harbour *mcr-1*. Sequence analyses of the intrinsic *mgrB* gene of the colistin-resistant *K. pneumoniae* isolates showed various deletions, premature stop codons or transposase insertions resulting in loss of functionality of this gene contributing to colistin resistance.

Conclusion: Our finding of plasmid-mediated colistin resistance gene *mcr-1* in ten *E. coli* clones from poultry products and four *E. coli* clones from human is of concern. Further surveillance is needed to investigate the spread of this resistance mechanism.

Presentation: Wednesday, 14 September 2016 from 9:00 – 9:15 in room Donausaal 3.

089/PRV

Is herbal tea from hot-water dispensers bad medicine for in-patients?

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Introduction: Herbal tea is frequently consumed in hospitals and preparation with water more than 90° C hot is thought to be the only acceptable method to eliminate bacteria present in tea bags. However, the compliance of medical personnel to use water still >90° C hot for the preparation of herbal tea might not be optimal. Another way to provide patients with herbal tea are hot-water dispensers (HWD) that allow patients to prepare herbal tea themselves. Again, a disadvantage could be that the appropriate water temperature might not be held for extended time periods.
Aim: To determine microbiological contamination of herbal tea brewed with water from HWDs in order to define the risk for consumers.

Method: Four different varieties of herbal tea were brewed in a sterile container using water from HWDs previously filled with hot sterile-filtered water. Herbal tea was prepared both shortly after

filling the HWDs and after 4-6 hours holding time. Microbiological tests of water and herbal teas included inoculation of 500 µl liquid on Columbia blood agar and Sabouraud agar, enumeration of bacteria in 1 ml liquid by the pour plate method and enrichment in thioglycolate broth (1 ml) and in Caso broth (100 µl). Samples of tea bags were tested for bacteria and fungi by dipping them into Caso broth.

Result: All herbal tea infusions were contaminated with aerobic spore forming bacteria, nonfermenting bacteria or yeasts. The bacterial load ranged from 7 to 1000 KBE/ml with no significant association to the holding time in the HWDs (freshly filled: 76 vs. 4-6 hours: 153KBE/ml; p=0.25). *Bacillus* spp. accounted for more than 90% of positive herbal tea samples. No toxin producing *B.cereus* was detected. Neither *Enterobacteriaceae* nor *Pseudomonas* ssp. were found. Water temperatures ranged from 67 to 80°C (mean: 73°C) and no association could be observed between temperature and bacterial growth (r=-0.02). Mean microbial contamination by variety of herbal tea was 156 (fennel), 146 (mixed herbs), 98 (peppermint), and 41 (camomille) KBE/ml. No bacterial or fungal growth was demonstrated from water samples from HWDs. Investigating teabags revealed relevant nosocomial pathogens, e.g. *Escherichia* ssp., *Enterobacter* ssp., *Enterococcus* ssp..

Conclusion: Herbal tea prepared with water from HWDs was microbiologically contaminated and therefore should not be used and consumed by high-risk patients, e.g. patients on intensive care units or patients after transplantation or high-dose chemotherapy. As all specimens fulfilled the German regulatory criteria for mobile water installations and no virulent bacterial species were detected in the HWDs might be suitable means to provide herbal tea for low-risk patients.

Presentation: Wednesday, 14 September 2016 from 9:15 – 9:30 in room Donausaal 3.

090/PRV

Monitoring of nontuberculous mycobacteriae in Heater-Cooler Units

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Introduction: Nontuberculous mycobacteriae (NTM) are a rare source of nosocomial infections. In 2015, a prolonged outbreak of *M. chimaera* infection was reported in open-chest surgery patients (1). Contaminated Heater Cooler Units (HCU) were accused as source for infection with *M. chimaera*.

Aim: Our aim was to test the efficacy of the manufacturers (LivaNova, formerly known as Sorin Group) protocol for disinfection of HCU to prevent or eliminate mycobacterial colonization. Peracetic acid (fortnightly) and hydrogen oxide (daily) were used as disinfectants. We monitored mycobacterial colonization of HCU from *M. chimaera* contaminated HCU and new HCU.

Materials and Methods: 6 HCUs were monitored during the observation period. 118 air (200l) and 39 water (100ml) samples were taken from 4 new HCU twice a month (August 2015 - February 2016). 20 water samples (1x100ml) were taken from 2 contaminated HCU (July - December 2015) twice a month. 8 air samples (200l) were taken further at the beginning of the observation. Water samples were decontaminated using the NALC-NaOH method. Culture was performed using Bactec MGIT liquid medium and Loewenstein-Jensen and Stonebrink solid media.

Air samples were taken with MAS-100 air monitoring system using 7H11 Middlebrook-Agar supplemented with Polymycin, Carbenicillin, Amphotericin B and Trimethoprim.

Result: All new HCU became colonized with *M. chimaera* (median 78 days) in spite of disinfecting HCUs as recommended by the manufacturer.

After the introduction of the disinfection protocol *M. chimera* persisted in contaminated HCU for 4 months. In individual cases *M. chimaera* was temporarily not culturable from new HCU by additional disinfection.

Conclusion: Regular disinfection using the manufacturers protocol does not reliably prevent or eliminate colonization of new HCU.

Reference

[1] Sax et al. Prolonged outbreak of Mycobacterium chimaera Infection After Open-Chest Heart Surgery, Clinical Infectious Diseases (2015) 61 (1) 67-75.

Presentation: Wednesday, 14 September 2016 from 9:30 – 9:45 in room Donausaal 3.

091/PRV

Co-occurrence of multiple β-lactamases and plasmid mediated quinolone resistance (PMQR) genes in *Enterobacteriaceae* from a polluted Nigerian wetland

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Introduction: Increasing evidence suggest that anthropogenic activities such as discharge of untreated wastewater significantly contributes to evolution and dissemination of clinically relevant antibiotic resistance genes in natural ecosystems. In developing countries such as Nigeria, there is an acute lack of sanitation and hygiene facilities and untreated wastewater is frequently discharged into aquatic ecosystems from point and non-point sources. However, few studies have investigated the impact of untreated wastewater on the development and spread of resistance within the Nigerian aquatic ecosystem.

Objective: This study investigated the occurrence of ESBLs and PMQR genes (usually found with ESBLs), and the genetic environment of *bla*$_{\text{CTX-M}}$ in bacteria isolated from a wetland polluted with untreated wastewater from hostel facilities within a University campus in southwestern Nigeria.

Materials and Methods: Thirty five bacterial strains isolated from wetland sediments collected monthly from October 2014 to January 2015 on ceftriaxone supplemented agar plates were investigated by PCR for integrons, ESBLs and PMQR genes. The genetic environments of *bla*$_{\text{CTX-M}}$ were also mapped by PCR.

Result: ESBLs and PMQR genes were detected in 12 isolates (34%) with *bla*$_{\text{OXA-30}}$, *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, *qnrB* and *acc (6')lb-cr* found in 12 (34%), 9 (26%), 4 (11%), 4 (11%) and 12 (34%) isolates respectively. ESBL and/or *bla*$_{\text{OXA-30}}$ co-occurred with PMQR genes in all the 12 isolates with 10 and 4 isolates carrying multiple ESBL and/or multiple PMQR genes. *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$ and *bla*$_{\text{OXA-30}}$ co-occurred with *acc (6')lb-cr* in 3 *Escherichia coli*, *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{OXA-30}}$ occurred with *acc (6')lb-cr* and *qnrB* in an *Enterobacter* sp., *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{OXA-30}}$ occurred with *acc (6')lb-cr* in 2 *Cronobacter sakazakii* and 3 *E. coli*, *bla*$_{\text{TEM}}$ and *bla*$_{\text{OXA-30}}$ occurred with *acc (6')lb-cr* and *qnrB* in an *Enterobacter* sp., and *bla*$_{\text{OXA-30}}$ alone occurred with *acc (6')lb-cr* and *qnrB* in 2 *Enterobacter cloacae.* All isolates were positive for class 1 integron and the MIC of tested antibiotics among them ranged as follows: imipenem (0.125->32µg/ml), ceftazidime (0.38-48µg/ml), aztreonam (0.047->256µg/ml), ciprofloxacin (0.38->32µg/ml) and sulphamethoxazole (32->1024µg/ml). All detected *bla*$_{\text{CTX-M}}$ were confirmed as *bla*$_{\text{CTX-M-15}}$ with the insertion sequence *ISEcp1* and *orf477* located upstream and downstream in an arrangement widely reported in medically important *bla*$_{\text{CTX-M-15}}$ carriers in many parts of the world.

Conclusion: The co-occurrence of multiple beta-lactamases and PMQR in different species of *Enterobacteriaceae* points to a possible stable selection of these gene combinations within this ecosystem. Further, the association of $bla_{CTX\text{-}M\text{-}15}$ with the active transposition unit highlights the important role of Nigeria's aquatic ecosystems as reservoir of bacteria carrying antibiotic resistance genes of interest to public health.

Presentation: Wednesday, 14 September 2016 from 9:45 – 10.00 in room Donausaal 3.

WORKSHOP 16
Bacterial metabolismus and virulence (FG MP)
14 Sept. 2016 • 08.30–10.00

092/MPV

Outer membrane vesicles increase transition fitness of a facultative pathogen via rapid surface exchange

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The adaption of bacteria to new environments is a key factor for infection, survival and colonization. So far a lot of studies focused on differential gene expression or proteome composition in various habitats. Less attention was drawn to the point of transition even though it is often essential to adapt rapidly to new conditions. The effective exchange of surface components such as proteins or LPS is crucial for many of such adaption processes.
Our hypothesis is that the formation of outer membrane vesicles (OMVs) plays a major role in the rapid surface exchange and is therefore a key feature of adaptation. To answer this question we used *Vibrio cholerae* as a model as the facultative human pathogen transits between the aquatic environment and the gastrointestinal tract.
We can show that vesiculation is upregulated mainly in early stages of infection via an iron dependent regulation. In vivo experiments revealed that a hypervesicular derivative of *V. cholerae* has a significant advantage in colonization of the murine model. We can demonstrate that the faster exchange of surface components due to increased OMV production counteracts the host derived antimicrobial mechanisms.
This study provides a first adaption mechanism, which is based on the faster exchange of outer membrane components due to vesiculation. These findings might also be applicable to other microorganisms which undergo rapid surface changes. Thus, it could be a global mechanism for adaption to new habitats.

Presentation: Wednesday, 14 September 2016 from 8:30 – 8:48 in room Donausaal 4-5.

093/MPV

CcpA as regulator of Streptolysin S in *Streptococcus anginosus*

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Streptococcus anginosus, as a member of the *Streptococcus anginosus* group, is a commensal of mucosal membranes, but also an emerging human pathogen. Some *S. anginosus* strains, including the type strain, show a prominent β-hemolytic phenotype. The genetic locus (*sag* gene cluster) of the hemolysin has recently been described and it exhibits high homologies to Streptolysin S (SLS) of *Streptococcus pyogenes*. Hemolytic activity as well as expression of the *sag* gene cluster is reduced in the presence of high glucose concentrations in *S. anginosus*. However, regulators controlling the expression of the SLS under theses conditions have not been elucidated.
The use of different sugars in reporter assays for hemolysin expression lead to the hypothesis that carbon catabolite repression (CCR), a well investigated regulation mechanism in $Gram^+$ bacteria controls hemolysin expression. A keyplayer in CCR of different streptococcal species is the regulator Catabolite control protein A (CcpA). *S. anginosus* harbors a close homologue of CcpA and we therefore chose to further investigate, the role of CcpA in expression of the *sag* gene cluster. A CcpA deletion mutant was constructed and transformed with the reporter plasmid

carrying the *sag* promoter in front of EGFP. Activity of the promoter was determined by FACS analysis at a range of different glucose concentration in the growth medium. The reduction of *sag* gene expression at high glucose concentration, which has been described for the wild type strain, is not present in the CcpA mutant, indicating that CcpA is involved in the regulation of hemolysin expression. The expression data could be verified in a functional hemolysis assay in which the CcpA mutant was able to lyse human erythrocytes in the presence of high glucose concentrations, in contrast to the wildtype *S. anginosus* strain. Based on these observations the promoter region of the *sag* gene cluster was analyzed bioinformatically for putative CcpA binding sites. Three potential CcpA binding sites could be identified. To determine the specific CcpA binding site, all three sites were altered by site directed mutagenesis. The putative binding sites were separately mutated or deleted and cloned in front of EGFP using a reporter plasmid. Promoters containing a mutation in the CcpA binding site should not demonstrate a glucose dependent reduction of EGFP-activity. This approach led to the identification of a specific nucleotide sequence, with good homologies to the consensus binding site of CcpA.
In summary this study characterized for the first time a regulator of the emerging pathogen *S. anginosus*. We could demonstrate that CcpA is involved in the regulation of SLS of *S. anginosus* and identified the putative CcpA binding motif in the SLS promoter region.

Presentation: Wednesday, 14 September 2016 from 8:48 – 9:06 in room Donausaal 4-5.

094/MPV
Utilization of host glycerophosphodiesters by *Staphylococcus aureus*.
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The facultative pathogen *Staphylococcus aureus* colonizes the human anterior nares and skin. Which carbon and energy sources can be utilized by *S. aureus* in these nutrient-poor habitats has remained largely unknown. We describe that *S. aureus* secretes a glycerophosphodiesterase (glycerophosphodiester phosphodiesterase, EC 3.1.4.46), GlpQ, degrading the glycerophosphodiester (GPD) head groups of phospholipids such as human phosphatidylcholine or phosphatidylinositol. Deletion of *glpQ* completely abolished the GPD-degrading activity in *S. aureus* culture supernatants. GPDs were detected in human nasal secretions probably as a result of phospholipid remodelling in the upper skin layers. Notably, GPDs promoted *S. aureus* growth under nutrient-poor conditions such as a synthetic nasal medium in a GlpQ-dependent manner indicating that GlpQ permits *S. aureus* to utilize GPD-derived glycerol-3-phosphate as a carbon and energy source. GPDs may be relevant nutrients for *S. aureus* and other skin-colonizing bacteria and inhibition of GlpQ might lead to new pathogen decolonization strategies.

Presentation: Wednesday, 14 September 2016 from 9:06 – 9:24 in room Donausaal 4-5.

095/MPV
Pathometabolism of Listeria monocytogenes
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A new term in infection biology is "pathometabolism" that describes the metabolic interactions between the pathogen and its host. When entering the human gastrointestinal tract, bacterial enteropathogens encounter nutrient competition with the host and its commensal microbiota. The main carbon, nitrogen and energy sources exploited by pathogens during infection, however, have been underinvestigated. During the last years, it revealed that most enteropathogens are equipped with a large set of specific metabolic pathways that allow them to overcome nutritional limitations,, thus improving their fitness within hosts. Those adaptations include the capacity to utilize *myo*-inositol, ethanolamine derived from phospholipids, fucose cleaved from mucosal glycoconjugates, and other metabolites not accessible for the gut microbiota or present in competition-free compartments.
The pathometabolism of facultatively intracellular bacteria is of special interest. In cooperation with Wolfgang Eisenreich (Garching, TU Munich), we applied ^{13}C-isotopologue profiling to study the carbon metabolism *Listeria monocytogenes* during growth in macrophages. GC/MS-based analysis of listerial amino acids derived from hydrolyzed proteins demonstrated an efficient utilization of glucose-6-phosphate for anabolic performances and of glycerol as energy supply, indicating a bipartite carbon metabolism within host cells. Using a set of ^{15}N-labeled substrates for *in vitro* growth experiments, we identified the branched-chain amino acids, methionine, ethanolamine and glucosamine as yet unknown nitrogen sources of *L. monocytogenes*. Moreover, we for the first time applied ^{15}N-isotoplogue profiling to an infection model using the nematode as a host, and showed that host-derived molecules are taken up and metabolized by the colonizing listeria. Taken together, our data allow unprecedented insights into metabolic fluxes of pathogenic bacteria during infection.

Presentation: Wednesday, 14 September 2016 from 9:24 – 9:42 in room Donausaal 4-5.

096/MPV
***Mycobacterium tuberculosis* requires lactate oxidation for growth in human macrophages**
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Mycobacterium tuberculosis (*Mtb*) is an airborne pathogen which caused the death of 1.5 million people worldwide in 2014 (WHO, Global tuberculosis report 2015). Its primary host cells are alveolar macrophages. Upon an infection, macrophages produce high amounts of lactate which might be used as carbon source by the pathogen. Therefore, our study focused on the question whether lactate utilization might provide a benefit for survival and replication of *Mtb* with in human macrophages.
By analyzing the *in vitro* growth of *Mtb o*n lactate, we demonstrated that the bacteria can utilize lactate as carbon source for metabolism. Moreover, we were able to identify the enzyme involved in lactate oxidation as LldD2, one of two potential lactate dehydrogenases annotated on the genome of *Mtb*. In contrast to LldD2, the potential lactate dehydrogenase LldD1 was not required for lactate oxidation in *Mtb*, which we proved by growth analysis of the knock-out mutants, enzyme activity assays and absolute quantitative real-time PCR. C_{13} metabolic flux analysis revealed that during [U-$^{13}C_3$]lactate utilization, carbon flow was directed into pyruvate metabolism, into the tricarboxylic acid (TCA) cycle, and subsequently into gluconeogenesis. Consequently, lactate metabolism depended on the activity of the phosphoenolpyruvate carboxykinase, a key enzyme in gluconeogenesis of TCA cycle intermediates. These findings indicate that lactate feeds into a central metabolic route, which also mediates lipid degradation in

Mtb. Furthermore, lactate oxidation proved to be important during growth of *Mtb* in human macrophages, as we found the Δ*lldD2* mutant to be profoundly impaired in intracellular proliferation in the macrophage infection model. We further observed that the inability to oxidize external lactate had a growth diminishing impact on the Δ*lldD2* mutant *in vitro*. Therefore the impaired intracellular growth might be based on an intoxication of the Δ*lldD2* mutant with lactate. Thus, lactate oxidation might possess a dual function: to remove a potentially toxic substrate from the environment, and to provide pyruvate as a carbon source for metabolism.

Presentation: Wednesday, 14 September 2016 from 9:42 – 10.00 in room Donausaal 4-5.

WORKSHOP 17
Interactions of Eukaryotic Pathogens with the Host (FG EK & FG II)
14 Sept. 2016 • 08.30–10.00

097/EKV

CYLD Aggravates Experimental Cerebral Malaria by Impairing CD8$^+$ T cell Responses and Fostering Blood-Brain Barrier Damage

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Introduction: Cerebral malaria is a severe complication of human malaria and may lead to death of *Plasmodium falciparum* infected individuals. Cerebral malaria is associated with sequestration of parasitized red blood cells within the cerebral microvasculature resulting in damage of the blood brain barrier and brain pathology. Although CD8$^+$ T cells have been implicated in the development of murine experimental cerebral malaria (ECM), several other studies have shown that CD8$^+$ T cells confer protection against blood-stage infections. CYLD, is a deubiquitinating enzyme which negatively regulates the immune response by inhibiting various signalling pathways including nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB), signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinases and T cell receptor.
Aim: To investigate how the deubiquitinating enzyme CYLD influences the outcome of ECM.
Materials and Methods: C57BL/6 WT and C57BL/6 Cyld$^{-/-}$ mice were either infected with *Plasmodium berghei* ANKA (*Pb*A) sporozoites or (*Pb*A)-parasitized red blood cells. ECM associated brain-pathology was studied by histopathological analysis. The cytokine and chemokine levels in the brain were determined by quantitative RT-PCR. The CD8$^+$ T cell response in the blood and brain was analysed by flowcytometry. Reciprocal bone marrow chimeras of WT and Cyld$^{-/-}$ mice were generated to identify the relevance of CYLD expression in the haematopoietic and parenchymal cells.
Result: Upon infection with *Plasmodium berghei* ANKA (*Pb*A) sporozoites or (*Pb*A)-infected red blood cells, 90% of the C57BL/6 Cyld$^{-/-}$ mice survived the infection, whereas all the wildtype mice succumbed within 9 days to ECM, with impaired parasite control and disruption of the blood brain barrier integrity. CYLD deficiency prevented widespread brain pathology with numerous hemorrhagic lesions, enhanced activation of astrocytes and microglia, infiltration of CD8$^+$ T cells and pronounced apoptosis of endothelial cells. Furthermore, the pathogen-specific CD8$^+$ T cell response was augmented in the blood of Cyld$^{-/-}$ mice with increased production of interferon (IFN)-γ and granzyme B. The enhanced CD8$^+$ T cell response in the Cyld$^{-/-}$ mice was accompanied by increased activation of Protein kinase C (PKC)-θ and NF-κB. Importantly, the accumulation of CD8$^+$ T cells in the brain of Cyld$^{-/-}$ mice was significantly reduced compared to the WT mice. Finally, bone marrow chimera experiments showed that the protection of Cyld$^{-/-}$ mice against ECM was not only due to the immune system but also due to radioresistant Cyld$^{-/-}$ parenchymal cells including the endothelial cells, which did not undergo apoptosis.
Summary: Taken together, our data identify CYLD as ECM promoting and thus, as a potential therapeutic target during ECM.

Presentation: Wednesday, 14 September 2016 from 8:30 – 8:45 in room Konferenz 2-3.

098/EKV

Dynamics of *Leishmania major* containing compartments in human primary macrophages

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The uptake of pathogens, such as *Leishmania* parasites, and the subsequent compartment biogenesis are highly dynamic processes. We could already demonstrate the *Leishmania* infectious inoculum to comprise a mixture of viable and apoptotic parasites. The latter one was found to be crucial for the survival of the overall population in human macrophages. Now we hypothesize that processing of apoptotic parasites influences compartmentalization of viable parasites in human primary macrophages.
First we assessed the kinetics of parasite uptake, using flow cytometry. We could demonstrate apoptotic parasites to be taken up more efficiently by anti-inflammatory macrophages (hMDM2) compared to pro-inflammatory macrophages (hMDM1). Moreover, preliminary results indicated apoptotic parasites to be internalized more quickly, compared to viable parasites. We already showed apoptotic parasites to reside in a single membrane compartment, to which the autophagy marker LC3 is recruited. To investigate the compartment dynamics, a lentiviral transduction system was applied to transduce human primary monocyte-derived macrophages with eGFP-LC3. Using live cell-imaging, we confirmed apoptotic parasites to reside in a LC3 positive compartment, in which they appear to be degraded. Interestingly, viable parasites also recruited LC3 to the membrane, for a limited time, after which parasites survived. In line, we could show compartments harboring apoptotic parasites to acidify faster as assessed by Lysotracker staining, suggesting parasite degradation. A more detailed look on the ultrastructure of infected macrophages, using 3D-electron microscopy, revealed an opening from the compartment to the extracellular space of the hMDM, strengthening the need of advanced techniques to assess host pathogen interactions in more detail.
Taken together, these data suggest apoptotic promastigotes to be internalized and processed more rapidly by hMDMs. Subsequently, viable parasites are able to "silently" enter the cell and enable a sustained infection. By shedding light on the uptake and biogenesis of compartments harboring *Leishmania* parasites, these data may contribute to the identification of novel therapeutic targets for the treatment of leishmaniasis.

References

[1] van Zandbergen, G., Bollinger, A., Wenzel, A., Kamhawi, S., Voll, R., Klinger, M., Müller, A., Hölscher, C., Herrmann, M., Sacks, D., et al. (2006). Leishmania disease development depends on the presence of apoptotic promastigotes in the virulent inoculum. Proc. Natl. Acad. Sci. U. S. A. *103*, 13837-13842.
[2] Crauwels, P., Bohn, R., Thomas, M., Gottwalt, S., Jäckel, F., Krämer, S., Bank, E., Tenzer, S., Walther, P., Bastian, M., van Zandbergen, G. (2015). Apoptotic-like Leishmania exploit the host's autophagy machinery to reduce T-cell-mediated parasite elimination, Autophagy 11, 285-297.

Presentation: Wednesday, 14 September 2016 from 8:45 – 9:00 in room Konferenz 2-3.

099/EKV

***Toxoplasma gondii* induces promiscuous DNA binding and impaired nucleocytoplasmic recycling of STAT1 during IFN-γ stimulation**

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Toxoplasma is one of the most common human intracellular parasites and an important opportunistic pathogen in immunocompromised individuals or after transplacental transmission. The parasite is able to establish a chronic infection with a dormant stage persisting for the host's life. This is facilitated by having evolved several strategies to dampen the host's immune response, including a global inhibition of the interferon (IFN-) γ response in infected macrophages (MΦ). The transcriptional response to IFN-γ is transduced by STAT1 (signal transducer and activator of transcription 1) and is pivotal for host defence against *Toxoplasma* and other intracellular parasites.
The activation of STAT1-responsive promotors is seriously impaired in *Toxoplasma*-infected MΦ, but the exact molecular mechanism of this inhibition remains elusive. Here we have analysed activation, subcellular distribution and DNA binding of STAT1 in RAW264.7 monocytes stimulated with IFN-γ. Despite impaired IFN-γ responsiveness, STAT1 surprisingly remains significantly longer in the nuclei of parasite-positive cells than in non-infected controls as revealed by quantitative immunofluorescence microscopy. Immunoblotting of subcellular protein fractions from *T. gondii*-infected and non-infected monocytes showed, that the increased amount of STAT1 in the nuclei of infected cells is fully activated by phosphorylation of Tyr701 and S727. DNase treatment of mild-detergent protein lysates from *Toxoplasma*-infected cells specifically leads to increased amounts of soluble phosphorylated STAT1 whereas STAT1 remained DNA-bound in the absence of DNase. These observations strongly suggest that an increased association of STAT1 with DNA in *Toxoplasma*-infected MΦ significantly reduces its nucleocytoplasmic shuttling during IFN-γ stimulation. Electrophoretic mobility shift assays unravelled that canonical binding of STAT1 to the gamma activated site (GAS) is inhibited by *T. gondii* infection. Instead, a non-canonical STAT1-GAS complex with lower electrophoretic mobility forms consistently, even in the presence of imperfect non-GAS sequences. This suggests a promiscuous binding of STAT1 to DNA in *Toxoplasma*-infected monocytes which may reduce the pool of activated STAT1 that can bind to GAS elements in IFN-γ-responsive promoters.

Presentation: Wednesday, 14 September 2016 from 9:00 – 9:15 in room Konferenz 2-3.

100/IIV

HIF-1α is actively stabilized by *H. capsulatum* but interferes with pathogen survival in human MΦ

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Histoplasma capsulatum (*Hc*) is the causative agent of histoplasmosis. Infection with *Hc* leads to severe mycoses in

immunocompromised patients. Inhaled spores perform a temperature-dependent switch into the pathogenic yeast phase and survive inside macrophages (MΦ) resulting in granuloma formation. *Hc*-induced granulomas were shown to be hypoxic in lung, liver and spleen of infected mice. Under hypoxia, the hypoxia-inducible factor (HIF)-1α is stabilized and regulates cellular metabolism and immune response. Recently, we showed that HIF-1α was crucial for resolution of sublethal infection in mice and that *Hc* stabilizes HIF-1α in human MΦ. Therefore, we investigated the impact of HIF-1α on host cell metabolism and pathogen survival in human MΦ.

Human monocyte-derived MΦ were infected with viable or heat killed (hk) *Hc* (MOI 5:1) in normoxia (21% O_2) or hypoxia (2% O_2) and analyzed 24h post infection (p.i.). Under normoxia, additional HIF-1α stabilization was achieved by the pharmacologic stabilizer DMOG. HIF-1α protein levels were investigated by western blot analysis. We measured GLUT1 and PDK1 gene expression as well as cytokine expression of IL-1β and TNF-α using qRT-PCR. Further, metabolic profiling of infected MΦ and *Hc* was assessed by Seahorse analyzer XF96. Survival of *H. capsulatum* was evaluated by plating yeast isolated from MΦ and counting colony forming units (CFU) 7 days later.

Only viable but not hk *Hc* stabilized HIF-1α in human MΦ 24h p.i. ($p<0.05$, n=3). Hypoxia-induced HIF-1α stabilization was further enhanced by *Hc* compared to infection in normoxia ($p<0.01$, n=3). In MΦ, expression of GLUT1 and PDK1 ($p<0.05$, n=3) as well as mitochondrial respiration and glycolysis were elevated by *Hc* infection ($p<0.001$, n=3). Regarding pathogen metabolism, *Hc* relied on mitochondrial respiration in liquid culture but was metabolic inactive inside the host cell under normoxia ($p<0.05$, n=3). *Hc*-induced upregulation of pro-inflammatory cytokines IL-1β and TNF-α ($p<0.05$, n=3). Pharmacologic stabilization of HIF-1α during infection further enhanced glycolytic gene and pro-inflammatory cytokine expression (n=3) while host cell glucose metabolism (*$p<0.05$, n=3) and mitochondrial respiration (***$p<0.001$, n=3) were decreased. Stabilization of HIF-1α along with infection resulted in decrease of *Hc* survival by 60 +/- 5% 24h p.i. but restored within 72h p.i. again (n=5). In contrast to that, stabilization of HIF-1α 6h before infection decreased *Hc* survival in the same manner but this effect was maintained up to 72h p.i. (n=5).

Our findings highlight an essential role of HIF-1α in fungal immunity of human MΦ. Future studies will focus on the putative role of metabolic intermediates in HIF-1α mediated fungal killing.

Presentation: Wednesday, 14 September 2016 from 9:15 – 9:30 in room Konferenz 2-3.

101/IIV

IgG4 subclass antibodies produced during lymphatic filariasis impair the activation of IgE-armed neutrophils and basophils but not eosinophils through interaction with FcγRI and II

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Introduction: Helminth parasites are remarkable for their ability to down-regulate host immunity by generating a modified Th2 immune response characterized by the presence of regulatory cytokines and high plasma levels of the non-cytolytic antibody IgG4. This particular isotype is described in both helminth and allergy models to display anti-inflammatory properties. How IgG4 molecules affect granulocytes activation and functions is still not well characterized.

Obejective: The present work aimed to clarify the mechanisms underlying the suppression of granulocytes' functions by IgG/IgG4 molecules in the physiopathology of Lymphatic filariasis

Patients and Methods: Using isolated granulocytes and affinity purified IgG and IgG4 fractions from plasma of endemic normals (EN), lymphatic filariasis pathology patients (CP), asymptomatic microfilaraemic (MF+) and amicrofilaraemic (MF-) individuals from endemic regions in Ghana, we analyzed the effect of bulk plasma and IgG positive or negative fractions on IgE/IL-3 stimulated granulocytes (CD66b+/CD63+/HLADR-) and granulocyte neutrophils (CD15+/CD16+), eosinophils (CD11b+/Siglec8+) and basophils (CD123+/CD203c+) by flow-cytometric analysis and ELISA assessment of histamine, eosinophil cationic protein and neutrophil elastase in culture supernatants. In addition, the ability of purified IgG4 antibodies from patients to bind on granulocytes and the associated downstream intracellular signaling pathways were investigated by FcγRs blocking, immunofluorescence and western blot.

Result: Granulocytes activation and granules content release were significantly inhibited by plasma of EN and MF+ individuals. This inhibitory capacity was abrogated upon depletion of IgGs from the plasma of MF+ individuals but persisted in EN plasma. Interestingly, in contrast to IgG4 from CP, affinity-purified IgG4 molecules from EN, MF+ and MF- significantly inhibited neutrophils and basophils but not eosinophils activation after interaction with FcγRI and FcγRII and modulation of Src, AKT and MEK phosphorylation.

Conclusion: Our data indicate that, during helminth infections, MF+ individuals display IgG4 antibodies with potent inhibitory activities on granulocytes and especially neutrophils and basophils. In addition, we have identified possible functional differences between IgG4 molecules from patients.

Presentation: Wednesday, 14 September 2016 from 9:30 – 9:45 in room Konferenz 2-3.

102/IIV

Surface-modified yeast cells: A novel eukaryotic carrier for oral application

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Vaccination is one of the most successful and important means of modern medicine leading to a significant decrease of mortality caused by a number of infectious microbes in the last decades. However, due to an increasing resistance of known pathogenic microorganisms as well as the appearance of new pathogens, there is still a need for the development of novel vaccines. Most human pathogens like bacteria and viruses enter the body via the mucosal surfaces of the gastrointestinal and respiratory tracts. Therefore, newly developed vaccines should be able, beyond the induction of a protective cellular and humoral immune response, to provoke also a mucosa-associated immune response resulting in the production of IgA by the mucosal surfaces.

The effective targeting and subsequent binding of particulate carriers to M cells in Peyer's patches of the gut is a prerequisite for the development of oral delivery systems. We have established a novel carrier system based on cell surface expression of the β_1-integrin binding domain of invasins derived from *Yersinia enterocolitica* and *Y. pseudotuberculosis* on the yeast *Saccharomyces cerevisiae*. The cell surface expression of all invasin derivatives was verified via indirect immunofluorescence and recombinant yeast cells showed improved binding to both human HEp-2 cells and M-like cells in vitro. Among the different derivatives tested, the integrin-binding domain of *Y. enterocolitica* invasin with a length of 198 amino acids ($Y.ent_{198}$) proved to be the

most effective and was able to target Peyer's patches *in vivo*. After feeding of mice with fluorescent labeled yeast cells it was shown via fluorescence microscopy that Y.ent$_{198}$ expressing yeast were considerably more effectively taken up in Peyer's patch tissue than the respective control yeast.
The results of this study confirm that the binding properties of recombinant yeast can be improved with regard to their oral administration. In particular, the expression of the integrin-binding domain of the *Y. enterocolitica* invasin significantly increased the binding to human epithelial cells in two different *in vitro* model systems and also *in vivo*. Our data implicate that invasin-expressing yeast cells can be used to target M cells specifically *in vivo* and lay the basics for the development of a novel yeast-based carrier for antigens or drugs.

Reference
[1] Kenngott et al. (2016) J Control Release 224; 1-7.

Presentation: Wednesday, 14 September 2016 from 9:45 – 10.00 in room Konferenz 2-3.

WORKSHOP 18
Antimicrobial, probiotic and immune mechanisms of the commensal microbiota (FG PW)
14 Sept. 2016 • 08.30–10.00

103/PWV
The cystic fibrosis lower airways microbial metagenome
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Cystic fibrosis (CF) is a life-shortening, debilitating, autosomal recessive disease that is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene. The basic defect predisposes to chronic airway infections with opportunistic pathogens which determine most morbidity in people with CF. Epidemiological data drawn from culture-dependent diagnostics of respiratory specimens indicated that CF patients become colonized in their airways with *Haemophilus influenzae* and *Staphylococcus aureus* during early childhood followed by *Pseudomonas aeruginosa* and sometimes by organisms such as *Burkholderia cepacia* complex or atypical mycobacteria later in life. We applied shotgun metagenome sequencing to resolve the complex poly-microbial communities in CF airways.
DNA libraries were prepared from sheared DNA of induced sputa. Sequencing was performed on a SOLiD 5500XL system in color space. The raw sequence reads were trimmed and then (in this order) low quality reads, human reads, non-human low-complexity reads and non-human reads encoding mobile genetic elements were removed. The remaining microbial reads were normalized by GC content and genome length. This curated data set was then used for the identification of taxa and their absolute abundance per human cell, principal component analysis, search for mutations in antimicrobial resistance genes and analysis of clonal diversity of the *S. aureus* and *P. aeruginosa* populations in the respiratory secretions.
Result: More than a 1,000 bacterial species belonging to 21 phyla were identified in the airways of children, adolescents and adults with CF by high-throughput shotgun metagenome sequencing of induced sputum samples. Actinobacteria, bacteroidetes, firmicutes, fusobacteria and proteobacteria constitute more than 99% of the bacterial community in CF airways. The DNA virome mainly consists of bacteriophages and occasionally of human pathogens such as adeno- or herpes viruses and the mycobiome mainly consists of Candida and Aspergillus species. The clonal populations of the common CF pathogens *S. aureus* and *P. aeruginosa* in CF airways are more complex than presumed to date. Besides one or two major clone types which are amenable to standard culture-dependent diagnostics several rare clone types and subclonal variants co-exist in CF lungs which could rapidly expand as a response to antimicrobial chemotherapy or invasion of novel microbes.

Presentation: Wednesday, 14 September 2016 from 8:30 – 8:45 in room Konferenz 1.

104/PWV
Interaction of prophages and colicin Ib at the single cell level in *Salmonella enterica* serovar Typhimurium
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Colicins are protein toxins produced by and toxic for members of the *Enterobacteriaceae* family (i.e. *E. coli, Salmonella spp.*). Colicins are only expressed by a fraction of the (genetically identical) population (phenotypic heterogeneity). Upon colicin release, bacteria are lysed, while the remaining part of the population survives and gains a fitness advantage against colicin-sensitive competitors. This scenario is referred as *division of labor* and can eventually increase the overall fitness of a colicin-producer in complex ecosystems. Importantly, colicins can be subdivided in two groups (A and B). Among other differences, group A colicins encode for a cognate lysis protein and release colicin by lysis protein-mediated cell lysis. In contrast, group B colicins do not encode for a cognate lysis protein and the release mechanism of group B colicins remained unclear. We characterized expression and release of pore-forming group B colicin Ib (ColIb) by the human enteric pathogen *S.* Typhimurium SL1344 (*S.* Tm). We could show that ColIb (*cib*) confers a fitness benefit to *S.* Tm in competition against ColIb susceptible *E. coli* strains in mouse model for *S.* Tm induced colitis. Moreover, we recently demonstrated that, in contrast to group A colicins, ColIb is released in the course of temperate phage cell lysis. Our data reveal a new mechanism for colicin release and point out a novel function of temperate phages in enhancing colicin-dependent bacterial fitness. Here, we characterized *cib* expression and temperate phage-mediated cell lysis at the single cell level using *gfp*- and *rfp*-based fluorescent protein reporter tools, respectively. Using these reporters we could demonstrate by single cell analysis (i.e. FACS, microscopic image analysis and live cell microscopy) that *cib* expression and activation of the lysis genes of temperate phages are co-regulated within individual bacteria.

Presentation: Wednesday, 14 September 2016 from 8:45 – 9:00 in room Konferenz 1.

105/PWV

CD101 maintains intestinal immune homeostasis due to the restriction of bacterial replication and translocation

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Introduction: Inflammatory bowel disease (IBD) is driven by complex interactions of genetic susceptibility traits, environmental factors and enteric microbes. However, the mechanisms by which the mutual interplay of the mucosal immune system and the intestinal microbiota is disrupted and by which inflammation is propagated are not understood.
Aim: We have recently reported that an enhanced expression of the transmembrane, Ig-like domains containing glycoprotein CD101 is associated with less severe IBD and significant alterations in the composition of the intestinal microbiota. Thus, we elucidated in this study the mechanism(s) by which CD101 interferes with distinct bacteria and mediates protection from IBD.
Materials and Methods: The expression of CD101, the composition of the intestinal microbiota, bacterial replication and dissemination as well as the extent of intestinal inflammation was assessed in IBD patients and in mouse models of acute and chronic colitis.
Results: We observed that wildtype or *DAroA* mutant *Salmonella* strains replicated and disseminated faster in $CD101^{-/-}$ than $CD101^{+/+}$ littermates. Subsequently, $CD101^{-/-}$ mice succumbed earlier to acute infection and developed a more severe colitis during chronic infection which was paralleled by a reduced recovery of *Lactobacilli* and *Clostridia spp*. Similar results were obtained with dextran sodium sulfate (DSS)-treated $CD101^{-/-}$ mice, which revealed in addition an accumulation of *Enterobacteriaceae* in the gut. These alterations in the composition of the intestinal microbiota were critical for the protective effects mediated by CD101 as only a reconstitution of $CD101^{+/+}$, but not $CD101^{-/-}$ mice with *Lactobacilli* ameliorated DSS- and *Salmonella*-induced colitis and inhibited the translocation of *Enterobacteriaceae* into extra-intestinal organs. While *Lactobacilli* adhered to CD101 without affecting its expression, an interaction of host cells with various *Enterobacteriaceae* led to a loss of their CD101-expression. The expression of CD101 was also pivotal for *Lactobacilli* to inhibit an infection of myeloid and intestinal epithelial cells with *Enterobacteriaceae* in *in vitro* co-culture studies. Vice versa, as infected cells were predominantly CD101-negative, *Enterobacteriaceae* disrupt these protective effects of CD101 and commensal bacteria. Most importantly, in IBD patients a reduced CD101-expression on peripheral and intestinal $CD11b^{+}$ monocytes and $CD4^{+}$ T cells correlated with an enhanced intestinal permeability and disease activity.
Summary: In summary, our data suggest that commensal bacteria require CD101 to protect from severe enterocolitis and IBD. How certain bacterial species (that might be missing in IBD patients) promote the function of CD101, how CD101 restricts bacterial replication and how *Enterobacteriaceae* suppress CD101 is part of our ongoing analyses.

Presentation: Wednesday, 14 September 2016 from 9:00 – 9:15 in room Konferenz 1.

106/PWV

Probiotic *Enterococcus mundtii* isolate protects *Tribolium castaneum* against *Bacillus thuringiensis*

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Introduction: Storage food pests are eating, oviposit and defecating in the same environment resulting in a constant exposure to their own feces. Insect feces again are known to have protective function against pathogens, partly based on symbionts. Furthermore, newly hatched offspring can directly acquire beneficial microbes from this feces in the environment. Insects can be used as an alternative model for medical prescreening applications.
Objective: Isolation of protective microorganism from the feces of the Mediterranean flour moth larvae, *Ephestia kuehniella*, and further *in vitro* and *in vivo* characterization of the isolates.
Materials and Methods: *In vitro* assays to characterize the probiotic profile of the isolates. *In vivo* characterization of the isolates' protective effect and influence of fitness parameters was done in the model organisms *Tribolium castaneum*. Isolates and corresponding supernatant (CFS) were orally exposed to *T. castaneum* larvae and after additional infection with entomopathogenic bacteria survival was recorded and fitness parameters measured.
Result: Isolated *Enterococcus mundtii* strains showed antimicrobial activity against a broad spectrum of bacteria. The *in vitro* characterization of one of the isolates revealed a high auto-aggregation score, a hydrophilic cell surface, a tolerance for low pH, no hemolytic activity and susceptibility to all tested antibiotics. *T. castaneum* larvae exposed to *E. mundtii* or the corresponding CFS showed an increase survival after exposure to entomopathogenic bacteria. Heat treatment or treatment with proteinase K reduced the protective effect of the CFS.
Conclusion: Feces of food pests provide an alternative source for new probiotic strains. *E. mundtii* isolates showed good probiotic properties both *in vitro* and *in vivo*. *T. castaneum* is a suitable insect model for high throughput screenings of probiotic microorganisms.

Presentation: Wednesday, 14 September 2016 from 9:15 – 9:30 in room Konferenz 1.

107/PWV

Quantification and characterisation of trimethylamine-producing bacteria in the human gut

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Introduction: Microbial trimethylamine (TMA) formation in the gut and its subsequent conversion to trimethylamine-*N*-oxide (TMAO) in the liver is linked to the development of atherosclerosis and severe cardiovascular diseases.
Aim: The aims of this project were to establish databases for the key genes involved the main TMA-synthesis pathways and to develop assays that (*i*) quantify their abundance in intestinal communities and (*ii*) enable detailed compositional insights into this important functional community.
Materials and Methods: Databases for the key genes of both pathways were constructed following a multiparametric screening approach consisting of analyses of *(i)* sequence similarity to Hidden-Markov Models, *(ii)* phylogenetic distance and *(iii)* conserved amino acid residues. The databases served as a basis to develop gene-targeted assays to enumerate *cutC* and *cntA* genes, encoding choline TMA-lyase and carnitine oxygenase, by quantitative PCR and to characterise their diversity by sequencing on the Illumina MiSeq platform. These assays were applied on faecal samples derived from 50 individuals and results were correlated to 16S rRNA gene data. Moreover, a metagenomic screening approach, specifically targeting the TMA-producing gut microbiota, was established and applied on data from 154 samples provided by the Human Microbiome Project.
Result: The screening of 50 human faecal samples revealed that the majority (~80%) contained TMA producers. However, only a minority of the total bacterial community members encoded the respective activities. Genes similar to those previously observed in members of the phyla *Actinobacteria*, *Firmicutes* and *Proteobacteria* were detected, in particular *cutC* genes similar to those of *Clostridium* XIVa strains as well *cntA* genes of *Gammaproteobacteria* were predominant. The majority of amplified *cutC* sequences displayed low nucleotide identities to a reference, indicating that most of *cutC*-derived TMA is produced by strains of *Clostridium* XIVa yet to be isolated. In contrast, the majority of amplified *cntA* sequences was nearly identical to those previously observed in *Escherichia coli*, and clustering revealed only a low gene richness. Analysis of metagenomic data yielded a similar abundance pattern and taxonomic composition of TMA-producing communities as obtained by the gene-targeted assays.
Summary: This project established a framework to quantify the potential of TMA-producing intestinal communities and to investigate their catabolic gene structures in detail. It will assist in further detailed investigations allowing to develop specific strategies altering the microbiota in order to reduce TMA production.

Presentation: Wednesday, 14 September 2016 from 9:30 – 9:45 in room Konferenz 1.

108/PWV

Human commensals producing a novel antibiotic impair pathogen colonisation

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The vast majority of systemic bacterial infections is caused by facultative, often antibiotic-resistant pathogens colonising human body surfaces. Nasal carriage of *Staphylococcus aureus* predisposes to invasive infection, but the mechanisms permitting or interfering with pathogen colonisation have remained largely unknown. Whereas soil microbes are known to compete by production of antibiotics, such processes have rarely been reported for human microbiomes. We show that nasal *Staphylococcus lugdunensis* strains produce lugdunin, a novel thiazolidine-containing cyclic peptide antibiotic prohibiting colonisation by *S. aureus*, and a rare example of a non-ribosomally synthesized bioactive compound from human-associated bacteria. Lugdunin is bactericidal against major pathogens, effective in animal models, and not prone to resistance development. Importantly, human nasal colonisation by *S. lugdunensis* was associated with a significantly reduced *S. aureus* carriage rate suggesting that lugdunin or lugdunin-producing commensals could be valuable for preventing staphylococcal infections. Moreover, human microbiota should be considered as a source for new antibiotics.
Presentation: Wednesday, 14 September 2016 from 9:45 – 10.00 in room Konferenz 1.

111/INV

Food as Source of Norovirus Infections- Experiences from Outbreak Investigations

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Introduction: Norovirus infection is a major cause of human gastroenteritis in the Northern hemisphere. It is estimated that about 40% of norovirus infections had a foodborne origin. Fresh produce and especially soft berries are considered to be food items that are at high risk to be contaminated.
Objective: Foods suspected to be contaminated with food-borne viruses are subject of inspection. It is necessary to discover the source of food associated viruses by two reasons. Most importantly, all contaminations sources need to be eliminated and foodstuffs should be withdrawn from the market. Secondly, food business operator could have an interest that their products are not involved.
Materials and Methods: Official protocols exist for the analysis of food associated viruses are needed to be applied. The protocol for soft berries and leafy greens, is based on alkaline elution and PEG. In contrast to analysis of medical specimens, the contamination dose is low and inhibitors of molecular reactions occur frequently. Contamination of food by infected food handlers is being detected by analysis of swab samples from kitchen environments. The variety of matrices makes it necessary to monitor the analytical process by application of a process control. Positive samples should be further investigated by sequence analysis. Based on these data, comparison with virus strains from patients as final proof is possible. As viral RNA is often detected at the limit of detection, it is necessary to follow several approaches

like Whole Transcriptome Amplification and or nested PCR to generate nucleic acid sequences long enough for reliable results.
Result: The approach was used in the 2012 outbreak of norovirus caused by Chinese strawberries. In food samples and in stool samples, several genogroups and types of norovirus were detected. This could lead to the assumption, that sewage water was most likely the source of contamination. On site visits the year following the outbreak demonstrated some shortcomings in the official system in the exporting country.
In general, the approch is being applied to all food associated outbreaks where positive food samples are available. Not in every case the epidemiolocigal link can be closed. It became evident that the lack of harmonized sampling protocols based on statistics is a further problem facing food business operators in the framework of their autocontrols and official surveillance bodies. Further works is required to fill present gaps in this field as well.
Summary: Gastroenteritis outbreaks affecting a few up to more than 10000 people occurre in Germany. Epidemiological studies help to determine the vehicle of infection. Most outbreaks in which the food source was dicovered, could be traced back to frozen berries and leafy greens. Virus extraction from food and molecualr analysis of food samples is based on official methods which are quite cumbersome and costly, other protocols are not applicable. Nucleic acid sequence analysis is being applied to close links between food samples and human samples. It should be noticed that standardized sampling protocols applicable worldwide do not exist.

Presentation: Wednesday, 14 September 2016 from 11:45 – 12:15 in room Donausaal 3.

WORKSHOP 19
Food Microbiology and Hygiene (FG LM)
14 Sept. 2016 • 13.00–14.30

112/LMV
Biodiversity and counts of thermophilic spore formers in milk and milk powder
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Milk and whey powders are technically demanding, but highly valuable products contributing significantly to the economic success of dairy companies. Of particular interest are thermophilic spore-formers that have their growth optimum in the range of 50-70°C, which is exactly the temperature range used for concentration processes during manufacturing. Growth of these organisms during production may lead to elevated counts in the end product limiting its quality. Therefore, strategies aiming at reducing the amounts of thermophilic spore-formers and their spores in milk powders are urgently needed.
The first task of the project was the optimization of a method for spore quantification, as an official method is still lacking. It turned out that although the maximum growth temperature for some strains is 70°C, a heating step of 10 min at 80°C as used for mesophilic spore-formers is sufficient to inactivate vegetative cells of thermophilic spore-formers as well.
Thermophilic spores in milk are low in count (10-100 cfu/mL) and *Bacillus licheniformis* is the dominating species found. It is in fact a mesophilic organism having an extended growth range up to 55°C, which is the reason for its high abundance among the thermophilic counts. There have been detected some more mesophilic organisms such as different *Brevibacillus* spp. Strictly thermophilic species such as *Geobacillus stearothermophilus* or *Anoxbacillus flavithermus* have been found occasionally, but they represent only a minor fraction of the count at 55°C. In milk powder to the contrary, much higher counts are found and the biodiversity of species is strongly reduced. The average spore count is >1000 cfu/g and may reach levels of >100.000 cfu/g. The dominating species is *A. flavithermus* followed by *G. stearothermophilus*, indicating a shift from raw material to end product.
The reasons and dynamics of this change are to be analysed in order to develop effective strategies for a control and reduction of thermophilic spore counts.

Presentation: Wednesday, 14 September 2016 from 13:00 – 13:15 in room Donausaal 4-5.

113/LMV
Coagulase-negative staphylococci in the food chain and their enterotoxigenic potential
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Introduction: *Staphylococcus aureus* is among the leading causes of food-borne diseases causing mild to severe intoxications after ingestion of staphylococcal enterotoxins (SE) preformed in food stuffs, mainly milk and meat, and products thereof. More than 20 different SE types have been described, among them SE types A-E being most often involved in staphylococcal food-borne outbreaks. Recently, also coagulase-negative staphylococci (CoNS) were questioned to play a role as food-borne hazards due to findings of enterotoxin genes in CoNS. Besides, CoNS were also recovered

from bovine mastitis cases and possibly may enter the food chain. However, until now little is known about the characteristics of CoNS present in the food chain in Germany.
Aim: The aim of the study was to identify and characterize CoNS isolated from food and bovine mastitis cases in Germany and to study their enterotoxigenic potential by screening for staphylococcal enterotoxin genes.
Materials and Methods: In total, 62 CoNS isolates from food products (milk, cheese, and meat products) and 31 isolates from cases of bovine mastitis were analyzed. CoNS were identified by the presence of *tuf* and the absence of *nuc* gene using an in-house mPCR protocol. Species identification was done by MALDI-TOF MS (Bruker Daltonik) and compared to 16S rRNA sequencing. The presence of staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sep*, *ser*) was analyzed by applying two in-house mPCRs.
Result: Overall, 15 different CoNS species were identified. CoNS isolates comprised mainly *S. xylosus*, *S. saprophyticus* and *S. haemolyticus*. Among the isolates from milk, cheese and meat products 14 different CoNS species were detected. 7 different CoNS species were collected from cases of bovine mastitis. Both methods applied were reliable for staphylococcal species identification. In none of the 62 CoNS investigated genes encoding for the 'classical' SE-types A to E could be detected by standard molecular methods usually applied for the detection of *S. aureus* enterotoxins. Also other SE-encoding genes (*seg-j*, *sep*, *ser*) could not been detected among CoNS from food products and dairy mastitis isolates.
Summary: Different CoNS species were identified in food products and from mastitis cases in dairy cattle in Germany. Major SE-encoding genes were not detected within the CoNS isolates. Thus, the consumer health risk due to CoNS present in the food chain seems to be low. Currently, further studies are ongoing aiming to characterize the consumer health risk due to CoNS in the food chain, also including specified molecular methods adapted to the detection of SE-encoding genes of CoNS.

Presentation: Wednesday, 14 September 2016 from 13:15 – 13:30 in room Donausaal 4-5.

114/LMV
Selection and application of *Staphylococcus equorum* strains in raw ham production
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Introduction: Raw hams are currently produced without the application of starter cultures. One of the main problems in raw ham production is incomplete reddening. The application of starter cultures might be favorable for enhancing color formation and thus decreasing the amount of defective goods.
Aim: This study aimed at screening *Staphylococcus equorum* strains *in vitro* for their technological properties and at subjecting the strains to a safety evaluation. Subsequently, selected strains were applied in raw ham processing and effects were analytically as well as sensory evaluated.
Materials and Methods: Twenty-three isolates and the *S. equorum* type strain were taxonomically determined on the strain level by molecular methods and investigated for antibiotic resistances, hemolysis and the presence of toxin genes. The strains were also screened *in vitro* for selected metabolic traits, such as lipolysis, proteolysis and nitrate reductase activity. During raw ham fermentation with selected strains, viable counts, water activity, pH-value and nitrate reduction were measured. Color changes were determined by L*a*b measurements and by panelists.
Result: The species affiliation of all strains was confirmed based on *dnaJ* sequences, and they were differentiated on the strain level. Twelve strains were susceptible to all 17 tested antibiotics, while eight strains were multi-resistant. The susceptible strains did not harbor the *sea*, *seb*, *sec*, *sed*, *see*, *seh*, *eta*, and *tst-1* genes, but two strains displayed β-hemolysis. Two strains were positive for proteolysis, while seven were positive for lipolysis. Nitrate reductase activity was strain specific. Five strains were subsequently chosen for application in raw ham fermentation, and all strains showed stable viable counts throughout fermentation. As expected from the *in vitro* screening, nitrate reductase activity was strain specific in the product as well. Color changes were most pronounced in the a* values indicative of successful reddening.
Conclusion: The *in vitro* screening successfully identified *S. equorum* strains with different metabolic properties that are indeed suitable starter cultures for raw ham fermentation.

Presentation: Wednesday, 14 September 2016 from 13:30 – 13:45 in room Donausaal 4-5.

115/LMV
Whole genome sequencing and qPCR analysis of *Advenella*, *Psychrobacter* and *Psychroflexus* phylotypes for Austrian raw milk hard cheese rinds during ripening
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Vorarlberger Bergkäse (VB) is an artisanal raw milk hard cheese manufactured in Western Austria. The composition of its rind microbiota and changes during the ripening process has been described previously by using 16S and 18S rRNA gene cloning (Schornsteiner et al. 2014). To learn more about the functional contribution of abundant phylotypes to cheese ripening, we performed whole genome sequencing of *Advenella* (*Betaproteobacteria*), *Psychrobacter* (*Gammaprotoeobacteria*) and *Psychroflexus* (*Bacteroidetes*). Furthermore, total and species-specific bacterial numbers during ripening were determined using real-time quantitative PCR (qPCR). Cheese rind samples were taken from ripening cellars of two different cheese producing facilities (L, S) in Vorarlberg (Austria) at five different time points: right after production (day 0) and after 14, 30, 90 and 180 days of ripening. All samples were cultivated and one representative isolate was chosen for Illumina MiSeq mate-pair genome sequencing. For qPCR, total genomic DNA was extracted from all samples using a bead-based isolation kit. To determine shifts in the absolute abundance at different time points along the ripening process, the levels of total bacteria and three species were quantified with Eva Green® qPCR. Our results reveal that each of the genomes harbors many enzymes shown to be important for cheese ripening such as: Cystathionine beta or gamma-Lyases, many proteases and peptidases (including proline imminopeptidases), aminotransferases, esterases and lipases and Lactate dehydrogenases. Thus, all of the isolates clearly have to potential to contribute positively to cheese ripening. The level of total bacteria was found to be comparable between the cellars, ranging from 10^8 to 10^{10} BCE/1g cheese rind. The level was decreasing by one log unit in the first 30 days of ripening, and remained stable for the rest of the ripening process. *Psychrobacter* and *Psychroflexus* increased by one log unit in the first 30 days, then the levels decreased gradually to the initial abundance over the following five months of ripening. *Psychrobacter* is known to contribute to cheese ripening, for instance by the formation of flavour compounds. In contrast, the level of *Advenella* decreased in the first month of ripening, but then increased throughout ripening, particularly in cellar S. At the end of the ripening process, the levels of *Advenella* were similar in both cellars. In conclusion, the three species quantified were identified to be essential, stable community members throughout

the ripening process and suggests an important contribution of these bacteria to cheese ripening. Currently, in-depth comparative genome analyses are underway to characterize these genomes in more detail.

Schornsteiner et al. (2014). Cultivation-independent analysis of microbial communities on Austrian raw milk hard cheese rinds. Int J Food Microbiol, 180, 88-97.

Presentation: Wednesday, 14 September 2016 from 13:45 – 14:00 in room Donausaal 4-5.

116/LMV

Detection of toxigenic *Bacillus cereus* group isolates from spices from German stores

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Question: The aims of this study were (1) the investigation of spices from retail stores in Berlin as potential reservoir of bacteria from the *B. cereus* group, (2) the further caracterisation of the isolated strains and occurrence of toxin encoding genes: *hbl, cytk, nhe* and *ces*.

Method: A total of 70 spice samples (Herbs de Provence, marjoram, oregano, paprika and pepper) were processed accordingly to the EN ISO 7932:2004. Detection of enterotoxins and cereulid genes as well as the differentiation within the *B. cereus* group was done using real-time PCRs. Strains belonging to the *B. cereus* group were tested with commercial available tests for their ability to produce the NHE and HBL enterotoxins.

Result: Bacteria belonging to the *B. cereus* Group were detected in the investigated samples. Genes encoding for human pathogenic enterotoxins were found in different combinations within the isolated strains and their ability to produce HBL and NHE was confirmed. These Enterotoxins are associated with diarrheal food poisoning. The spices with the highest burden of *B. cereus* were Herbs de Provence, oregano and black pepper.

Conclusion: Food poisoning with *B. cereus* is of major public health concerns, the European Food Safety Authority (EFSA) recognize that these diseases “are likely to be considerably underdiagnosed” and “therefore presumably underreported in most countries” (4, 5).

Our results confirm the assertion of the EFSA concerning the frequent contamination of spices with the so-called presumptive *B. cereus* (3). This study contributes to elucidate the occurrence of toxigenic *B. cereus* in spices and to improve the genotyping of this bacterial group.

References

[1] Moreira, P.L., Lourencao, T.B., Pinto, J.P., Rall, V.L., 2009. Microbiological quality of spices marketed in the city of Botucatu, Sao Paulo, Brazil. J Food Prot 72, 421-424.

[2] Hara-Kudo, Y., Ohtsuka, K., Onoue, Y., Otomo, Y., Furukawa, I., Yamaji, A., Segawa, Y., Takatori, K., 2006. Salmonella prevalence and total microbial and spore populations in spices imported to Japan. J Food Protect 69, 2519-2523.

[3] EFSA (2013) Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 1 *EFSA J*, 3025

[4] EFSA (2013): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-bourne outbreaks. *EFSA J*, 3129.

[5] EFSA (2005): Opinion of the Scientific Panel on Biological Hazards *on Bacillus cereus* and other *Bacillus spp* in foodstuffs. *EFSA J.* 175.

Presentation: Wednesday, 14 September 2016 from 14:00 – 14:15 in room Donausaal 4-5.

117/LMV

The agr peptide sensing system of Listeria monocytogenes - autoregulation and identification of the autoinducing peptide

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Listeria monocytogenes (*Lm*) is an important human, food-borne pathogen and a model organism for intracellular pathogenesis. Important features including biofilm formation and virulence of *Lm* are subject to regulation by the *agr* peptide sensing system. The aim of the presented study was to identify the structure of the *agrD*-encoded native autoinducing peptide (AIP) of *Lm*, which is process to its active form by posttranslational modification before secretion into the extracellular space. Moreover, the molecular mechanisms of positive autoregulation of the *agr* system are investigated.

A range of synthetic peptides were tested for activation of the *agr* promoter using a luciferase reporter assays and the AIP was identified by mass spectrometry. Regulatory sequences motifs potentially were searched *in silico* and their role in autoregulation was confirmed by luciferase reporter assays. Electrophoretic mobility shift assays were performed to confirm binding of the AgrA response regulator to the *agr* promoter.

Screening of a range of synthetic peptides by luciferase reporter assays for *agr* promoter activation suggests, that the native AIP of *Lm* is a cyclic pentapeptide consisting of a thiolactone formed by the cysteine residue at position 32 with the carboxyl group of the valine at position 36 of the ArgD prepeptide sequence. An identical peptide was identified in culture supernatants of a recombinant *E. coli* strain expressing AgrBD. Upon deletion of *agrD*, *agr* promoter activity was completely abolished suggesting positive autoregulation that depends on the *agrD* encoded AIP and the LytTR family response regulator AgrA. *In silico* analysis identified potential LytTR binding motifs in the *agr* promoter. Binding of AgrA to this promoter was confirmed by EMSA and further luciferase reporter assays demonstrated that one of the potential LytTR motifs is required for positive autoregulation of the *Lm agr* system via its AIP.

Presentation: Wednesday, 14 September 2016 from 14:15 – 14:30 in room Donausaal 4-5.

WORKSHOP 20
Eukaryotic Pathogens (incl. DMykG Lecture) (FG EK)
14 Sept. 2016 • 13.00–14.30

118/EKV

DMykG Lecture: The fungal pathogen *Aspergillus fumigatus* exploits its virulence determinants to defend against environmental predators

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Systemic mycoses are severe infections of immunocompromised patients and frequent nosocomial threats which are aggravated by the limited options for diagnosis and therapy. Filamentous fungi, such as the classical mold *Aspergillus fumigatus*, are often coined "opportunistic" and represents one of the classical examples of an environmentally acquired pathogen. Its virulence potential has partially been attributed to its intrinsic resistance to the innate immune system. More specifically, the fungus has developed sophisticated mechanisms to either escape from human phagocytes or withstand their oxidative burst. The latter includes the peroxiredoxin Aspf3 which is essential for fungal virulence in immunosuppressed mice as wells as during the defence against external oxidative threats. But how these free-living organisms could have evolved multifactorial virulence strategies effective against animals and humans is not well understood.
In their natural environment, all fungi have been exposed to environmental predators, such as soil amoebae throughout their evolutionary history and probably well before the emergence of innate immunity. In the past the well-studied soil amoeba, *Dictyostelium discoideum* served as our initial model for investigating the general virulence determinants of *A. fumigatus*. During *in vitro* confrontations with fungal conidia, we could demonstrate that phagocytic interactions between both organisms showed similarities to encounters with macrophages. While white, unmasked conidia of a *pksP* mutant were rapidly ingested by *Dictyostelium*, uptake of those covered with the green pigment DHN-melanin was drastically reduced. Similar effects on the recognition by phagocytes were also observed for the convergent Asp-melanin of *A. terreus*. Mycotoxins like gliotoxin or the spore-borne polyketide trypacidin further contributed to the anti-phagocytic properties of *A. fumigatus*. To further expand our model and to demonstrate that such predatory interactions with fungi occur in natural habitats, we have recently isolated and identified the widely spread soil amoeba *Protostelium mycophaga*. The amoeba is characterized by strictly fungivorous life-style. Feeding experiments of *P. mycophaga* with different pathogenic fungi revealed a broad food fungal prey spectrum including most *Candida* species, but also filamentous fungi such as *A. fumigatus*. I will present our first insights to the highly efficient killing mechanisms of this amoeba.

Presentation: Wednesday, 14 September 2016 from 13:00 – 13:30 in room Konferenz 2-3.

119/EKV

Characterization of the putative seven-helix protein 2 in the transmission stages of the malaria parasite *Plasmodium falciparum*

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Background: The parasitic disease malaria is a leading cause of death worldwide with an estimated 200 million clinical cases annually. Particularly affected are children under five years of age and pregnant women in sub-Saharan Africa. Malaria is caused by unicellular parasites of the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes during a blood meal. The complex life-cycle of malaria parasites requires a high degree of tight coordination allowing the parasite to adapt to changing environments in the two different hosts. One of the major challenges is the rapid stage conversion of sexual precursor cells, the intraerythrocytic gametocytes, into gametes, which takes place immediately after transmission from the human to the mosquito. Gametogenesis is known to be triggered by environmental stimuli, including a drop of temperature and the presence of xanthurenic acid in the mosquito midgut. While some components of the signalling pathway leading to gametogenesis in the parasite have meanwhile been identified and hint to the involvement of seven-helix proteins, the receptor or receptors needed for perception of the environmental signals in the mosquito midgut have not yet been identified. Understand the molecular tools that enable the parasites to deal with large environmental variations will help to identify novel therapeutic targets.
Objective: The objectives of this work are to investigate the expression of the putative seven-helix receptor protein 7-Helix-2 (gene-ID: PF3D7_1204400) in the blood and transmission stages of the human malaria parasite *P. falciparum* and to decipher the function of 7-Helix-2 for gametogenesis.
Method: Transcript expression of 7-Helix-2 was studied via semi-quantitative reverse-transcriptase PCR. For protein expression analysis, mouse polyclonal antisera was generated and applied to indirect immunofluorescence assays and Western blot analysis. For functional studies, a gene-disruptant line (7-Helix-2(-)) has been generated via single crossover homologous recombination.
Result: 7-Helix-2 is specifically expressed in gametocytes, where it accumulated in granular structures. Gene-disruption proved that the protein is dispensable for erythrocytic replication of *P. falciparum*. Further phenotypical analyses will be presented.
Conclusion: This is one of the first reports on a putative seven-helix receptor protein in *P. falciparum*. The preliminary results indicate a function during malaria parasite transmission from the human to the mosquito vector.

Presentation: Wednesday, 14 September 2016 from 13:30 – 13:45 in room Konferenz 2-3.

120/EKV

Histone deacetylase inhibitor MS-275 increases expression of IFN-γ-regulated genes in *Toxoplasma gondii*-infected macrophages but does not augment cell-autonomous immunity

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Toxoplasma gondii infects a broad range of mammals and birds and is highly prevalent throughout the world. Infection of humans is usually benign or asymptomatic but leads to chronic infection for

the host's life. The parasite's ability to inhibit IFN-γ-regulated gene expression in infected cells is critical for evasion of host immunity and for establishing chronic infection. Such unresponsiveness of *T. gondii*-infected macrophages to IFN-γ is accompanied by defective chromatin remodeling at the promoters of distinct IFN-γ secondary response genes. Remarkably, this could be partially restored by treatment with histone deacetylase (HDAC) inhibitors. Here, we describe the effect of HDAC inhibitors on IFN-γ-regulated gene expression in *T. gondii*-infected monocytes on a genome-wide level.

High throughput mRNA sequencing confirmed the transcriptome-wide defect of *T. gondii*-infected RAW264.7 monocytic cells to respond to IFN-γ. Treatment of macrophages with the HDAC inhibitor MS-275 increased mRNA levels of the majority of IFN-γ secondary response genes but only a subset of IFN-γ primary response genes in both non-infected and parasite-infected cells. Importantly, MS-275 did not generally rescue RAW264.7 from the parasite-imposed inhibition of IFN-γ-regulated primary or secondary response genes. Transcript levels of a variety of secondary response genes including CIITA, GBPs and MHC genes were nevertheless strongly increased by MS-275 in *T. gondii*-infected cells as compared to controls. MS-275 also considerably modulated the IFN-γ-independent transcriptome of RAW264.7 cells. Surprisingly, this regulation was counterbalanced by *T. gondii* infection. Expression of immunomodulatory IL-10, IL-4 and SOCS2 was increased in infected and non-infected monocytic cells after treatment with MS-275. Since MS-275 thus modulated expression of IFN-γ-dependent and -independent genes in *Toxoplasma*-infected cells, we next determined whether it can improve the anti-parasitic defense in unstimulated, IFN-γ- or IFN-γ/LPS-stimulated host cells. FACS analysis and immunofluorescence microscopy revealed that MS-275 did not considerably diminish the number of parasite-positive cells or the intracellular replication in unstimulated or stimulated monocytic cells. Together, our results indicate that the HDAC inhibitor MS-275 is able to increase particular the expression of IFN-γ secondary response genes in *Toxoplasma*-infected monocytic cells but does not abolish the inhibitory effect of parasite infection on IFN-γ-mediated responsiveness. Thus, a supportive therapy using MS-275 appears inappropriate for treatment of toxoplasmosis.

Presentation: Wednesday, 14 September 2016 from 13:45 – 14:00 in room Konferenz 2-3.

121/EKV

***Candida albicans* EED1 links quorum sensing and hyphal maintenance**

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Morphogenesis in *Candida albica*ns requires hyphal initiation and maintenance, and both processes are regulated by the fungal quorum sensing molecule (QSM) farnesol. The mechanisms by which farnesol modulates filamentation and to which extend this QSM affects virulence are still not fully understood.

We discovered that the deletion of the *C. albicans EED1* gene, which is crucial for hyphal extension and maintenance, led to reduced filamentation at high densities. This effect was mediated by farnesol, while sensitivity to other fungal and bacterial QSMs was unaltered. Although exogenous farnesol reduced filamentation of an *eed1*Δ strain, it did not induce cell death, suggesting that reduced filamentation was not due to reduced cell viability. Genetic studies using hyperactive and deletion mutants of key factors in pathways known to be involved in farnesol signaling were performed. However, farnesol hypersensitivity in *eed1*Δ strain was independent of Ras1, cAMP-signaling, Nrg1 and Czf1, suggesting that further pathways might exist by which farnesol influences morphology in *C. albicans*.

Interestingly, spent supernatant of the *eed1*Δ strain revealed a higher quorum sensing activity than a respective wild type supernatant, which was associated with a rapid and increased farnesol production in the *eed1*Δ mutant. When *eed1*Δ was grown under continuous medium flow conditions, to remove accumulating QSMs from the supernatant, maintenance of *eed1*Δ filamentation, although not restored, was significantly prolonged, indicating a link between the farnesol sensitivity, production, and the hyphal maintenance-defect in the *eed1*Δ mutant strain.

Presentation: Wednesday, 14 September 2016 from 14:00 – 14:15 in room Konferenz 2-3.

122/EKV

Understanding azole-induced death of *Aspergillus fumigatus*

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The mold *Aspergillus fumigatus* is the most frequent causative species of invasive aspergillosis, a systemic infection with high mortality rates in immunocompromised patients. The antifungal voriconazole is currently recommended for first-line treatment of invasive aspergillosis. This drug belongs to the azole class of antifungal drugs. Azoles interfere with the ergosterol biosynthesis pathway by inhibiting the lanosterol 14-α-demethylase. Surprisingly, the antifungal activity of azoles significantly differs in different fungal pathogens. While azoles are primarily fungistatic in *Candida* species, they typically exhibit a fungicidal activity in *Aspergillus* species. The nature of the fungicidal activity on *Aspergillus* is not known. By exploiting new reporter systems we analyzed the effects of azoles on *A. fumigatus*. We identified the reason of fungal death and found irregular depositions of cell wall polymers which indicate a defect in cell wall biogenesis. Here we report our new insights in the mode of action of azole antifungals on *A. fumigatus*.

Presentation: Wednesday, 14 September 2016 from 14:15 – 14:30 in room Konferenz 2-3.

WORKSHOP 21
Infection Immunology (FG II)
14 Sept. 2016 • 13.00–14.30

123/IIV

Janus-faced effects of Dasatinib on the immune response against tuberculosis

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Question: Due to the emergence of multidrug resistant strains, new strategies to treat tuberculosis are urgently needed. Since the Abl tyrosine kinase inhibitor Imatinib leads to phagosomal acidification and increased killing of intracellular *Mycobacterium tuberculosis* (*Mtb),* the Abl tyrosine kinase represents an attractive target for host-directed therapy. In this study we aimed to dissect the influence of the second generation Abl tyrosine kinase inhibitor Dasatinib on the innate and adaptive immune responses against *Mtb* in primary human cells.
Method: Primary human cells were incubated with virulent mycobacteria or mycobacterial antigens in den presence or absence of Dasatinib. Immune responses were evaluated by ELISA, flow cytometry, immunoprecipitation, Western blotting, antimicrobial and degranulation assays.
Result: Intracellular mycobacterial growth is restricted through Dasatinib treatment (86%), even though it did not induce acidification of lysosomes in untreated macrophages. While Dasatinib supported the antimicrobial effects in macrophages, *Mtb*-specific release of IFNg and TNFa by human T-cell lines was almost completely abolished by treating the cells with Dasatinib. As a possible mechanism, we identified the inhibition of lymphocyte-specific protein tyrosine kinase (Lck) phosphorylation. Similarly the proliferation and degranulation of cytotoxic granules in response to mycobacterial antigens was suppressed by Dasatinib. Accordingly, preliminary experiments indicate that the cytotoxic effector molecules perforin and granzyme B accumulate in intracellular vesicles rather than being released to exert their biological function.
Conclusion: These results indicate that Dasatinib has differential effects on macrophage and T-cell function: antimicrobial activity of macrophages alone is supported whereas the essential T-cell help required for optimal macrophage activation is severely hampered. On-going experiments are designed to evaluate the net effect of Dasatinib on *Mtb* infected macrophages co-cultured with effector T-cells.

Presentation: Wednesday, 14 September 2016 from 13:00 – 13:15 in room Konferenz 1.

124/IIV

A serological evaluation of the host immune response during Necrotizing Soft Tissue Infections caused by *Streptococcus pyogenes*

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Introduction: The gram positive human pathogen *Streptococcus pyogenes* can cause severe Necrotizing Soft Tissue Infections (NSTI), requiring intensive care along with rigorous medication, ablative surgery and adjunctive therapies such as intravenous immunoglobulin administration (IVIG). The efficacy of IVIG is speculated to be mediated by various mechanisms like opsonization of the bacterial pathogen and neutralization of bacterial toxins; however, its effectiveness *in-vivo* is not well characterized.
Objective: Considering the efficacy of IVIG, the study aims to elucidate if a lack of protective antibodies against the bacteria and/or the bacterial exotoxins represents a risk factor for the development of a severe NSTI.
Methodology: The role of bacterial and exotoxin-specific antibodies during the NSTI pathogenesis was evaluated by a serologic approach using plasma samples and bacterial isolates of clinical NSTI cases collected during the early and late stage of infection in frame of the EU-funded project INFECT. Additionally control cases were included where patients suffered from severe skin and tissue infections that did not develop necrosis. The protection potential of the antibody content of the collected plasma samples against the corresponding bacterial pathogens was measured by an Opsono-Phagocytosis assay. The antibody mediated protection against pathogen specific exotoxin sets identified by a multiplex PCR screening was analyzed by ELISA in combination with specialized functional assays.
Result: The analysis of 16 INFECT cases clearly showed that the adaptive immune system of all analysed patients was not able to provide efficient protection against the corresponding bacterial isolate during the early stage of infection, however the applied IVIG treatment was able to compensate this susceptibility. In contrast, the plasma samples of the non-NSTI control cases mediated effective opsonisation and bacterial killing. The determination of protective antibody titers against the detected isolate specific exotoxin pattern by ELISA, combined with functional assays not only confirmed the crucial role of the adaptive immune system during the confrontation with *S. pyogenes*, but also demonstrated the potential of an IVIG treatment to compensate this stage of immunologic susceptibility.
Conclusion: A lack of protective antibodies against streptococcal exotoxins and against the bacteria itself represents a significant risk factor for the development of a severe NSTI. However, this serologic susceptibility is overcome by IVIG administration thereby demonstrating the potential of IVIG as potent adjunctive immunogenic therapy.

Presentation: Wednesday, 14 September 2016 from 13:15 – 13:30 in room Konferenz 1.

125/IIV

Sensing of *Legionella pneumophila* by the cGAS-STING pathway is affected by the HAQ variant of STING

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Legionella pneumophila is an important cause of pneumonia and a model organism to study intracellular bacteria. We previously showed that *L. pneumophila* activates type I IFN production by macrophages depending on cytolsolic DNA sensing, and that type I and II IFNs are key regulators of gene expression and antibacterial defense during lung infection. In this study we aimed at elucidating the molecular mechanism behind *L. pneumophila*-mediated type I IFN responses. We demonstrate that *L. pneumophila* is sensed by the cGAMP synthase (cGAS)-STING pathway in murine and human cells leading to type I IFN responses as well as production of pro-inflammatory cytokines. Upon intranasal infection, STING-deficient animals showed reduced cytokine production and a diminished antibacterial defense. Importantly, cells of human individuals carrying the frequent HAQ variant of STING were largely defective in mounting type I IFN responses to *L. pneumophila* infection, bacterial DNA or cyclic dinucleotides (CDNs). Collectively, the cGAS/STING pathway contributes to the antibacterial response in *L. pneumophila* infection, and individuals

expressing HAQ STING might have an altered susceptibility towards *L. pneumophila* and various other infections.

Presentation: Wednesday, 14 September 2016 from 13:30 – 13:45 in room Konferenz 1.

126/IIV

Staphylococcal autolysin and lipoteichoic acids recruit platelet factor 4 to the bacterial surface

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Background: The platelet-derived chemokine platelet factor 4 (PF4) binds to Gram-negative and Gram-positive bacteria. Gram-negative endotoxin has been characterized to interact with PF4, thus potentially inducing a misdirected host defense mechanism in heparin-induced thrombocytopenia in the wake of a primary anti-PF4/polyanion antibody response (1). The surface structures mediating PF4 binding to Gram-positive bacteria are as yet unknown.
Aim: To identify PF4 binding partner(s) of Gram-positive staphylococci.
Method: PF4 binding to *Staphylococcus aureus* (*S. aureus*) was analyzed by flow cytometry using biotinylated PF4 and fluorescently labeled streptavidin. The interaction of PF4-biotin and purified wall teichoic acids (WTA) and lipoteichoic acids (LTA) was assessed with peroxidase-conjugated streptavidin by enzyme-linked immunosorbent assay (ELISA). Staphylococcal surface proteins were separated by two-dimensional gel electrophoresis followed by a ligand overlay assay with PF4 and identification of the target protein by mass spectrometry. The PF4-autolysin interaction was further characterized by ELISA and surface plasmon resonance using heterologously expressed autolysin domains.
Result: Lack of WTA strongly enhanced PF4 binding to *S. aureus* as indicated by flow cytometry. In accordance, purified WTA did not interact with PF4, while PF4 bound to immobilized *S. aureus* LTA and preincubation of PF4 with increasing LTA concentrations inhibited PF4 binding to *S. aureus*. Pretreatment of *S. aureus* with proteolytic enzymes such as pronase E or trypsin effectively reduced PF4 attachment suggesting proteinaceous binding partners as well. A PF4 ligand overlay assay and mass spectrometry identified the surface-exposed protein autolysin as PF4 binding protein. Notably, PF4 preferentially bound to R1R2-repeats of autolysin.
Conclusion: LTA and proteins mediate PF4 recruitment to the surface of Gram-positive bacteria. Specifically, the surface protein autolysin was identified as staphylococcal PF4 binding partner. This study further supports a role of PF4 in the antibacterial host defense.
*equal contribution

Reference

[1] Krauel, K, Weber C, Brandt S, Zähringer U, Mamat U, Greinacher A, Hammerschmidt S. Platelet factor 4 binding to lipid A of Gram-negative bacteria exposes PF4/heparin-like epitopes. Blood. 2012; 120(16):3345-3352.

Presentation: Wednesday, 14 September 2016 from 13:45 – 14:00 in room Konferenz 1.

127/IIV

Hypoxia-inducible factor-1α stabilization supports the expression of human β-defensin 2, thereby inhibiting growth of *M. tuberculosis* in human macrophages.

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Introduction: Hypoxia-inducible factor-1α (HIF-1α) is known to be a key oxygen sensor that globally controls gene expression to adapt cellular metabolism to hypoxia. Pharmacological inhibition of prolylhydroxylases stabilizes HIF-1α and thereby artificially mimics cellular hypoxia, leading to increased expression of more than 300 genes, amongst them erythropoietin. This has led to the development of HIF-1α stabilizers, e.g. Molidustat, for treatment of renal anemia in chronic kidney disease. However, the role of HIF-1α in the immune response to microbial pathogens is not well studied.
Aim: We recently showed that hypoxia enhances antimicrobial activity against *Mycobacterium tuberculosis* (*Mtb*) in human macrophages. Here, we aimed to identify the mechanism underlying this observation by measuring the effect of HIF-1α stabilization on the expression of antimicrobial effector molecules.
Materials and Methods: HIF-1α expression was analyzed by immunoblot in PBMC and GM-CSF macrophages using increasing concentrations of Molidustat. Cytotoxic side effects of Molidustat treatment were excluded by FITC Annexin V staining. Effects of Molidustat on TNFα levels were checked by enzyme-linked immunosorbent assay. Quantitative LightCycler PCR was used to detect human β-defensin 2 (hBD2) and vitamin-D-receptor (VDR) mRNA levels. The effects of Molidustat on intracellular *Mtb* were measured by quantification of mycobacterial growth.
Result: In preliminary experiments we demonstrated that Molidustat ranging from 0.1 nM to 10 µM was not toxic for freshly isolated PBMC or primary human macrophages. Molidustat treatment resulted in a dose-dependent stabilization of HIF-1α in PBMC and macrophages. Stabilization of HIF-1α by Molidustat induced a significant upregulation of VDR and hBD2, which define an antimicrobial effector pathway. Molidustat (10µM) inhibited the *Mtb*-antigen triggered release of TNFα, a cytokine closely involved in protection to tuberculosis. Despite reduced TNFα-levels, Molidustat induced a significant upregulation of hBD2 and VDR (hBD2: 20±7.9; VDR: 11±4.5) as compared to untreated samples. To test whether this upregulation of the antimicrobial peptide hBD2 was functionally relevant, we determined the effect of Molidustat on the intracellular proliferation of virulent *Mtb* in human macrophages. In three out of three experiments, Molidustat limited *Mtb* growth by 93±3% on average.
Summary and Conclusion: Our results provide evidence that increased HIF-1α levels correlate with improved control of virulent *Mtb* in human macrophages. Therefore, Molidustat and possibly other prolylhydroxylase inhibitors, which increase the endogenous levels of HIF-1α may constitute a novel host-directed therapy to treat drug-resistant tuberculosis.

Presentation: Wednesday, 14 September 2016 from 14:00 – 14:15 in room Konferenz 1.

128/IIV

Macrophage activation by corynebacteria: contribution of TLR2 and Mincle-FcRg-dependent recognition of cell wall glycolipids

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Non-toxigenic *Corynebacterium diphtheria* and *C. pseudotuberculosis* can cause invasive disease in humans and animals. How corynebacteria are recognized by innate immunity is largely unknown. The cell wall of phylogenetically related mycobacteria contains the glycolipid trehalose-6,6-dimycolate, which activates macrophages through the C-type lectin receptor (CLR) Mincle. Here, we explored innate immune cell activation by different corynebacteria species and their cell wall glycolipids, using macrophages from mice deficient in Mincle, its adapter protein Fc receptor gamma chain (FcRγ), or the Toll like receptors (TLR) TLR2 and TLR4. Macrophages were stimulated with whole corynebacteria or chloroform-methanol extracts, followed by measurement of nitric oxide (NO) and G-CSF. NO release induced by whole bacteria was comparable in wildtype, TLR4-, Mincle- and FcRγ-deficient macrophages, but strongly reduced in macrophages lacking both TLR2 and TLR4. In contrast, TLR2/4- and Mincle-FcRγ-deficiency led to reduced NO and almost abrogated G-CSF after stimulation with lipid extracts. Glycolipid preparations were bound by recombinant Mincle-Fc fusion protein, indicating a direct interaction with the receptor on macrophages. Interestingly, lipid extracts from *C. diphtheriae* strain DSM43989 failed to induce significant NO or G-CSF release, which correlated with a lack of mycolic acid esters as revealed by thin layer chromatography and of binding to Mincle-Fc. Together, the data indicate that whole corynebacteria trigger both TLR and CLR pathways, with a pivotal role for TLR2, whereas Mincle-FcRγ-signaling is required for the response to the corynomycolates of the corynebacterial cell wall. These findings suggest an important role for these innate receptors in corynebacterial infection in human and veterinary medicine.

Presentation: Wednesday, 14 September 2016 from 14:15 – 14:30 in room Konferenz 1.

POSTERSESSION 01
12 Sept. 2016 • 10.30-11.00

Diagnostic Microbiology (StAG DV)

129/DVP

Multiplex species-specific DNA detection of Helicobater bilis and hepaticus

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Background: We would like to investigate the association of biliary tract disease with *H.bilis* and *H.hepaticus* infections in humans by serology and PCR. Available PCR, however, are based on singleplex 16S-rRNA detection displaying a rather low sensitivity, low species-specificity, are time-consuming and restricted to a subset of *Helicobacter*-species.
Aim: To develop a multiplexed high-throughput assay (Helicobacter DNA finder, HDF) for the simultaneous and specific detection of *H.bilis*, *H.hepaticus* and closely-related or human-associated *H.muridarum*, *H.pylori*, *H.heilmannii*, *H.typhlonius*, *H.cinaedi*, *H.pullorum* and *Campylobacter jejuni* in biological samples by targeting gyraseA and gamma-glutamyltransferase gene.
Method: Isolated DNA is amplified by species-specific primers in a multiplex PCR. The biotinylated amplicons are detected via hybridisation to specific oligonucleotide probes coupled to spectrally distinct sets of fluorescent beads (Luminex). A feasibility study was performed with biological samples (liver, duodenum, faeces) of 203 mice with *Helicobacter*-status defined by routine 16S-rRNA-based PCR.
Result: Lower detection limit for each species was <100 copies/PCR. Of 203 murine samples, 40 were *Helicobacter*-negative and 163 positive for *H.hepaticus*, *H.typhlonius* and/or *H.bilis*. 201 samples (99%) were concordantly *Helicobacter*-negative or -positive (kappa value 0.97; 0.93-1) by HDF and routine PCR. On species level 247 infections were identified by HDF (including single/multiple infections), of these 169 (68%) in agreement with routine PCR.
Conclusions and Outlook:Newly developed HDF allows a multiplexed high-throughput detection of *H.bilis*, *H.hepaticus* and seven other *Helicobacter*-species, probably with higher analytical sensitivity than routine PCR. In future, human samples will be analysed and compared to serological results to identify human infections and assess their potential association with biliary tract cancer.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

130/DVP

Solid phase microbead array for multiplex O-serotyping of E. coli

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Introduction: O Serotyping of Escherichia coli (E. coli) is complex concerning the presence of 184 O antigens. Agglutination tests employing anti E. coli polyclonal antiserum or antibody coated latex particles are the main diagnostic tools in the laboratory. Visible antigen-antibody complexes are formed which allow easy determination by the eye.
Question: A major drawback is that bacteria can be analyzed with regard to only one antiserum / antibody at a time. Here we present a proof of concept of a planar microbead array for a multiplex O serotyping in E. coli.
Method: As representative candidates, ten clinically relevant E. coli pathotypes that are associated with high risk for diarrhea in humans were examined (O26, O55, O78, O118, O124, O127, O128, O142, O145 and O157). The antisera were assigned to specific microbead populations, which can be distinguished by their size and fluorescent encoding. Antibodies of the antisera were coupled to microbeads. Fluorescence-stained bacteria bound to antibodies. Automated image processing and data analysis were conducted by our fully automatic microscopic platform VideoScan.
Result: Homogenous antibody coating of the microbeads was demonstrated by an intra-population CV ranging from 3.3 % to 6.3 % and an interpopulation CV of 9.5 %. Fluorescence signal intensities showed that E. coli cells of a certain serogroup bind highly specific ($p < 0.001$) to microbeads with the matching antiserum.
Conclusion: In summary, we established a new diagnostic tool for an automated multiplex serotyping of E. coli that enables to simultaneously screen for different O antigens in a high throughput manner. The techniques used to prepare the microbeads can also be utilized for testing other pathogens.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

131/DVP

Real-Time Differentiation of *Mycobacterium chimaera* from *Mycobacterium intracellulare* by MALDI Biotyper Software

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Introduction: The *Mycobacterium avium* complex (MAC) has been constantly growing in its number of species. *M. intracellulare* is part of this complex since long time and was often associated with pulmonary disease. In contrast, the species *M. chimaera* was described in 2004 and a lower pathogenicity is assumed. However, heater cooler devices used in cardiac surgery were recently supposed to be the source of invasive *M. chimaera* infections reported from Europe and the US.
A reliable differentiation of these two closely related species in daily routine is challenging, *e.g.* GenoType® Mycobacterium CM (Hain Lifescience, Nehren, Germany) identifies both species as *M. intracellulare*, 16S rRNA gene differs in only 1 base pair. MALDI Biotyper (Bruker Daltonik GmbH, Bremen, Germany) standard algorithm based workflow groups both species into one complex.
Objective: A reliable differentiation of both *Mycobacterium* species by MALDI-TOF MS is possible based on species-specific peaks. Here, we present three methods for species assignment using these characteristic masses including a first evaluation of the prototype of a new software module which needs no additional user input.
Materials and Methods: *M. chimaera* strains (n = 45) and *M. intracellulare* strains (n = 14) were cultured on solid Löwenstein-Jensen medium or in liquid BD BACTEC™ MGIT™ tubes (BD, Heidelberg) according to standard procedures. All study strains were identified by ITS sequence analysis as reference method. Mass spectra were recorded and compared to Mycobacteria Library 4.0 using MALDI Biotyper Compass software (Bruker Daltonik, Germany). Characteristic peaks were checked manually, by an

external software-based tool and by a module integrated into MBT Compass software.
Result: The MALDI Biotyper standard algorithm resulted in correct identifications at *M. chimaera*/*intracellulare* group level for all 59 tested strains. For species differentiation, the acquired mass spectra were reviewed manually for four species-characteristic peaks. Using this model, 56 isolates (94.9 %) were assigned to the correct species. For a more convenient approach, software for real-time identification was developed and tested. The calculated log(IQ) value allowed correct classification of all isolates (100 %). In addition, first data obtained with a new MALDI Biotyper integrated software module will be shown.
Summary: Differentiation of *M. chimaera* and *M. intracellulare* is challenging In daily routine. Here, we present three possibilities to obtain definite species identification results from MALDI-TOF mass spectra including software for real-time classification.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

132/DVP
FunResDB - a web-based service to analyse *Aspergillus fumigatus* drug resistance
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Background: Treatment of invasive aspergillosis (IA) primarily relies on antifungal drugs of the azole class. Due to the emergence of azole resistant *Aspergillus fumigatus* it is nowadays important to perform fungal drug susceptibility testing to optimize treatment of IA. On the molecular level, azole resistance is most frequently associated with mutations in the CYP51A protein.
Method: We set up the database FunResDB (www.nrz-myk.de/funresdb) to gather all available information about CYP51A-dependent azole resistance and developed a web-based service that allows the analysis of cyp51a sequences from clinical isolates to predict the susceptibility of azoles.
Result: By manual curation of more than 150 publications, we obtained data about the genotype and the resistance of several azoles including itraconazole, voriconazole, posaconazole and isavuconazole. In summary, the screening resulted in 79 distinct *Aspergillus fumigatus* CYP51A genotypes, which contain a total of 59 non-synonymous mutations. A mutation search tool is implemented to identify mutations and resistance for novel clinical sequences.
Conclusion: This curated web-based service integrates a database and essential tools to study and monitor fungal drug susceptibility based on cyp51a sequences. Therefore, FunResDB can be applied in routine work in clinical microbiology labs which have access to cyp51a sequencing.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

133/DVP
Direct Detection of Nontuberculous Mycobacteria with MALDI-TOF MS from Blood Culture Bottles
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Introduction: Diseases caused by nontuberculous mycobacteria (NTM) are increasing and an early detection and identification help physicians to start immediately with appropriate therapy. In clinical laboratory workflow, a positive blood culture with mycobacteria is transferred to fresh media. This cultivation step prior to identification is time consuming. In addition, molecular methods are widely used for species identification which is cost-intensive compared to MALDI-TOF MS.
Objective: In this proof-of-concept study, an adapted preparation method was tested to identify NTM directly from positive blood culture material. If proven successful, the skipping of a cultivation step helps to save time which can be crucial for therapy of these slowly growing microorganisms.
Materials and Methods: Mycobacterial strains (n = 35) from clinical routine (n = 12) and from interlaboratory tests (n = 23) were inoculated on solid Löwenstein-Jensen (LJ) medium (BD, Heidelberg, Germany). Suspensions of the respective mycobacteria were inoculated in BACTEC™ Myco/F lytic bottles (BD). Bottles flagged positive were processed immediately by MALDI Sepsityper® KIT (Bruker Daltonik, Bremen, Germany) according to the instructions of the manufacturer. The resulting bacterial pellet was further processed according to the MycoEX protocol (Bruker Daltonik). A blood agar plate was inoculated as check for potential contamination of the blood culture. In parallel, liquid BD BACTEC™ MGIT™ tubes (BD) and LJ media were inoculated with positive BACTEC™ Myco/F lytic culture. Mass spectra were acquired in a microflex LT instrument and compared to Mycobacteria Library 3.0 using MALDI Biotyper Compass software (Bruker Daltonik).
Result: All inoculated media became positive. Mass spectra were acquired from each grown culture. All identification results were correct. Log(score) values from BACTEC™ Myco/F varied between 1.94 and 2.41. The same correct species result was gained from LJ, MGIT and blood medium.
Summary: A commercial automated detection system (BACTEC Myco/F Lytic bottles) was spiked with mycobacteria and processed with a combination of Sepsityper® kit and MycoEX protocol. Identification results were of the same quality as results obtained from additional subcultures. These data show a promising way to get an identification result several days earlier and for lower costs than by following the conventional procedure.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

134/DVP
Molecular typing of *Vibrio parahaemolyticus* isolates originating from different sources by DNA microarray analysis
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Introduction: *Vibrio parahaemolyticus* is one of the 12 pathogenic species of the genus *Vibrio*. Its natural habitat is the marine environment and the pathogen is a leading cause of seafood borne gastroenteritis. In most cases, genes encoding the thermostable direct hemolysin (TDH) and the TDH-related hemolysin (TRH) were found in clinical strains and are considered as typical virulence factors of this species. We were interested to identify additional virulence determinants that have impact on the pathogenic potential of this *Vibrio* species. The aim of this study

was to describe parameters that allow us to distinguish between environmental *V. parahaemolyticus* isolates and pathogenic strains, which can cause human infections. To achieve this, we have analyzed more than 60 *V. parahaemolyticus* strains originating from different sources.
Method: The DNA microarray used was developed and optimized by our group in the past years. The array enables us to detect non-cholerae *Vibrio* spp. and to investigate the presence of virulence determinants in a fast and easy way. The detection system uses simultaneous amplification and biotinylation of relevant target sequences by linear multiplex PCR. Positive hybridizations to specific DNA probes are visualized by an enzyme-mediated precipitation reaction. Analysis and evaluation of densitometry measurements is performed on an automated array scanner.
Result: More than 60 *V. parahaemolyticus* strains were analyzed. With the aid of 222 DNA probes, spotted in duplicate on the solid array matrix, 93 target genes of different *Vibrio* spp. can be detected, chosen from genes encoding adherence and invasion mechanisms, capsule synthesis, exoenzymes and toxins, type III secretion systems and resistance mechanisms. Based on the hybridization results, we established molecular fingerprints of human, environmental and food isolates of *V. parahaemolyticus*, thus comparing the genetic relationship within these *V. parahaemolyticus* isolates. Completely sequenced *V. parahaemolyticus* strains were taken as positive controls.
Summary: The investigation of a series of *V. parahaemolyticus* isolates with this newly developed DNA microarray allows for a very detailed characterization of these strains, based on molecular fingerprints. The results will help us to establish a risk matrix that can be used for pathotyping of individual *V. parahaemolyticus* isolates.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

135/DVP
Characterisation of bacterial volatile compounds as biomarkers for species identification and metabolic profiling.
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Introduction: Biochemically active bacteria are known to produce a number of bacterial volatile compounds (bVOC), which are primary and secondary metabolites delivered by different metabolic pathways. Their release depends physiological and physicochemical conditions such as growth rate, cell density or the availability of carbon and energy sources. These compounds can be grouped in several chemical classes including hydrocarbons, aldehydes, ketones, alcohols, esters, aromates, terpens, fatty acid derivates, sulphur and nitrogen containing compounds [1]. More than 1,000 bVOC have been described so far [2]. In principle, they are well-suited biochemical markers for the characterisation of living bacteria using non-invasive sampling methods in order to show the presence or absence of certain bacteria species in a sample or even in infected biomaterials (food, parts of the human body) [3]. In the pilot study presented here, adapted HS-SPME-GC/MS methods for the analysis of characteristic bVOC emitted into the headspace of prototypic bacterial cultures were used.
Method: First, in a non-targeted analytical approach peak patterns of released volatile compounds were identified. Bacteria species of relevance for biotechnological or medical questions (*E. coli*, *B. thuringiensis*, *B. glumae*) were cultivated in LB medium and analysed in airtight sealed serum flasks for the release of volatile compounds using different SPME fibre types. Each fibre type allows the adsorption of compounds with certain physicochemical properties such as polarity or within a range of molecular masses. Desorption of bVOC and subsequent analyses of peak retention times and fragmentation patterns were performed on an Agilent 6980 GC system coupled to a 5973N mass selection detector.
Result: Initial screenings for characteristic volatile compounds released by different bacteria species cultivated in liquid medium revealed a number of suitable bVOC, which could be used as biomarkers for the biochemical characterisation of bacterial cultures under the conditions described. Additionally, variations in GC peak intensities were observed which might reflect physiological challenges in the batch cultures analysed here.
Conclusion: In the next steps optimisation of the cultivation conditions as well as the analytical method will be combined with in-depth database research in order to set-up the full chain of sampling, measurement and computer-assisted data evaluation.

References
[1] Schulz S and Dickschat JS. Bacterial volatiles: the smell of small organisms. Nat Prod Rep, 24(4): 814-42, 2007.
[2] Audrain B, Farag MA, Ryu C-M, Ghigo J-M. Role of bacterial volatile compounds in bacterial biology. FEMS Microbiol Rev, 39(2): 222-33, 2015.
[3] Bos LDJ, Sterk PJ, and Schultz MJ. Volatile metabolites of pathogens: a systematic review. PLoS Pathog, 9(5):e1003311, 2013.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

136/DVP
Comparison of Two Automated *Treponema*-Specific Antibody Assays
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Introduction: The laboratory diagnosis of syphilis is commonly based on a combination of serological tests which detect specific antibodies against *Treponema pallidum* (TP) and non-TP antibodies. However, systematic comparative analyses of the performances of available automated diagnostic assays are lacking.
Objective: The aim of this study was to compare two automated TP-specific immunoassays to a gelatin particle agglutination assay (TPPA) and to each other.
Method: A total of 501 routine samples were analyzed. All specimens were leftovers from the daily operations of our diagnostic laboratory.
Result: The overall qualitative agreement, analytical sensitivity and analytical specificity of the assays compared to the TPPA (Serodia-TPPA, Fujirebio) were as follows: Architect Syphilis TP (Abbott), 99.4%, 97.2% and 100%; Elecsys Syphilis (Roche), 99.2%, 98.2% and 99.5%. Direct comparison of the Architect Syphilis TP to the Elecsys Syphilis test showed a high overall qualitative agreement of 99.4%. Linear and polynomic regression analyses revealed good correlations and a strong monotonic relationship between antibody titers by TPPA, signal-to-cutoff ratios (S/CO, Abbott) and cutoff indices (COI, Roche), as obtained by the automated immunoassays (R^2=0.80-0.93, ρ=0.58-0.74, P <.0001). Five samples were associated with discrepant results: Two positives by TPPA were negative with both automated immunoassays and later found to contain antibodies against *Borrelia burgdorferi*. After eliminating these samples from the analysis, overall qualitative agreement, analytical sensitivity and analytical specificity were: Architect Syphilis TP, 99.8%, 99.1% and 100%; Elecsys Syphilis, 99.6%, 100% and 99.5%. The remaining 3 discrepant samples were subject to complementary

analysis by TP-immunoblot (Mikrogen). One was TmpA- and Tp17-positive, the second was TmpA- and Tp453-positive. Both were only detected in the Elecsys Syphilis test. The third sample was only TmpA-positive and was detected by both, the Elecsys Syphilis and the TPPA assay.

Summary: Our study demonstrated that both TP-specific immunoassays showed a high level of agreement with each other and with the reference results obtained by the "Gold Standard" TPPA. High sensitivities and specificities were observed with very rare cases of false-positive and false-negative results as compared to the TPPA. In summary, both automated immunoassays are suitable for use in screening for syphilis and for monitoring of antibody concentrations (e.g. after treatment). Nevertheless, the specific serological results should always be interpreted in context of the individual patient's clinical situation and treatment history.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

137/DVP

Detection and identification of Rickettsia based on fluorescence in situ hybridization

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Rickettsia are a group of obligate intracellular bacteria and most of the known species are pathogenic to humans. These bacteria are found worldwide and are usually transmitted via an arthropod that is both its reservoir and vector. *Rickettsiae* are fastidious to culture and isolation attempts from vectors or human samples are frequently affected by bacterial contamination. Serological tests have been commonly used for the diagnosis of rickettsioses, but usually yield no positive result when the patient is acutely unwell. Therefore, the gold standard for the diagnosis of an acute rickettsial illness is qPCR. However, despite being very sensitive, this method is accompanied by well-known drawbacks such as the lack of multiplex species differentiation. *Rickettsia*-specific qPCR also provides no information about the presence, quantity and quality of other bacterial contaminations.

In order to circumvent these pitfalls and to establish a new method to detect and to differentiate *Rickettsia* in clinical samples and cultures, we report on the development of a fluorescence *in situ* hybridization (FISH) based assay for the rapid identification of *Rickettsia*. Although some published *Rickettsia* probes exist, a comprehensive probe set for the simultaneous detection and differentiation of medically relevant *Rickettsia* species is hitherto not available. Probes were designed using the arb software package and probe specificity was evaluated by formamide series. If adequately fixed material was not available, probes were evaluated using clone-FISH. For this, plasmids carrying rickettsial ribosomal RNA genes were transformed into *E. coli* and transcription of the heterologous RNA was induced. The resulting group-specific probes were used to detect rickettsiae and differentiate between members of the spotted-fever and typhus-group of *Rickettsia* in a first step. In the following hybridization, species-specific probes were used to identify the human pathogens *R. prowazekii, R. typhi* and *R. rickettsii* as well as several other medically-relevant rickettsial species. The development of a FISH-based method is useful in overcoming the drawbacks of serology and qPCR. The ability of this new assay to visualise rickettsiae inside host cells within only four hours and the highly specific nature of the test makes it a valuable tool for the diagnoses of acute rickettsial infection.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

138/DVP

Comparison of cultural and molecular-biological methods of the detection of vaginitis causes

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Introduction: The flora of the portio and vagina from healthy women with normal hormone status and in fertile age exists above of lactobacilli. A reduction of lactobacilli can be a reason for a bacterial miscolonisation and effected infections (1). The common bacterial miscolonisation is caused by *G. vaginalis* and releases the bacterial vaginosis. The bacterial vaginosis increased the risk for an infection with sexually transmitted agent and inflammations can caused on the basis of bacterial miscolonisation from other pathogenic agents. Mycoplasma and Ureaplasma can live as a component of the vaginal flora peacefully or cause infections.

Aim: Pathogenic vaginitis agents were detected culturally and compared with a molecular-biological method (PCR).

Materials and Methods: Vaginal swabs of patients were inoculated on a defined culture media, incubated overnight and identified by Maldi Biotyper (Bruker). The Kit MYCOPLASMA DUO (Bio-Rad) was used for identification and differentiation of genital Mycoplasma.

Vaginal swabs were checked with the multiplex PCR FTD Urethritis plus. The DNA extraction occurred after instructions of the High pure Kit PCR Template Preparation (Roche). Moreover the PCR was performed and interpreted after instructions by FTD Urethritis plus with LightCycler 480 instrument II (Roche).

Result: A judgement of the whole vaginal flora could be carried out with the cultural method. Thus, *S. agalactiae* can be identified from pregnant women. Moreover, there can be performed an antibiotic testing. The multiplex PCR FTD Urethritis plus can only detected a few agents.

Between the cultural and the molecular-biological method exists a discrepancy. The biggest inequality exists at the identification of Ureaplasma and Mycoplasma. More Ureaplasma could be found with the multiplex PCR Urethritis plus of FTD. The same could be determined for the Mycoplasma. However, a few percentages Ureaplasma and Mycoplasma were only detected with the MYCOPLASMA DUO Kit from Bio-Rad.

Summary: A big difference exists between the cultural and the molecular-biological method. Advantages of the cultural method are the evidence of accompanying bacterial flora (*E. coli* or *S. agalactiae*), so there is a judgement of the vaginal flora possible. Moreover, the antibiotic testing can occur. Disadvantageous is the longer incubation time. Also, the specificity of this method is lower in relation to the PCR. The advantages of the molecular-biological method are the time factor and the specificity. The patient can obtain on the same day the results. The disadvantages are the restricted agent identification, as well as the missing general view of the vaginal flora.

Reference

[1] Neumeister B.; Geiss H. K.; Braun R. W. & Kimmig P. (2009): Mikrobiologische Diagnostik: Bakteriologie-Mykologie-Virologie-Parasitologie; 2. vollständig überarbeitete Auflage; ISBN: 978-3-13-743602-7; Stuttgart: Thieme

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

139/DVP

Optimization and Evaluation of MRSA Detection by Peak Analysis of MALDI-TOF Mass Spectra

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Background: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a widely used method for identification of microorganisms in medical microbiology.
Recently, Josten et al. observed the presence of a peak related to a small peptide called PSM-mec in mass spectra of certain MRSA strains. With the described peak in a detection window of m/z 2411-2419 this group of MRSA strains could be detected with high sensitivity and specificity [1].
Objective: We have automated and optimized the detection of the previously described MRSA-specific peak and evaluated the method with data from routine diagnostics of a medical microbiology laboratory. A prototype software for real-time classification was developed.
Materials and Methods: MALDI-TOF mass spectra of *Staphylococcus aureus* isolates from medical microbiology routine diagnostics were screened for the peak related to the PSM-mec peptide. The analysis also included mass spectra of *Staphylococcus aureus* ATCC 33591 (MRSA) presenting the respective peak. Data derived from MALDI-TOF mass spectra were correlated to information about antimicrobial resistance from the laboratory information system (LIS).
Analysis of data was performed using custom Python scripts, the R statistics package, Microsoft Excel, a custom database system for MALDI-TOF MS data and flexAnalysis software (Bruker Daltonik, Germany). Mass spectra of *Staph. aureus* isolates acquired in the years 2011-2014 with a microflex LT instrument (Bruker Daltonik, Germany) were analyzed retrospectively.
Result: After analysis and recalibration of spectra the peak detection window could be narrowed down to m/z 2411-2415 resulting in an increased specificity of MRSA detection.
In total N = 1304 mass spectra of *Staph. aureus* isolates were analyzed. 211 (16.8%) of these belonged to methicillin-resistant strains. Of these MRSA strains, 49 (23.2%) presented the PSM-mec related peak. None of the 1093 MSSA strains showed the described peak (specificity 100%).
The optimized detection method was implemented in a software tool for real-time identification of these strains.
Conclusion: An optimized MALDI-TOF based detection method for certain MRSA strains based on the description of Josten et al. [1] was successfully evaluated with routine data and implemented as a software tool for real-time classification. Simultaneous detection of the PSM-mec related peak during identification in medical microbiology routine would allow early adaptation of therapy and measures of infection control.

Reference

[1] Josten et al., Int J Med Microbiol. 2014 Nov; 304(8): 1018-23

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

140/DVP

Comparison of two modern molecular biological systems (SeptiFast, Roche; Unyvero, Curetis) with conventional microbiological diagnostics in ICU patients with sepsis, septic shock or septic nosocomial pneumonia

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Background: In surgical ICU patients postoperative complications with serious infections and sepsis can occur. Besides primary sepsis, sepsis can also develop from nosocomial pneumonia, particularly in ventilated patients. In the context of sepsis diagnostics two CE-certified test systems have been established in addition to the classical microbiological methods. The SeptiFast test (Roche) is used for the detection of pathogens from whole blood. The Unyvero system (Curetis) enables rapid diagnosis of pathogens causing nosocomial pneumonia, including frequent resistance genes. Investigations on a combination of both methods with respect to key parameters such as detection rates, days on adequate treatment, sensitivity and specificity compared to conventional methods might represent a very interesting approach in establishing a more rapid molecular diagnosis especially for septic ICU patients.
Materials and Methods: This is a prospective controlled single centre observational study including patients from a surgical ICU. Study evaluation was blinded in respect to the results of combined application of the commercially available test systems Unyvero and SeptiFast versus conventional microbiological diagnostics such as swab and blood culture (BC) on the ICU. Only patients fulfilling the current international sepsis criteria and suffering from clinically diagnosed septic nosocomial pneumonia, sepsis, or septic shock were included. A control group of patients with no signs of infection or sepsis from the same ICU was included.
Result: In this study 73 septic patients (m/f 37/36, mean age 68 years) were included. 24 out of 73 septic patients revealed positive blood cultures, with an average time to gram-stain report to the clinician in charge of 25 hours.
For the SeptiFast system sensitivity and specificity turned out 67 % and 87 %, respectively, in regard to BC-proven bloodstream infections. Furthermore 27 (37 %) of ICU patients with sepsis or septic shock showed significant benefit either because more sensitive detection of causative pathogens (11, 15 %) and/or a significant reduction in turnaround time (TAT), (15, 21 %).
In 29 septic patients with a supposed pneumonia bronchial secretions were analyzed by the Unyvero method (sensitivity: 93 %, specificity: 87 %). Of these patients 19 (66 %) turned out positive, 9 negative, and 1 invalid. In 14 (48 %) of the positive patients pathogens recovered by the Unyvero system could be verified by culture, additional pathogens could be identified in 7 patients, 5 (17%) patients showed negative results upon conventional culture.
Conclusion: Compared to the results of the routine microbiological diagnostic methods both molecular test systems turned out superior in respect to TAT, sensitivity, and specificity. Consequently, molecular pathogen detection should be regarded as a valuable tool to complement classical microbiological diagnostics in patients suffering from severe sepsis on the ICU.

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141/DVP

Estimation of ampicillin resistance of microbial cells suspensions by electro-acoustical analytical method

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Introduction: Study of the microbial adaptation to antibiotic action and differentiation of bacterial strains susceptible and resistant to antibiotic is still important theoretical and applied problem. At the same time it's well-known that the action of antibiotic agents on bacterial cells is closely connected with the changes in their biochemical processes (metabolic pathways) as well as in cell morphology (cell lengthening and swelling, their bending, chain or ball formation, lysis), which leads in some cases to changes in membrane permeability to specific ions or molecules with probable subsequent changing cellular electro-physical properties. So using the electro-acoustic analytical method, based on registration of bio-specific reactions in liquid suspension that contact with the piezoelectric material surface, such functional changes can be determined.
Objective: Obtaining the effect of ampicillin on the electrophysical characteristics of ampicillin- sensitive and ampicillinresistant Escherichia coli cells by electro-acoustic analytical method.
Method: Studying of all possible changes in mechanical and electrical properties of microbial suspensions that could take place at antibiotics interactions was carried out by using a specially-made sensor based on a piezoelectric resonator with a lateral electric field with frequency range of 6 - 7 MHz. For performing corresponding analysis previously prepared microbial cells with or without antibiotics addition were placed in the container for liquids and real and imaginary parts of electrical impedance of resonator were measured by using the precision LCR meter (4285A, Agilent Technologies Inc., Santa Clara, CA).
Result: The biological activity of betalactam antibiotics is mainly connected with their ability to interact with microbial cell surface and so to change barrier properties of the cytoplasmic membrane. Ampicillin, as a representative of such antibiotics, demonstrates activity against several gram-negative genera, including coliforms - so for the research Escherichia coli cells were used. The effect of ampicillin on the sensitive and resistant Escherichia coli strains cells using electroacoustic method of analysis (piezoelectric resonator with a lateral electric field) has been for the first time in microbiology studied. Under the ampicillin action on sensitive E.coli K-12 and B-878 strains, resistant E. coli K-12 (pUC-18) strains occurred different electro-acoustical effects.
Conclusion: Thus, registration of microbial suspensions electro-acoustical effects analysis is promising method for applying in microbiology, medicine or veterinary as a method of microbial cells antibiotic resistance estimation test. The work is financially supported by the grant of RFBR № 16-07-00818.
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142/DVP

Over expression of gamma glutamyl cysteine synthetase gene is associated with natural antimony resistance in *Leishmania tropica* isolates

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Introduction: Antimonials compounds remain the treatment of choice for anthroponotic cutaneous leishmaniasis (ACL); and increasing rates of antimony resistance is becoming a serious health problem in some endemic regions. Therefore, unraveling molecular markers for monitoring of drug resistant parasites is crucial. Different studies have been suggested the importance of gamma glutamyl cysteine synthetase (γ-GCS) gene in the synthesis of glutathione and trypanothione, the main reducing agents in antimonials metabolism.
Objective: According to the vital role of γ-GCS in antimony resistance, in present study we investigated the RNA expression of γ-GCS gene in sensitive and resistant *Leishmania tropica* isolates using real-time RT-PCR.
Materials and Methods: The clinical isolates, including 8 resistant and 8 sensitive samples, were collected from ACL patients. Amastigote sensitivity tests were conducted by *in vitro* infection of cultured murine macrophages. After RNA extraction and cDNA synthesis, gene expression analysis was performed by quantitative real-time PCR using SYBR® Green.
Result: Drug sensitivity test revealed that the average of IC_{50} values of resistant isolates was five times higher than that of sensitive stains. The results of real time RT-PCR revealed a significant up regulation (2.1 fold) in the average expression of γ-GCS gene in resistant isolates compared to sensitive ones.
Conclusion: Our findings suggest that γ-GCS can be considered as potential molecular markers for screening of antimony resistance in clinical isolates. Moreover, over expression of it as a defense mechanism could possibly protect cell from oxidative stress induced by antimonials.

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143/DVP

Fast, economic and simultaneous detection of carbapenem resistance genes via multiplex real-time PCR for blaKPC, blaNDM, blaVIM and blaOXA-48

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Rapid molecular identification of genes that mediate carbapenem resistance in Gram-negative bacteria is crucial for infection control, prevention, surveillance, rapid confirmation of other diagnostic test procedures and epidemiological purposes. Multiplex and multi-color channel real-time PCR provides very accurate and reproducible quantitation of gene copy numbers from a single sample. The method measures PCR product accumulation through dual-labeled TaqMan probes. Each probe uses a different wavelength for the determination of fluorescence signals and allows the quantitative detection of each target.
A novel multiplex real-time PCR was bioinformatically designed, developed and validated to detect simultaneously the most clinically important carbapenemase genes, blaKPC, blaVIM, blaNDM and blaOXA-48. Each target was evaluated in single- and multiplex reactions.
Specifically, the assay was tested with 24 different carbapenemase positive Gram-negative reference strains and verified in a blind

study using 50 carbapenemase positive and 50 carbapenemase negative isolates via colony PCR. All qPCR data were compared with data obtained by microarray-based genotyping (CarbDetect AS2, Alere, Jena, Germany). Additionally, the phenotypes of all isolates were determined using the VITEK-2 system. Furthermore, spiked swabs with different numbers of colony forming units of carbapenemase producing bacteria were used to determine the sensitivity of this multiplex real-time PCR assay.
Using a dilution series of all reference strains (107 to 101 gDNA/µl) the sensitivity and specificity of the multiplex reaction targeting all four carbapenemase genes were not inferior to the corresponding singleplex reactions. Colony PCR with carbapenem positive and negative isolates were in 100% concordance with results received by microarray-based genotyping. Therefore, this assay could be routinely used for the confirmation of phenotypically detected resistance and detection of clinically important carbapenemase genes in native patient samples (e.g., rectal swabs).
Unlike other quantitative PCR methods, the assay does - due to the closed tube system and multi- wavelength fluorescence detection - not require post-PCR sample handling, preventing carry-over contaminations of amplicons and additional manual efforts. Therefore it is accurate, economic, fast, and it is less labor-intensive than other current quantitative PCR methods.

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ID 318/DVP

Direct, specific and rapid detection of Carbapenemases from culture material or in clinical samples using a multiplex antibody microarray

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Carbapenems are last line antibiotics and can be administered only intravenously. They are mainly used in intensive care settings. The spread of multi-resistant Gram-negative bacteria with acquired and transferable carbapenemase genes constitutes a major public health concern worldwide. Routine detection of carbapenemases is done by culture, possibly on selective media, followed by standard susceptibility testing. In order to rule out other mechanisms of carbapenem resistance, confirmatory tests need to be performed after isolate identification with reduced susceptibility to carbapenems. Options include the Modified Hodge Test, various disk diffusion or epsilonmeter-based protocols, the molecular detection of known carbapenemase genes by PCR or the detection of carbapenem degradation by mass spectroscopy.
In order to facilitate the development of a fast and economic confirmatory test, monoclonal antibodies were raised against the currently four most relevant carbapenemases (KPC, NDM, OXA-48 and VIM) using phage display technology. They were selected because of their prevalence, and relevance, on a global level as well as with regard to a European setting.
For each target, eight antibodies were produced and tested with purified recombinant and native antigens. The most sensitive and most specific carbapenemase antibody pairs were determined and selected to be spotted onto microarrays as capture antibodies and for visualisation as a labelled 4plex detection mixture. The detection limits for all four targets were determined using purified recombinant antigens with about 100 pg/ml. Subsequently, native carbapenemases were detected directly from bacterial cells. Altogether, 459 clinical isolates were tested that previously also have been genotyped by DNA microarray (Alere CarbDetect test). These included 78 NDM-positives, 64 KPC-positives, 80 VIM-positives, 114 isolates with OXA-48 or OXA-48-like variants (OXA-54, OXA-162, OXA-163, OXA-181, OXA-204, OXA-232,) and 126 isolates with OXA alleles other than OXA-48. For 457 out of these 459 isolates, results were concordant. The two non-concordant isolates harbored OXA-60 and OXA-40(72)+OXA-51. Furthermore, the assay was applied for the detection of carbapenemases directly from urine samples from patients with a known CRE urinary tract infection. Five out of nine urines were detected positive without any prior sample preparation or culture. Beside a use for culture confirmation, the array platform can be used to screen further antigens and the antibodies described herein could also be investigated for a future use in rapid bed-side tests such as latex/particle agglutination or lateral flow strips. Antibody based tests such as latex/particle agglutination or lateral flow strips or devices could facilitate a rapid, cheap and highly specific detection of known carbapenemases that does not require additional hardware.

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320/DVP

Performance of the new GenoType NTM-DR, compared to genome sequencing and phenotypic methods to determine subspecies and drug resistance in M. abscessus complex

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Introduction: Recently a new line probe assay, the GenoType NTM-DR (HAIN Lifescience, Nehren, Germany) has been developed for subspecies differentiation and detection of resistance for macrolides and aminoglycosides of Mycobacterium abscessus ssp. We studied the performance of the test compared to sequencing and phenotypic resistance results.
Method: Clinical isolates sent to our National Reference Centre for Mycobacteria collected between 2015 and 2016 were analysed for subspecies identification, erm(41)-characterisation rrl- and rrs-mutations by sequencing (1-3), as well as phenotypic resistance test according to the CLSI recommendations for clarithromycin and amikacin. The results were compared to those of the new GenoType NTM-DR for subspecies differentiation and the erm(41)–, rrl- and rrs-gene mutations.
Results: 45 clinical isolates were analysed, of whom 34 were identified as M. abscessus abscessus, 4 as M. abscessus bolletii and 7 as M. abscessus massiliense by sequencing. There was agreement of Genotype NTM-DR results and sequencing in 91% of subspecies identification. GenoType NTM-DR matched 100% (41/41) of erm(41) sequencing results, the 4 subspecies-disagreements excluded, and 96% (43/45) of the rrl-sequencing results, all 45 strains included. GenoType NTM-DR and phenotypic resistance test results matched in 96% for clarithromycin resistance and in 93% amikacin resistance.
Conclusion: The new GenoType NTM DR assay is a valuable test for subspecies identification and detection of defined mutations leading to amikacin and clarithromycin resistance.

References

[1] Nash KA, Brown-Elliott BA, Wallace RJ, Jr. A novel gene, erm(41), confers inducible macrolide resistance to clinical isolates of mycobacterium abscessus but is absent from mycobacterium chelonae. Antimicrob Agents Chemother 2009;53:1367-1376.
[2] Bastian S, Veziris N, Roux AL et al. Assessment of clarithromycin susceptibility in strains belonging to the mycobacterium abscessus group by erm(41) and rrl sequencing. Antimicrob Agents Chemother 2011;55:775-781.
[3] Prammananan T, Sander P, Brown BA et al. A single 16s ribosomal rna substitution is responsible for resistance to amikacin and other 2-deoxystreptamine aminoglycosides in mycobacterium abscessus and mycobacterium chelonae. The Journal of infectious diseases 1998;177:1573-1581.

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322/DVP

Isolation of obligate anaerobic bacteria from lower respiratory tract of patients with cystic fibrosis and the identification potential of "Matrix Assisted Laser Desorption Ionisation – Time of Flight Technology"

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Background: Lower respiratory tract infections (LRTI) are considered major cause of morbidity and of early mortality in patients with Cystic fibrosis (CF). The role of aerobic and facultative anaerobic bacteria in CF is well documented. In contrast, little is known about the significance of strictly anaerobic bacteria in such infections. Anaerobic bacteria are not routinely considered in the microbiological diagnostic procedure.
Patients and Methods: In this study sputum of 53 CF patients (45 adults and 8 patients <18 years old) were tested for the prevalence of strictly anaerobic bacteria. The sputa were collected from the patients and immediately transported to the microbiology lab using the Port-A-Cul transport system. The cultured anaerobic bacteria were identified using the Matrix Assisted Laser Desorption Ionisation – Time of Flight mass spectrometry (MALDI-TOF MS). The results were compared with those of classical biochemical (api rapid 32a und api 20A) and 16s-rRNA sequencing with regard to the identification's potential, turnaround time and expenses.
Results: Anaerobic bacteria were cultured from the sputa of 41 patients (77%). Monocultures were found in 71% of all cases. In total, 84 anaerobic bacteria were isolated. Prevotella was the most frequent genus, and Prevotella melaninogenica the most frequent species, followed by Veillonella spp.. Anaerobic bacteria were found in sputa of patients from all ages and both genders. The lung functions didn't affect the outcome. 16s-rRNA sequencing possessed the highest ID potential on species level as compared to biochemical procedures and MALDI-TOF MS. However, it was significantly more expensive and more time consuming. Using MALDI-TOF MS, 52 (62%) of the 84 isolated bacterial strains were identified at the species level, 26 (31%) at the genus level. In 6 (7%) isolates the ID results were not reliable. Notably, 95% of the species ID results of the MALDI-TOF MS were consistent with those of the 16srRNA-sequencing. The ID results of the biochemical procedures were inferior to and more expensive than those of MALDI-TOF MS.
Conclusions: Anaerobic bacteria should be considered in the routine microbiological diagnostic of CF using suitable transport and culturing procedures and, if indicated, in possible antibiotic therapy. The MALDI-TOF MS is a reliable, fast and cost-efficient technique for the identification of strictly anaerobic bacteria.

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323/DVP

Comparison of two molecular assays for diagnosis of *Clostridium difficile versus* the implemented diagnosis algorithm

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Background: *Clostridium difficile* (*C.difficile*) is the major cause of antibiotic associated pseudomembranous colitis and the most frequently identified cause of nosocomial diarrhea. The aim of this study was to compare the conventional *C. difficile* diagnostic algorithm consisting of GDH antigen and toxin ELISAs from stool as well as culture with two different molecular test systems. One of the molecular assays detects the PaLoc encoding both *tcdA* and *tcdB* genes (illumigene® Test, Bioscience Europe) and the other one detects the *tcdB* gene of *C. difficile* (BD MAX™ Cdiff Assay).
Materials/Methods: The study was carried out in the laboratory of Microbiology of Max von Pettenkofer Institute (Munich, Germany) from March 10th to May 17th, 2016. 113 stool specimens submitted to the laboratory for *C. difficile* diagnosis which were GDH positive were tested in parallel with both nucleic acid amplification technique, illumigene® Test (Bioscience Europe) and BD MAX™ cDIFF. The results were compared to the ones obtained with the current implemented diagnostic algorithm, which consists of 1) RIDASCREEN® *Clostridium difficile* GDH assay from the stool sample; 2) RIDASCREEN® *Clostridium difficile* Toxin A/B enzyme immunoassay from the stool sample; 3)anaerobic culture of the the stools on Braziers *Clostridium difficile* Selective Agar (HPA) (Oxoid®) for 48 h at 37ºC and growth is observed after 48 hours; 4) RIDASCREEN® *Clostridium difficile* Toxin A/B enzyme immunoassay from the culture.
Results: A total of 113 samples from 78 patients (40 (51.3 %) women, 38 men) were included in the study. Median age: 57.6 (range 5 months to 89 years). All the samples but three were unformed. We observed 96.2 % accordance between illumigene® Test (Bioscience Europe) and BD MAX™ Cdiff. Four discordant results between both nucleic acid amplification technics were detected. In seventeen samples the culture of the stools was negative but both BD MAX Cdiff assay and illumigene® Test assay (n= 16) or one of them (n=1) were positive; just six of these 17 samples belonged to patients with clinical symptoms of gastrointestinal disease. In nine samples, the *C. difficile* Toxin A/B enzyme immunoassay from the culture was negative but both nucleic acid amplification technics were positive (92% accordance between culture and both nucleic acid amplification technics); two of this nine samples belonged to patients who had clinical symptoms of gastrointestinal disease. illumigene® Test assay: 2 instrument failures, 3 PCR inhibitions; BD MAX™ Cdiff assay: 1 instrument failure, 3 PCR inhibitions.
Conclusion: Considering the culture as the gold standard for the *C. difficile* diagnosis, the PCR shows a little higher accordance with the culture compared to the LAMP. A rapid diagnosis of *C. difficile* infection is important to implement appropriate therapy and infection control measures. The main advantage of the DNA-based methods is the rapidity in the diagnosis, but the clinical significance of detecting DNA of *C. difficile* in absence of the toxin is not clear. A nucleic acid amplification positive assay does not necessarily indicate the presence of viable organisms. Just eight patients from the twenty six who had a positive molecular test and a negative culture for *C. difficile* or a negative *C. difficile* Toxin A/B enzyme immunoassay from the culture had clinical symptoms of *C. difficile* infection. Clinicians should send a sample to the laboratory for *C. difficile* diagnosis only when the patient presents clinical symptoms of gastrointestinal infection. Otherwise, the diagnostic could lead to an over-diagnosing of *C. difficile* infections.

Free Topics (FT)

144/FTP

Analysis of risk factors for invasive fungal infections in patients after liver transplantation

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The incidence of fungal infections in solid organ transplant (SOT) has increased during the past decade. Especially in the immediate postoperative course following liver transplantation (LT) invasive fungal infections (IFI) pose a vital threat to a significant percentage of these pharmacologically immunosuppressed patients. The objective of this study was to identify risk factors for the development of IFI in patients after LT.

Method: During 2004-2013 LT was performed in a total of 780 adult patients at our center. All patient records were analyzed for incidence and outcome of fungal infection during the initial hospital stay following LT. IFI was diagnosed based on current EORTC criteria. Mandatory symptoms at the time of diagnosis were fever, elevated serum inflammation parameters, vasopressors, enhanced respiratory support with or without pneumonia.

Result: In 119 transplant recipients microbiological proven fungal infection/colonization was diagnosed. The underlying diseases requiring LT were HCC (n=24), HBV (n=22), HCV (n=20), Ethyl toxic cirrhosis (n=18), cryptogenic cirrhosis (n=15), all other (n=20). In 52 transplant recipients fungal colonisation was diagnosed due to the absence of the above mentioned symptoms. IFI based on EORTC criteria was detected in 67 patients. Most IFIs were caused by Candida species (albicans 46%, non-albicans 18%), followed by Aspergillus species (27%) and other molds (8%). The overall mortality of patients with IFI due to Aspergillus ssp. was 42%, in patients with IFI due to Candida albicans 29% and in patients with IFI due to Candida non-albicans was 46%. Anti-fungal therapy consisted of Caspofungin+Fluconazole in 22%, Voriconazole in 22% and Fluconazole in 14% for the Candida non-albicans patients; Fluconazole 48%, Caspofungin 14% and Voriconazole 11% for the Candida albicans recipients. The most significant individual risk factors (as calculated by multivariate analysis) for the development of IFI were bacteriological positive intraoperative microbiological swaps ($p=0.003$) and an initial non-function of the liver graft after LT ($p<0.001$).

Conclusion: The results shown confirm the high incidence of IFI in patients following LT suggesting the more wide spread use of prophylactic anti-fungal treatment in LT patients with perioperative bacterial infection and/or early liver dysfunction.

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145/FTP

Bactericidal and wound healing properties of cold argon plasma

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Introduction: Cold plasma is a flow of partly ionized gas with a temperature less than 10^5K, active components of which are charged particles, neutral active species (ozone, hydrogen peroxide, nitric oxide) and UV radiation. Plasma sources are developed for different medical purposes, including treatment of infected wounds [1].

Objective: The aim of this work was to evaluate bactericidal and wound healing properties of cold plasma *in vivo*, using a mice model of infected wounds and *in vitro*, using human cell lines of keratinocytes and fibroblasts.

Materials and Methods: Two microwave generators of argon plasma were used in the experiments: MicroPlaSter β [2] for *in vivo* experiments and Plasma 200 (JIHT RAS, Russia) for *in vitro* ones.

To evaluate the bactericidal effect of plasma *in vivo* mice Balb/c were immunosupressed by ciprofloxacin, the full thickness skin wound was infected with 10^7 CFU/100μl *Staphylococcus aureus* 78 and in 3 days wounds were treated by plasma for 5 min in a distance of 20 mm during 7 days. The bactericidal effect was measured as an amount of bacteria survived after plasma treatment. The speed of wounds contraction was established by measuring of wound square on 1-9, 12 and 14 days of treatment.

Human keratinocytes HaCaT and human dermal fibroblasts were used in *in vitro* experiments. The cytotoxicity and proliferation index (cell index) were evaluated in real time using xCELLigence (Roche). Cells were seeded at a concentration of 20 000 cells/well and treated by plasma once for 2 and 5 min from a distance of 7 mm. 1 mm of medium was retained in wells before treatment, and immediately after the plasma application the medium was added.

Result: The amount of the survived bacteria after 5 min plasma treatment of wound surfaces was 2.3% for *S.aureus* 78 ($p<0,05$) from the initial concentration. The increase of wound healing since the 2nd till the 4th day of plasma treatment was statistically significant. But further plasma applications did not cause a promotion of healing, so it was decided to investigate a potent proliferation effect of argon plasma to keratinocytes and fibroblasts, after the single treatment. 2- and 5-min plasma treatment did not cause any proliferation changes of keratinocyte. But at the same time single treatment of fibroblasts for 2 and 5 min resulted in log-phase prolongation for 30 h and increase of cell numbers up to 42.6% in the stationary and degradation phases ($p<0,05$) (Fig.1)

Conclusion: The application of microwave argon plasma could increase a wound healing due to its bactericidal effect and increase of fibroblast's proliferation.

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References

[1] Nosenko T. et al. Free Radical Biology and Medicine 2009;47:128.
[2] Shimizu T. et al, Plasma Process. Polym. 2008; 5: 577.

Figure 1

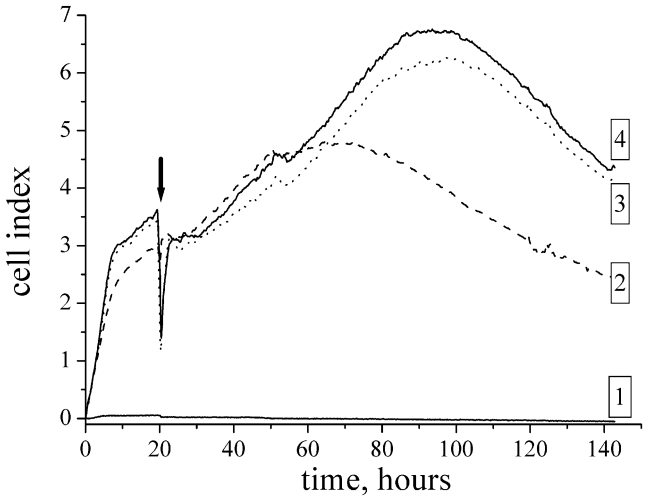

Fig. 1. Cell index of fibroblasts after plasma treatment. 1: control without cells, 2: non-treated control, 3: 2-min treated cells 4: 5-min treated cells. Arrow - plasma application.

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146/FTP

Anti-tubercular activity of synthetic derivatives of a natural stilbene

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Question: Tuberculosis (TB) and multidrug- and extensively drug-resistant TB in particular are remaining a major global health challenge. Thus, efficient new drugs against TB are urgently needed. This study investigated the anti-tubercular activity of synthetic derivatives of a natural stilbene against *M. tuberculosis.*
Method: Synthetic derivatives of isopropylstilbene were analyzed for anti-tubercular activity using MGIT 960 instrumentation, software EpiCenter, equipped with TB eXiST module (Becton Dickinson) against *M. tuberculosis* ATCC 27294 and multidrug- as well as extensively drug-resistant clinical isolates. Cytotoxic effects of drug candidates were determined by a MTT dye reduction assay using A549 adenocarcinomic human alveolar basal epithelial cells.
Result: Quantitative drug susceptibility testing was performed for two substances. Growth of *M. tuberculosis* ATCC 27294 was inhibited by synthetical derivatives DB55 and DB56 at 25µg/ml each. Growth of clinical isolate MDR *M. tuberculosis* was inhibited by DB55 and DB56 at 25µg/ml and 50µg/ml, respectively. Growth of clinical isolate XDR *M. tuberculosis* was inhibited by DB55 and DB56 at 25µg/ml each. Toxicity in terms of IC_{50} values of DB55 and DB56 were determined to be 12.15µg/ml and 16.01µg/ml, respectively.
Conclusion: Synthetical derivatives of stilbene might be effective candidates as anti-tubercular drugs. However, toxicity of these substances as determined by IC_{50} values might limit therapeutic success *in vivo*. Further investigations should address lowering the toxicity by remodeling stilbene derivatives.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

147/FTP

Analysis of the antimycotic effect of the ionophoric acting yeast killer toxins Zygocin and K1

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The increase in local and systemic fungal infections and also in antifungal drug resistance is still a major concern in clinical medicine. Unlike bacteria, eukaryotic yeast and fungal cells are closely related to mammalian cells and, therefore, the treatment of mycosis is often accompanied and hampered by severe side-effects. Furthermore, most of the antimycotics in use either target fungal ergosterol biosynthesis (which in its enzymatic steps is highly analogous to mammalian cholesterol biogenesis) or interfere with yeast or fungal cell wall components; however, none of these drugs efficiently kills a broad spectrum of pathogenic yeast and fungi. Furthermore, the molecular mechanisms of yeast cells' adaptation processes leading to antimycotic insensibility are poorly characterized. For this reason, the application of selected broad-spectrum yeast killer toxins as potential antifungal seems a promising approach to fight fungal pathogens. Particularly interesting toxin candidates are zygocin, a monomeric protein toxin secreted by the spoilage yeast *Zygosaccharomyces bailii*, and K1, an A/B toxin of viral origin produced by killer strains of the budding yeast *Saccharomyces cerevisiae*. Although both toxins differ in primary sequence and subunit structure, both are postulated to act as ionophors and kill a broad spectrum of human as well as plant pathogenic yeast and fungi, including *Candida albicans* and *C. glabrata*. In this study, biochemical and structural properties of zygocin and K1 are being characterized with a focus on dissecting the molecular mechanisms of K1 toxicity and protecting immunity in K1 producing killer cells. These studies include purification of both protein toxins and detailed analyses of their killing spectra. K1 immunity is investigated by expression of various suicidal toxin variants (e. g. V5 tagged and/or truncated) in different compartments of sensitive yeast.
Recent in vivo killing assays verified the broad-range killing spectrum of both toxins, with K1 bearing a pronounced antifungal activity that is even stronger than that of zygocin. However, both toxins are capable of efficiently killing diverse clinical *C. glabrata* isolates. Purification of the monomeric protein toxin zygocin was successful and without loss in activity. Currently, heterologous expression of both toxins in the methylotrophic yeast *P. pastoris* is in progress to increase the overall yield for preparative toxin purification and subsequent structural analysis. Results will be presented demonstrating that the intracellular expression of the cytotoxic α subunit of K1 results in the strongest suicidal phenotype in yeast, even after expression of a V5-tagged variant of K1α. Further coexpression studies with different K1 constructs in conjunction with the unprocessed toxin precursor (preprotoxin) will shed light onto the mechanism of toxin action and protecting immunity.

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148/FTP

Sensitive detection of Botulinum Neurotoxin combining affinity enrichment and enzyme activity detection

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Introduction: Botulinum neurotoxins (BoNT) are the most poisoneous biological toxins with LD50≥ 1ng/kg body weight. Therefore, diagnostic systems have to be extremely sensitive. Since, especially when dealing with samples intentionally

contaminated with BoNT, PCR detection of specific nucleic acids is not possible, highly sensitive protein detection is needed. For the detection of BoNT their enzyme activity as metalloproteinases is often used to enhance signal-to-noise ratio leading to low limits of detection. This systems mainly use expensive equipment like mass spectrometers.

Objective: To develop of a test system for the sensitive detection of BoNT in food chain matrices. Therefore, magnetic enrichment of the toxins is combined with protease activity detection using FRET assay. This detection is performed using standard real-time PCR equipment.

Materials and Methods: To ensure a broad applicability we used FRET detection of BoNT mediated cleavage of substrate peptides on Real-Time PCR equipment routinely available in diagnostic laboratories and even in mobile laboratory settings. Prior to enzyme activity determination the toxins are enriched by magnetic affinity purification.

Result: FRET peptides harbouring cleavage sites for BoNT subtypes were generated and assay conditions optimised. For the applicability with complex matrices, i.e. food samples, a magnetic enrichment of the toxins is used.

This further enhances sensitivity and, especially, minimises matrix effects potentially inhibiting metalloproteinase activity of BoNT. We found not only toxin specific antibodies but also cellular receptors of BoNT, i.e. SV2c for BoNT/A, effective for the enrichment of BoNT. With this combination the toxin was detected in spiked food chain matrices, i.e. juices and milk, using standard Real-Time PCR equipment.

Conclusion: Using affinity enrichment followed by of BoNT protease activity testing using FRET assays the sensitive detection of BoNT even in complex matrices can be achieved by just using broadly distributed Real time PCR systems.

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149/FTP

'CREDIBLE' - A Cre-mediated Double Reporter System to Study Penetration of Physiological Barriers by Cell-penetrating Effector Proteins

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Bacterial pathogens including *Yersinia* and *Salmonella* express effector proteins that are translocated into the host cell via a type three secretion system (T3SS) during infection. Previous studies identified the effector protein YopM of *Yersinia enterocolitica* as a novel bacterial cell-penetrating effector (CPE) that is able to translocate across the host cell plasma membrane independently of *Yersinia*'s T3SS. The two N- terminal α-Helices (2αH) have been identified as the protein transduction domain (PTD) of YopM, which mediates autonomous translocation and has the ability to intracellularly deliver molecular cargos like GFP. In order to further study and characterise the mechanisms of cell penetration by CPEs and further cell-penetrating peptides (CPPs) *in vivo*, we have generated a Cre-mediated double reporter ('CREDIBLE') system. Transgenic mice harbouring the 'CREDIBLE' construct express two reporter genes, namely near-infrared fluorescent protein (iRFP) and luciferase upon Cre/loxP-recombination. The 'CREDIBLE' system has been proven to be functional and both reporters are expressed upon recombination *in vitro*. Furthermore, crossing transgenic mice with PGK-Cre mice, which express Cre-recombinase lead to recombination events, indicating the functionality of the system *in vivo*. In order to analyse the distribution of CPP/CPEs *in vivo*, we have constructed different PTD-Cre fusion proteins, including 2αH-Cre. The recombinant proteins will be administered via different routes into the transgenic mice and their distribution can be analysed in real time by non-invasive live optical imaging. In addition, the system described here can be applied in a variety of studies using drug delivery systems such as exosomes or targeted drug delivery in order to characterise their efficacy *in vivo*. Furthermore, the 'CREDIBLE' system can be used to monitor bacterial or viral infections *in vivo* and, in particular, to gain a more detailed insight into the role and function of various virulence factors during infection (e.g. secreted bacterial effector proteins, outer membrane vesicles).

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150/FTP

Anticipating the second-line antibiotic era: drug resistant tuberculosis strain drives epidemic in Central Asia

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Multidrug resistant (MDR) and even extensively drug resistant (XDR) *Mycobacterium tuberculosis* complex (MTBC) strains have emerged worldwide and represent a serious challenge for global tuberculosis (TB) control. High rates of MDR-TB have been associated with particular phylogenetic lineages of the MTBC such as the Beijing lineage and the strong clonal expansion of MDR strains in Eastern Europe. Detailed data on the genetic diversity of outbreak strains, on the evolutionary history and possible effects of treatment programs, e.g. DOTS (Directly Observed Therapy, Short Course) on the relative transmission success is only sparsely available and largely speculative.

We applied whole genome sequencing on 277 MDR-TB strains from Nukus, Uzbekistan covering the years 2001 to 2006 to analyze the population structure and precisely assess the extent of transmission networks. Using Bayesian coalescent analysis, we determined the emergence of resistance variants in the history of the most successful strain types and changes in the effective population size of over time.

Transmission success is not equally distributed among the MDR strain population and can differ even among closely related outbreak strains. MDR-TB clones with particular combinations of multiple first and second-line resistance conferring and fitness related mutations existed prior the introduction of programmatic second-line treatment in Uzbekistan in 2003. These variants were most likely selectively promoted during the first introduction of WHO endorsed DOTS strategy in 1998 and accounted for 75% of all MDR-TB cases in 2005/2006.

We conclude that ineffective first-line and probably self-administered second-line drug regimens resulted in a large diversity of highly resistant TB strains before the initiation of formal MDR-TB treatment programs in Nukus, Uzbekistan. Our data strongly argue for a rapid and precise molecular resistance diagnosis to prevent further transmission and amplification of drug resistance, especially during the introduction of new antibiotic regimens including Bedaquiline and Delamanid.

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151/FTP

Whole genome based genotyping of Mycobacterium tuberculosis complex isolates using a standardised and easily expandable genome-wide MLST approach

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In recent years, the success of dropping total case numbers of tuberculosis has become challenged by the increasing incidence of multiple (MDR) or extensively resistant (XDR) TB. As there is virtually no environmental reservoir of the MTBC bacteria, the disease can be controlled by public health interventions. This necessitates reliable genotyping of bacterial isolates for monitoring treatment success, local outbreak detection, and regional surveillance. The results of traditional typing methods such as spoligotyping or MIRU-VNTR can be easily expressed in a number format similar to a barcode, automatically grouping the isolates in a meaningful way by simply collecting those isolates with an identical barcode pattern in a clonal complex. Thereby, the results of newly analysed isolates can easily be combined with existing data and shared between laboratories.
Several studies have already shown that the use of whole genome sequencing (WGS) allows for a much higher resolution and simultaneous resistance phenotype prediction. However, at present, the key limiting factors for widespread use of WGS genotyping are non-standardised analysis pipelines and the problems inherent in the commonly used SNP based analysis for data exchange between laboratories in an easily extendable classification scheme. In fact, with SNP based pipelines, the addition of new isolates into an existing study usually requires extensive recalculation.
One solution is the use of a multi locus sequence type (MLST) scheme encompassing the entire core genome set of genes (cgMLST). This strategy has been successfully employed for several bacterial species. Previously, we demonstrated the usefulness of a core genome MLST scheme for M. tuberculosis, with sufficient resolution to resolve individual outbreaks. In this study, we introduce and evaluate the performance of a cgMLST scheme for the whole MTBC using a reference collection reflecting the known diversity. From our results, the suggested MLST scheme consisting of 2891 genes was able to reliably classify isolates, with at least 97% of the genes reliably identified in all MTBC groups, and allowing for in depth resolution of individual outbreaks.

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General and Hospital Hygiene (StAG HY)

152/HYP

Validation of DST/MPN-kits for quantification of bacterial counts in drinking water and assessment of the impact of pre-analytical environmental conditions

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Introduction: Microbiological analysis of drinking water samples is an important component of water safety. Enteric bacteria, Legionella and Pseudomonas are quantified using membrane filtration methods, which are laborious and time consuming. Commercial kits employing defined substrate technology (DST) in combination with the most probable number approach (MPN) are designed to facilitate the analysis and to reduce the processing time.
Aim: To validate under routine laboratory conditions the performance of commercial DST/MPN-tests; to test whether environmental conditions affect the test results based on substrate metabolism.
Materials and Methods: Pseudalert®, Colilert®-18, and Enterolert®-DW / Quanti-Tray® were purchased from IDEXX Laboratories. Results were read after 18-24h of incubation. Filtration of water samples was performed according to DIN EN ISO 9308-1, 7899-2, 16266, 11731, and 11731-2. Biofilms were generated in microtiter plates for short term experiments or in glass separating funnels for a period of 90 days.
Result: Using 184 routine water samples of various sources the PPV of Pseudalert® was determined to be 1, the NPV was 0.97, specificity was 1, and the sensitivity was 0,57. There were 6 false negative samples, all of which with low bacterial counts of 1-5 CFU/100ml as determined by the gold standard (water filtration). Due to the low number of positive samples, only the excellent NPV was confirmed for Colilert®-18, and Enterolert®-DW. The coefficients of determination varied between 0.84 and 0.94, if the DST/MPN-assays were compared with serial dilution and plating of artificially contaminated water samples. Growth in LB-broth, LB-broth diluted 1:5 with PBS, or M9 medium supplemented with 0.4% glucose did not result in a discrepancy between Pseudalert® and serial dilution/plating. Growth temperature before inoculation (24, 37, and 42°C) also had no effect. Biofilms grown for 24 h in M9/glucose medium yielded comparable CFU when measured by Pseudalert®, filtration, and serial dilution/plating. The same held true for biofilms maintained over 90 days in glass separating funnels and sterilized drinking water.
Conclusion: DST/MPN-assays allowed a rapid and reliable quantification of water microorganisms. Limitations were a slightly unsatisfying sensitivity of Pseudalert®, as well as the fact that positive results with Colilert®-18, and Enterolert® should be confirmed by re-cultivation as evidenced by inoculation experiments with various bacterial species (data not shown). Pseudalert® proofed to be a robust assay not affected by pre-analytical environmental conditions. DST/MPN-assays reduced laboratory turnover and hands-on time.

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153/HYP

Discrimination of *Klebsiella pneumoniae* phylogroups by MALDI-TOF Mass Spectrometry

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Background: *Klebsiella (K.) pneumoniae* is a commonly encountered pathogen, which can cause a wide range of infections. Antibiotic resistant strains have emerged over the last years, which are frequent causes of nosocomial outbreaks. Recent reports describe three *K.pneumoniae* phylogroups (KpI, KpII, and KpIII). KpI is thought to be associated with higher pathogenicity and hospital outbreaks, whereas KpII and KpIII are found as colonizers in humans, animals, and plants. In addition, the core chromosomal *SHV*, *OKP* and *LEN* beta-lactamases (*bla*) genes evolved in parallel with the three groups. Rapid typing of *K. pneumoniae* isolates is desirable in order to detect potential pathogenic strains and also transmission events. However, commonly used typing methods are costly and/or labor intensive. MALDI-TOF mass spectrometry (MS) is routinely used in microbiological laboratories for rapid species identification. The goal of this study was to establish markers in MALDI-TOF spectra that allow for rapid distinction of *K. pneumoniae* phylogroups.

Method: 40 strains of *K. pneumoniae*, which were previously analyzed by whole genome sequencing, were grouped according to the presence of *bla* genes (corresponding to KpI, KpII and KpIII). MALDI-TOF spectra of the strains were recorded in octuplets on three days on a MALDI Biotyper system (Bruker Daltonics) and spectra were imported into the BioNumerics 7 software (Applied Maths). Summary spectra were then calculated for each isolate and these spectra were again summarized according to *bla* groups. The amino acid sequence of ribosomal proteins was calculated from the corresponding genes in the annotated genome sequences using the ExPASy online tool.

Result: *K. pneumoniae* isolates could be assigned to *bla*SHV (KpI) or *bla*LEN (KpIII) groups. Summary MALDI-TOF spectra of bacterial isolates revealed 15 mass peaks specific for *bla*LEN strains and 13 peaks specific for *bla*SHV. Nine of these specific peaks could be found in both groups in a <100 Da range, potentially representing protein isoforms with one (or more) amino acid substitution(s). The calculated masses of the ribosomal proteins L31p, and de-methionated L28p, and S15p were found to differ between both *bla* groups and these masses matched three of the specific peak masses for the respective group. Three more peak masses could be identified as the double charged ions of these proteins. Unbiased comparison of all open reading frames in the genomes additionally revealed that the single and double charged isoforms of the stress-response protein CsbD matched two more specific peak masses.

Conclusion: MALDI-TOF spectra contain markers that allow to distinguish *K. pneumoniae* phylogroups, which could be used in future as a quick screening tool to detect the more virulent KpI group or be used to detect transmission events.

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154/HYP

Proteomic analysis of *esbl-producing escherichia coli before, during and after continuous exposure to subinhibitory concentrations of* benzalkonium chloride

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Introduction: Disinfectants are widely used in different areas and an inappropriate use might cause the emergence of disinfectant resistances. Benzalkonium chloride (BC) represents an important biocidal agent widely used as a disinfectant in the food-producing industry. Exposure to a serially increasing concentration induces adaptation to higher BC concentrations, which in turn contribute to antibiotic resistance through cross-reactive mechanisms.

Objective: The aim of the investigation is to assess the protein expression of *Extended spectrum beta-lactamase* (ESBL)-producing *E. coli* after continuous exposure to increasing but still subinhibitory concentrations of BC.

Materials and Methods: Adaptation to BC was induced in the *E. coli strain* ($bla_{CTX\text{-}M1}$, $qacE\ \Delta1^+$, $qacH^+$) by continuous sub-culturing starting from 10 ppm and stepwise increasing concentration by 1 ppm / day for 30 days. For proteomic analysis, cells were harvested at mid-logarithmic growth phase at initial unexposed (control), 30 days adapted and after 10 days of subsequent culturing without BC (stability 10). The whole cell proteins were extracted and digested with trypsin. The resulting peptides were desalted and separated using reverse-phase nanoLC. The eluted peptides were ionized on-line by electrospray ionization and transferred into an LTQ Orbitrap Velos, which was operated in the positive mode to measure full scan MS spectra (from m/z 300-1700) followed by isolation and fragmentation by collision-induced dissociation of the twenty most intense ions. Mass spectra were analysed using the MaxQuant software by searching against a Uniprot reference database of *E.coli*. Following statistical analysis using the Perseus software, all the identified proteins were assigned with KEGG orthology identifiers and functional hierarchies mapped using KEGG Automatic Annotation Server.

Result: About 60% of the identified proteins appeared to be influenced during the course of BC adaptation and stability passage. The majority of proteins that were differentially expressed after 30 days of induction were reverted to normal or oppositely regulated after the stability passage. Compared to the un-induced control, bacterioferritin, chaperonin GroES and an uncharacterized protein YggE were found to be consistently down-regulated during the induction and stability checking, while twelve proteins mainly consisting of enzymes, binding proteins and stress-related proteins were consistently found to be up-regulated. Up-regulation of g-type lysozyme supports earlier reports that the BC adaptation might occur mainly through secretion of proteins that protect the cell against damaging agents.

Conclusion: Continuous step-wise exposure to sublethal concentrations of BC resulted in stably increased MIC values. The cellular response and adaptation might occur through various mechanisms such as protection of the cell through enhanced general stress tolerance.

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155/HYP

Evaluation of Infection Control Newsletters (NL) written by infection control nurses (ICN) participating in the HygPfleg-Project

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Background: The HygPfleg-Project developed and pilot-tested a curriculum for infection control nurses (ICN). ICN were trained to link didactic and psychological skills with infection control, enabling them to provide in-house training for infection control link nurses (ICLN). Strategies to ensure long-term collaboration with ICLN were included in the curriculum, one being the implementation of an infection control newsletter (NL). Participants were tasked to write a NL.
Aim: The study was undertaken to evaluate the intention, potential and quality of the Hygpfleg-Project participants' NL.
Method: NL were analysed in three categories consisting of formal, content-related and purpose-related criteria. It was determined whether behavioural or technical changes were intended and if an explanation and motivation was given. Criteria were assessed on a threefold scale ranging from high, middle to low. The selection of topics was evaluated.
Result: At the date of investigation 30 NL were available from 126 participants (response rate 24%). Data are preliminary, more NL will be obtained. Formal analysis yielded that 77% were one-page NL (maximum 11 pages). 66% contained 1-2 topics (maximum 5 topics). 36% were considered easily recognisable, 36% were judged as clearly structured.
Content-related criteria showed that in 30% the relevance of the chosen topics was apparent from the text. In 23% the relevance did not become apparent. 3% were considered highly entertaining, 50% were considered not entertaining. 43% had high content of information (COI), 43% had medium COI. 10% were considered highly motivating, 40% had no elements of motivation. 70% intended behavioural changes (e.g. improved hand hygiene, personal hygiene), 23% intended technical changes (e.g. order new aprons). 7% were informational with no apparent intention for changes. In 27% strong explanations were given for the intended changes, in 30% some explanation was found. 43% did not give an explanation.
53% contained information on pathogens (e.g. MRSA, Norovirus, Influenza), 30% focussed on personal or hand hygiene, 20% informed about new products and 13% contained internal epidemiological data.
Conclusion: Three quarters of the NL were of high or medium quality by formal criteria. If changes are intended, NL should contain convincing explanations and elements of motivation. NL can be a tool of achieving even behavioural changes, if a strong emphasis is put on content-related criteria (1). To support ICN in using NL as a tool for establishing long-term collaboration with ICLN, distributing information or motivating infection control actions, a content-related structure should be provided.

Reference

[1] Peter.D; Kugler.Ch; Meng.M; Galante.R; Braun.G; Mattner.F: Evaluation of an Infection Control Newsletter (NL) as one HygPfleg-Project-Strategy to ensure sustainable collaboration with infection control link nurses (ICLN).

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156/HYP

When cleaning makes things worse: pyrosequencing and confocal microscopy analysis of bacterial communities in used kitchen sponges

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Domestic habitats often represent reservoirs of microbes, frequently including opportunistic pathogens. Microbial "hot-spots" are wet and nutrient-rich, such as dishwashers and kitchen sponges, since nutrient accumulation supports consistent bacterial growth. Here, we analyzed the bacterial microbiota of 15 kitchen sponges collected in diverse households in the greater area of Villingen-Schwenningen. For each sponge, bottom and top parts were analyzed separately. Our aim was to test several intrinsic and extrinsic factors (number of users, frequency of both use and replacing, special cleaning and brand), in order to identify possible drivers of bacterial assemblage in kitchen sponges. To investigate diversity and structure of the bacterial microbiota, we used 454–pyrosequencing of 16S rRNA gene libraries, followed by analysis with the QIIME software pipeline. In addition, the bacterial colonization pattern in the sponges was investigated by fluorescence in situ hybridization coupled with confocal laser scanning microscopy (FISH–CLSM). Our results showed that the most abundant operational taxonomic units (OTUs) belong to the genera Acinetobacter, Enhydrobacter, and Pseudomonas (class Gammaproteobacteria) and Chryseobacterium (phylum Bacteroidetes). Interestingly, all of these genera include also opportunistic pathogens, such as Acinetobacter baumannii and Chryseobacterium hominis. Alpha– and beta–diversity of bacterial microbiota did not correlate with any of the investigated factors, with the exception of the factor "special cleaning", indicating that the user were applying special methods to clean their sponges. This factor significantly affected beta–diversity metrics (weighted Unifrac–distances), by surprisingly increasing the relative abundance of an OTU, whose 16S sequence is 99.8% identical to that of Chryseobacterium hominis (Fig. 1). This demonstrated that cleaning measures of kitchen sponges might paradoxically increase the risk of relatively enriching potential dangerous species. FISH–CLSM confirmed the sequencing results by showing a dominance of Gammaproteobacteria. Sponges showed the typical network–texture, and bacteria were ubiquitously distributed, mainly concentrating in the internal cavities where they formed a biofilm–like layer on the solid/liquid interface (Fig. 2, arrow). We suppose that these liquid–filled cavities accumulate nutrients and might favor quorum sensing by facilitating molecular exchange. Isolated colonies were also detected in other niches of the sponges (Fig. 2 arrowhead). Our study sheds more light on the microbial ecology of sponges, and provides information to improve kitchen hygiene. More research is needed to increase our knowledge of domestic habitats and "in-vironmental" microbiology, in order to better understand the potential threats and the impact on human health

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Clinical Microbiology and Infectious Diseases (StAG KM)

157/KMP

Investigation of *E. coli* pathotypes for biofilm formation in association with their phenotypic and genotypic characteristics

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Introduction: Biofilms are microbial communities living in a self-produced extracellular polymeric matrix consisting of exopolysaccharides, proteins and DNA adhered to abiotic or biotic surfaces. Bacterial biofilms can cause chronic and device-related infections due to their high resistance to antibiotics and the host immune system. There is a growing presumption that biofilm formation is associated with the pathogenicity of bacteria.
Objective: The aim was to investigate the potential of biofilm formation of different *E. coli* pathotypes and the correlation of genotypes and phenotypes from *E. coli* pathotypes to biofilm formation. The pathotypes include HFEC, UPEC, ETEC, EPEC, EAEC, SAEC, CAEC and AFEC.
Materials and Methods: We enhanced our previous published VideoScan technology [Rödiger et al. 2013], which is based on fully automated fluorescence microscopy to perform high-throughput screening of biofilms. We analysed 187 *E. coli* strains for biofilm formation using 4 different media in a 96 well plate format. By using the Syto 9 staining and the VideoScan technology fluorescence intensities of the biofilms were measured and overview images from each well were taken. Strains were additionally examined for biofilm formation by using crystal violet assay. Furthermore we examined motility and performed PCR for biofilm associated genes (e.g. *agn43*, *bcsA*, *csgA*, *fimH*, *fliC*). Curli and cellulose production were analysed using Congo red and calcofluor agar method.
Result: By screening we found a high prevalence of genes, which are considered to play a role in biofilm formation (e.g. *bcsA* 96,3%, *csgA* 99,5%). In total 74,3 % of all strains are motile. The Congo red assay revealed 27,8% of rdar, 32,6% of bdar and 39,6% of saw morphotype while incubating the strains at 28°C. At 37°C only half as much of the strains (14,4%) produced the rdar morphotype. In total curli expression was less prevalent, when incubating the strains at higher temperatures, what is in agreement with the fact, that curli expression is a temperature regulated process. However, we could not find a significant correlation between genotype or phenotype and the biofilm formation of the different pathotypes. With our VideoScan technology we could identify strong biofilms formers belonging to the group of EAEC. These results could be confirmed by using crystal violet assay.
Conclusion: With our VideoScan technology it is possible to evaluate biofilm formation for a large number of strains in a fully automated screening. With this image-based technique we could detect a considerable number of biofilm formers out of 187 *E. coli* strains with EAEC being the pathotype exhibiting the strongest biofilm formers. For pathogenic bacteria biofilm formation is an important step in the host infection process and our results show that the ability to form biofilms is rather correlated with the pathotypes than with the genotype or phenotype of a strain.

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158/KMP

Is the current *Neisseria gonorrhoeae* antimicrobial resistance testing in Germany sufficient to guide the treatment?

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Background: *Neisseria gonorrhoeae* (NG)-infections are not reportable in Germany and only limited data on NG epidemiology and antimicrobial resistance (AMR) are available. Since 2013 the first line therapy according to German guidelines includes ceftriaxone or cefixime together with azithromycin. If susceptible, cefixime, ciprofloxacin, ofloxacin or azithromycin can be used as a single medication. E-test is recommended for NG AMR testing, especially for ceftriaxone and cefixime. With Gonococcal Resistance Network (GORENET) we monitor the NG AMR patterns and resistance testing in Germany.
Aim: We described NG AMR resistance testing in the participating laboratories to assess need of quality assurance.
Method: Between April 2014 and December 2015 data on NG AMR tests were collected from participating laboratories nationwide. Laboratories were asked to send isolates to the consiliary laboratory (CL) for repeated AMR testing by using E-Test.
We described the reported samples by used tests, tested antibiotics (ceftriaxone, cefixime, azithromycin, ciprofloxacin, and penicillin), and result interpretation standard. For samples tested with E-test and reported minimum inhibitory concentration value (MIC), we compared values of the MIC between reported results and results from CL. We described deviance as difference in the dilution steps.
Result: In total 23 laboratories submitted data on 1,530 NG AMR tests. Resistance to ceftriaxone was tested in 95%, cefixime in 92%, azithromycin in 92%, penicillin in 97%, and ciprofloxacin in 98% samples. Proportion of samples tested by E-test was 93% (ceftriaxone), 94% (cefixime), 81% (azithromycin), 78% (penicillin), and 74% (ciprofloxacin). Rest of the samples was tested by agar diffusion. In 77% AMR testing results were interpreted by using EUCAST and 23% by CLSI.
As not for all samples MIC were reported and only part of the isolates were submitted to CL, reported MIC and MIC from CL testing could be compared for 372 isolates, figure 1. The deviation in results led to different interpretation of AMR test result in 2% (ceftriaxone), 15% (cefixime), 57% (azithromycin), 32% (penicillin), and 5% (ciprofloxacin).
Conclusion: Not all NG AMR tests included recommended antibiotics and were performed by E-test. Different standard for the interpretation of AMR are used. Deviations between reported MIC and testing in CL were observed.
This might lead to suboptimal therapy and thus further development of AMR is possible. To facilitate optimal response to development of NG AMR in Germany, a test panel including antibiotics recommended for therapy, validated quantitative NG AMR test methods, and unified interpretation standard should be used. Reasons for deviances between results in laboratories and CL should be further investigated. External quality assurance for NG AMR testing and training of the laboratory technicians should be implemented.

Figure 1

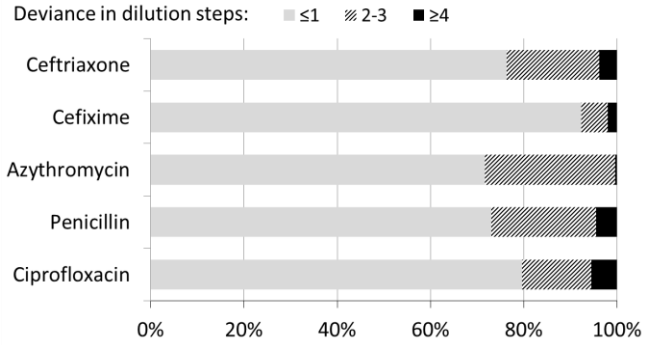

Figure 1: Deviance in dilution steps by antibiotic

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

159/KMP

Antimicrobial resistance of *Neisseria gonorrhoea* towards ceftriaxone and cefixime remains low in Germany

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Background: *Neisseria gonorrhoeae* (NG)-infections are not reportable in Germany and limited data on NG-epidemiology and antimicrobial resistance (AMR) are available. The first line therapy in Germany is ceftriaxone together with azithromycin. With Gonococcal Resistance Network (GORENET) we monitor the NG-AMR in Germany.
Aim: We analysed data on NG-AMR testing to guide treatment algorithms and targeted prevention strategies.
Method: Between April 2014 and December 2015 data on NG-AMR-tests and patient related information was collected from laboratories nationwide.
Laboratories were asked to send isolates to the consiliary laboratory for AMR-testing towards ceftriaxone, cefixime, azithromycin, ciprofloxacin, and penicillin by using E-test, and beta-lactamase by using Nitrocephin test. AMR-results were interpreted according to EUCAST 4.0.
We calculated proportions, medians, and interquartile range (IQR). We compared medians by Wilcoxon-Mann-Whitney-Test and proportions by Chi-squared test or Fisher's exact tests, where applicable.
Result: In total 23 laboratories submitted data on 729 samples collected between April and December 2014 and 927 samples collected between January and December 2015. Altogether, 90% isolates were from men. Median age of tested men was 33 (IQR 25-44) and women 27 (IQR 22-40) years (p-value<0.001). Most frequently tested materials among men were urethral (96%) and rectal swabs (2%), among women mainly endocervical or vaginal swabs (84%). Samples from men were submitted by urologists (74%) and from women by gynecologists (80%).
Consiliary laboratory tested 261 isolates from 2014 and 276 from 2015, table 1. In 2014 62% and in 2015 78% of the isolates showed minimum inhibitory concentrations (MIC) of ≤ 0.016mg/L to cefixime. MIC towards cefixim resistant strains was 0.19 mg/L. MIC of towards azithromycin resistant strains, except one, showed low level resistance and were mostly arranged near the breakpoint. In 2015 one isolate displayed high-level resistance to azithromycin (MIC 256mg/L). High-level plasmid mediated resistance to penicillin (penicillinase producing NG-PPNG) were found in 25% of all tested strains in 2014 and in 14% in 2015.
Table 1. Proportion of NG isolates tested susceptible, intermediate and resistant towards ceftriaxone, cefixime, azithromycin, penicillin and ciprofloxacin
Conclusion: Isolates tested for NG-AMR were mostly from men. NG AMR to ceftriaxone was not detected and to cefixime remains low, while resistance and intermediate susceptibility to azithromycin, ciprofloxacin and penicillin is substantial. Except decrease in AMR towards ciprofloxacin and penicillin, no substantial changes in AMR pattern between 2014 and 2015 could be detected.
Monitoring of NG-AMR should be highly prioritised and number of collected and tested isolates increased.

Figure 1

	Sensitive		Intermediate		Resistant	
Antibiotic	2014	2015	2014	2015	2014	2015
Ceftriaxone	100%	100%	-	-	0%	0%
Cefixime	98%	99%	-	-	2%	1%
Azithromycin	55%	62%	33%	28%	12%	10%
Penicillin*	9%	14%	62%	67%	29%	19%
Ciprofloxacin*	27%	41%	0%	0.4%	73%	58%

** Chi² test p-value<0.05*

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160/KMP

Education in Biosecurity & Dual Use Issues in the Life Sciences: Promoting Awareness Raising and Self-Governance within the Scientific Community.

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Most research shows the potential to generate information, technologies, materials or knowledge that could be misused for malevolent purposes. This dual use dilemma can be recognised in many if not all scientific disciplines. In some cases dual use research will provide results that could be immediately misused and could cause great harm. Such activities are termed dual use research of concern (DURC). The public debate on gain-of-function experiments using artificially created airborne, human-transmissible H5N1 influenza viruses (published in 2012) raised the question of whether such research poses unnecessary risks for biosafety, biosecurity and public health. Some critics even postulated a pandemic potential of these virus strains in the case of an accidental or intentional release implying a lack of awareness of the threat potential of that work. How to deal with the immanent dual use dilemma in the life sciences? How to keep the balance between freedom of research and the responsibility to protect humans and the environment? Although a couple of respective codes of conducts already exist (e.g. on working with highly pathogenic microorganisms, released by the German Research Foundation (DFG) in 2008) it was not clear if more regulation measures were needed to cope with hazards originating from DURC-related experiments. On behalf of the German government the German Ethics Council prepared an opinion on that matter published in 2014 recommending the implementation of educational programmes on biosecurity, a code of conduct for responsible research as well as improvements of legal regulations. The leading question is whether or not self-governance in the scientific community will be sufficient to manage research projects showing a significant misuse potential. The German National Academy of Sciences Leopoldina and the DFG jointly published recommendations for handling security-relevant research in 2014:

key prerequisites for successful self-governance in the sciences is sensibilisation and raising awareness of potential risks among professionals and especially students. But how to integrate adequate education in already densely packed curricula in the life sciences? One practical example is the integration in an obligatory course on biomedical ethics. This offers the possibility discussing biosecurity-relevant questions in the broader context of ethical implications of biomedical research. In a multi-step approach students learn to understand why certain subclasses of experiments fall into the category of DURC and how to do an appropriate risk assessment. Teaching involves theoretical (lectures) and participative (presentations, simulations) elements, which promote sensibility of the students for misuse potentials in the life sciences and encourage them to take responsibility for their own work - without impeding necessary scientific progress.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

161/KMP

Local antibiotics in prosthetic joint and bone infections - why, when and how should they be used?

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Introduction: With an incidence rate of 1-2% in primary and up to 5% in revision procedures prosthetic joint infection (PJI) is a rare pathology of joint replacement surgery. Treatment of PJI is often complex and increasingly challenging as a consequence of patient risk factors and the growing prevalence of multi-resistant pathogens in the orthopaedic ward. In order to prevent bacterial colonization and biofilm formation on the "vulnerable" prosthesis or to support the septic treatment of a PJI, a combination of systemic and local antibiotics has become surgical standard. The best studied and most widely used delivery system for local antibiotics is bone cement. However, in some countries the practice of using antibiotic-loaded bone cement (ALBC) is started to be critically discussed on the background of the rising antibiotic resistance problematic.
Aim: The benefit and risks of using ALBC or other delivery systems for local antibiotics are discussed on the basis of a metaanalysis of the available clinical evidence.
Method: A metaanalysis of available clinical data including RCTs, retrospective cohort studies, single centre observational studies and pharmacokinetic analysis has been performed for antibiotic-loaded bone cements, collagen fleeces and synthetic bone graft substitutes.
Result: Pharmocokinetic studies have shown that the level of antibiotics released from bone cements and other antibiotic carriers may reach bactericidal in situ-concentrations which are 100 - 1000 fold higher compared to concentrations of systemically administered antibiotics. Typically, this is not associated with a higher burden of systemic side effects. According to several arthroplasty registries the combined use of systemic and local antibiotics may reduce the risk for PJI and/or the incidence of PJI relapses up to 50%. Gentamicin is the prophylactic local antibiotic of choice, because of its broad-spectrum and strict concentration-dependant bactericidal effect. In order to support eradication of an already established PJI in the joint cavity or in the bone, an antibiogram-adapted combination of two or more antibiotics in a cement spacer should be considered after surgical debridement of the infected tissue because of their synergistic action. However, not any antibiotic is suitable for using with bone cement. For the microbiologist and orthopaedic surgeon it is therefore important to know which basic chemical and physical requirements an antibiotic chosen for admixing to bone cement powder must fulfill. These include often not easily predictable parameters such as water-solubility, heat-stability, no chemical interaction with cement polymerization etc.
The available literature doesn't support concerns about deleterious effect of antibiotics on mechanical resistance of bone cement, if not exceeding the 10% rule (max. 10% of antibiotic admixed to cement powder), implant survival and development of bacterial resistance.
Conclusion: The regular use of ALBC has proven effective in PJI prophylaxis and septic revision treatment. However, selection of inappropriate antibiotics, selection of a bone cement with bad elution properties and incorrect surgical techniques can limit this effect on the infection rate. Gaining deeper knowledge about the properties and the application of ALBC is important not only for the orthopaedic surgeon, but also for the microbiologist and/or infectious disease specialist.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

162/KMP

Ranalexin - an antimicrobial peptide from *Rana catesbeiana*
The peptide ranalexin was synthesized via solid phase peptide synthesis and tested against different bacterial species.
A strong activity against multiresistant isolates of *Acinetobacter baumanii* was shown.

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Introduction: An overview of ranalexin is given in this area, including the structure formula, the mechanism of action and a picture of *Rana catesbeiana* is also this area.
Related antimicrobial peptides: This area contains a table with the amino acid sequence of related antimicrobial peptides of the genus *Rana*.
Derivatives of antimicrobial peptides in modern medicine: In this are daptomycin and colistin are described, including their structure formula
Result: The results of antimicrobial activity testing, via microdilution, against 22 bacterial species are shown in a table.
Further research outlines: The plans for the further research on ranalexin are shown here.

References

[1] Clark, Durell, Maloy and Zasloff. "A novel antimicrobial peptide from bullfrog (Rana catesbeiana) skin, structurally related to the bacterial antibiotic, polymyxin." The Journal of biological chemistry, 1994.
[2] Simmaco, Mignogna, Barra. "Antimicrobial peptides from amphibian skin: What do they tell us?" Biopolymers, 1999.
[3] Desbois, Sattar, Graham, Warn, Coote. "MRSA decolonization of cotton rat nares by a combination treatment comprising lysostaphin and the antimicrobial peptide ranalexin." Journal of antimicrobial chemotherapy, 2013.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

163/KMP

Doxycycline treatment interferes with the metabolism of *Chlamydia trachomatis*

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Urogenital infections with the obligate intracellular bacterium *Chlamydia trachomatis* are the most common bacterial sexual transmitted diseases worldwide. In most cases the infection is asymptomatic, but recurrent infections are associated with ectopic pregnancy, pelvic inflammatory disease and infertility. Doxycycline and azithromycin are the recommended treatment for

C. trachomatis. As it is known doxycycline leads to a metabolic switch from oxidative phosphorylation to glycolysis in human cell lines, we wondered whether there is an effect on chlamydial metabolism. Therefore, we analyzed the metabolic activity of *C. trachomatis* and host cells during doxycycline treatment.
Two-photon microscopy was used to analyze the metabolic activity of *C. trachomatis*-infected HeLa cells during doxycycline treatment. Thereby, we used fluorescence lifetime imaging (FLIM) of NAD(P)H to separately characterize the metabolic activity of *C. trachomatis* and its host cell. HeLa cells were infected with *C. trachomatis* L2 (0.5 IFUs/cell). At 20 hours post infection, infected cells were treated with 1 or 2 µg/ml doxycycline for 4 hours. Growth and progeny were analyzed by quantification of recoverable IFUs.
We observed that doxycycline treatment resulted in a significantly decreased amount of infectious progeny compared to untreated samples. Interestingly, enhanced metabolic activity of *C. trachomatis* was indicated by increased NAD(P)H intensity and relative amount of protein-bound NAD(P)H in the inclusions either with 1 or 2 µg/ml doxycycline compared to non-treated cells.
Our results indicate that doxycycline leads to an upregulated metabolism in the inclusions, although it inhibits the proliferation of *C. trachomatis*. There might be an increased uptake of nucleotides under the treatment with doxycycline. In further experiments we analyze the effect of prolonged doxycycline treatment on the metabolism of *C. trachomatis* and the activity of mitochondrial respiratory chain complex.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

164/KMP
Upon transition into the dormant state Mycobacteria accumulate porphyrins
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Introduction: Tuberculosis, which is caused by a bacterial pathogen *Mycobacterium tuberculosis* (MTB) is a leading cause of mortality among infectious diseases. MTB uses diverse strategies to survive in a variety of host lesions and to evade immune surveillance. Development of latent TB infection is based on the ability of this genus to persist in the dormant state (Wayne, 1994). As a model for studying of the mechanisms of TB long-term persistence, a rapidly growing nonpathogenic microorganism *Mycobacterium smegmatis* is widely used.
We have recently developed a model of transition of *M. smegmatis* cells into the dormant state, based on gradual acidification of the culture medium (Kudykina *et al.*, 2011). These cells displayed the ovoid morphotyp with a thickened cell wall, decreased metabolic activity and elevated resistance toward the heating and antibiotics; moreover upon transition into the dormant state, these bacteria begun to accumulate and to excrete a maroon-colored pigment (Fig. 1). Assuming that pigmentation might contribute to the resistance of the bacteria toward unfavorable ambient conditions, we aimed at elucidating the chemical nature of the pigment and understanding its role in mycobacterial physiology.
Results and Discussions: Application of MALDI-TOF to the analysis allowed us to demonstrate, that cells accumulate a hydrophobic methylated (esterified) form of coproporphyrin III, while to supernatant bacteria excrete hydrophilic, water soluble coproporphyrin III. In our further experiments we have found, the increased amounts of porphyrins were associated with a low catalase activity (0.080.02U/min for ovoid pigment-producing bacteria vs 0.210.01 U/min for actively growing cells) and low peroxidase activity (0.460.02 U/min for ovoid pigment-producing bacteria vs 0.840.02 U/min for actively growing cells). This could mean that stored porphyrins either could act as inhibitors of catalase/peroxidase activity, or they could adopt functions of a cytoprotector - protecting the dormant cells from the reactive oxygen intermediates in macrophages, and demonstrating the function of antioxidants.
We for the first time, demonstrated accumulation and localization of the esterified hydrophobic porphyrins in mycobacterial dormant cells, which in their turn, are essential for survival of bacteria in stress conditions. The enzymes involved in porphyrins biosynthesis, thus could be considered as potential targets for development of new strategies against TB.

References
[1] Kudykina, Y.K., *et al.*, 2011. Generation of dormant forms by Mycobacterium smegmatis in the poststationary phase during gradual acidification of the medium. Microbiology 80, 638-649.
[2] Wayne, L.G., 1994. Dormancy of Mycobacterium tuberculosis and latency of disease. Eur. J. Clin. Microbiol. Infect. Dis. 13, 908-914.

Figure 1

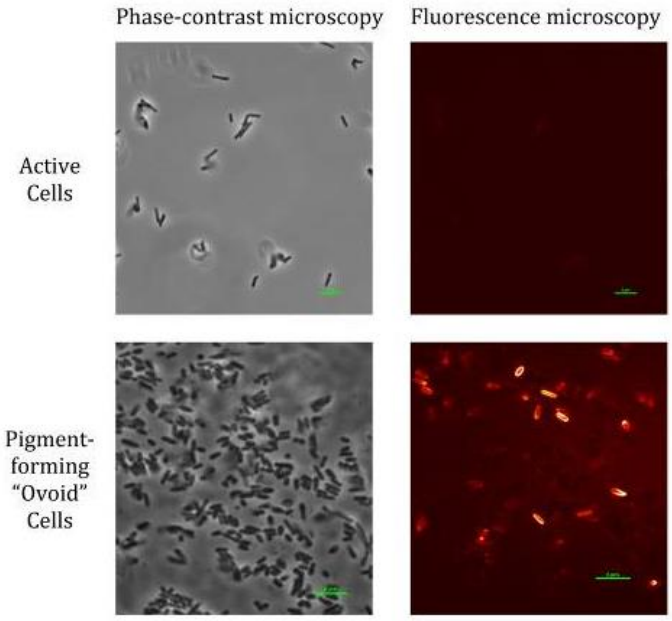

Fig. 1. Phase-contrast and fluorescence microscopy of M. smegmatis cells. Accumulation of a fluorescent pigment among the dormant ovoid cells is visible

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

165/KMP
Livestock-associated methicillin-resistant Staphylococcus aureus in the airways of cystic fibrosis patients
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Objective: Cystic fibrosis (CF) patients suffer from persistent recurrent bacterial airway infection, which often lead to lung insufficiency and decreased life expectancy. One of the most prevalent pathogens recovered from CF airways is Staphylococcus aureus. While the impact of methicillin-susceptible S. aureus (MSSA) on lung function and disease progression is less clear, it has been shown that patients with methicillin-resistant S. aureus (MRSA) experience lung function decline, more severe disease and reduced life expectancy compared to patients infected by MSSA.

Recent epidemiological studies of MRSA determined the prevalence of community- and hospital acquired MRSA in CF patients, however there is no information about the prevalence of livestock-associated (LA) MRSA in CF.
Method: We determined the prevalence of MRSA in two certified CF centers in Muenster using our CF data base. MRSA strains were molecular typed by spa-typing. Sequential LA-MRSA isolates from individual patients were subjected to DNA microarray analysis (IdentiBAC Microarray, Alere Technologies GmbH, Jena, Germany).
Result: MRSA was recovered from 14 of 189 (7,4%). LA-MRSA was identified in 5 patients (35%), all belonging to CC398. LA-MRSA persisted in one patient 9 years, in 2 patients LA-MRSA was detected within the last year with a persistence now of one year and in 2 patients LA-MRSA were identified only once without further persistence. Thirteen LA-MRSA isolates from 3 patients were subjected to DNA microarray analysis. All isolates carried the mecA and tetM resistance genes, while ermA was detected in all isolates of patients 1 and 2, ermC in both isolates of patient 3, tetK in all isolates of patients 1 and 3 and the aminoglycoside resistance gene aadD in 4 of 8 isolates of patient 2 and in both isolates of patient 3. Neither enterotoxin genes nor the hlb-converting phage was detected in the isolates leading to the presence of a functional hlb gene. While the leukotoxins lukF and S were present in all isolates, lukX was present in 10/13 and lukY in 9/13 isolates. All isolates belonged to capsule type 5. While the proteases sspA, B and P, the biofilm genes icaA, C and D were present in all isolates, the protease aur was missing in one, the adhesin fib was present in the first isolate of patient 1 only and sdrD in 10/13 isolates.
Conclusion: LA-MRSA can be isolated from the airways of CF patients and can colonize/infect the airways persistently. Although LA-MRSA were isolated from one patient for 9 years and in 2 patients for one year, the strains did not acquire the hlb-converting phage, which carries important genes interfering with host response. The resistance pattern was different and stable for the individual patients isolates, while few virulence genes differed during persistence.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

166/KMP

Betreuung von Patienten mit *Staphylococcus aureus*-Bakteriämie durch das Antibiotic Stewardship Team am Universitätsklinikum in Goettingen - Epidemiologie und Prognose

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Einleitung
Staphylococcus (S.) aureus ist ein häufiger Erreger einer ambulant bzw. nosokomial erworbenen Bakteriämie. Eine Kolonisation mit *S. aureus* in Verbindung mit einer Hautläsion (z. B. Verletzung oder invasiver Eingriff +/- Nichteinhalten von Standardhygienemaßnahmen) kann bei Gesunden als auch chronisch Kranken zu einer *S. aureus*-Bakteriämie (SAB) führen.
Material und Methoden
Seit der Umsetzung von Antibiotic Stewardship (ABS) an der Universitätsmedizin Goettingen (UMG) Anfang 2013 erhalten alle Patienten mit einer SAB (etwa 120 bis 140 pro Jahr) ein Konsil durch den klinischen Infektiologen des ABS-Teams. Ein standardisiertes Behandlungsprotokoll, das die empfohlene Antibiotika-Therapie, die Therapiedauer und weitere durchzuführende diagnostische Maßnahmen enthält, soll dabei den behandelnden Arzt auf Station in der Betreuung des Patienten mit SAB unterstützen.
Ergebnisse
Von den prospektiv seit 2013 konsultierten Patienten sind bisher 92 (36 weibliche und 56 männliche Patienten) vollständig in die Datenbank aufgenommen. Das Medianalter der weiblichen Patienten beträgt 73 Jahre (Range 0-89; Mean 69,3 Jahre). Das Medianalter der männlichen Patienten beträgt 64 Jahre (Range 0-90; Mean 62,0 Jahre).
Die SAB wurde erworben: ambulant 12,0% (11/92), durch ambulante Gesundheitsversorgung (Dialyse / Chemotherapie) 5,4% (5/92), im Pflegeheim 2,2% (2/92), durch stationären Aufenthalt in den letzten 90 Tagen für >48 Stunden 18,5% (17/92), nosokomial UMG 48,9% (45/92), nosokomial anderes Krankenhaus 7,6% (7/92), Rezidiv einer SAB >4 Wochen <3 Monaten 1,1% (1/92), Rezidiv einer SAB >3 Monaten 4,3% (4/92).
Als Ursache der SAB fanden sich: kein Fokus 9,8% (9/92), Besiedlung mit *S. aureus* 6,5% (6/92), infizierte periphere Venenverweilkanüle 15,2% (14/92), infizierter zentraler Venenkatheter 21,7% (20/92), Pneumonie 15,2% (14/92), Knochen-/Gelenkinfektion 10,9% (10/92), infiziertes Fremdmaterial 3,3% (3/92), Haut-/Weichteilinfektion 10,9% (10/92), Endokarditis 5,4% (5/92), spontan-bakterielle Peritonitis 1,1% (1/92).
Die 30-Tage-Gesamtmortalität betrug bei den weiblichen Patienten 47,2% (17/36) und bei den männlichen 26,7% (15/56).
Schlussfolgerung
Am häufigsten wird die SAB an der UMG nosokomial erworben, vor allem durch infizierte zentrale Venenkatheter und periphere Venenverweilkanülen. Die 30-Tage-Gesamtmortalität ist hoch, mit einem Trend zu einer höheren Sterblichkeit bei weiblichen Patienten. Die Auswertung weiterer Patienten wird zeigen, ob sich dieser Trend fortsetzt.

Presentation: Monday, 12 September 2016 from 10:30 – 11:30 in room Donauhalle.

POSTERSESSION 02
12 Sept. 2016 • 17.00-18.00

Infection Prevention and Antibiotic Resistance (FG PR)

167/PRP

Results of self-reporting survey of physicians about the behavior to prescribe antibiotics in 7 hospitals 2014 and comparison to data from 2009

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Introduction: The importance of multiresistant gram-negative bacteria as the cause of life-threatening infections is increasing and the development of new antibiotics is not in sight. Hence, antibiotic stewardship activities are of foremost importance.
Objective: To compare knowledges of physicians in antibiotic prescribing between 2009 throughout Germany and 2014 in 7 German hospitals belonging to the mdro-network.
Method: A questionnaire was sent to all 380 employed physicians of 7 hospitals in 2014. Primary, secondary and tertiary care hospitals were included. The following items were investigated: demographic data, position in the hospital, certified specialization, presence of antibiotic prescription rules and behavior to follow them. 119 received questionnaires of 2014 were compared to 3613 obtained in 2009.
Result: 119 of 380 (31%) questionnaires were returned (Secondary care hospital (42.9%), tertiary care 29.4% and primary care 26.9%). 64 (53.8%) were interns. The half of them had a certified specialization, the other half was still in training. 39 (32.8%) were consultants and 15 (12.6%) were chief physicians. 22.6% were internists, 18.4% surgeons, 10.9% urologists and 9.2% anaesthetists. The majority prescribed antibiotics on a daily routine, even among the interns 66%. Chief physicians (75%) and consultants (65%) advice antibiotic therapy every day. 25 % of interns reported they would advise colleagues daily and 65% at least once a week. Indications for antibiotic use were antibiotic prophylaxis in surgery (35), urinary tract infection (32) and pneumonia (29). Standard duration of antibiotic therapy reported were 7 days for pneumonia, 5 days for urinary tract infections and 1 day for antibiotic prophylaxis in surgery, while 50% of surgeons widened the antibiotic prophylaxis to 3, 5, 7,8 and 10 days.
93.3% physicians reported the use of therapeutic guidelines depending on availability either from their own hospital or from medical societies. 95% of all answers declare that they estimate the importance of antimicrobial resistances for their work as high. Only 59.3% were provided with the antimicrobial resistances data of their hospital. 52.1 % would not ask a consultant for advice before prescribing a reserve antibiotic.
In comparison to 2009 lacking knowledge about antimicrobial resistances, the use of guidelines, and consultation of a consultant improved about 5%-points each.
Conclusion: The survey detected obvious weaknesses in the prescribing of antibiotics in hospitals. Compared with the answers from 2009 in 2014 a slight improvement was shown for the knowledges of guidelines and the use of consultation of more experienced colleagues. However, interns advice themselves within their groups and the half of all surgeons still use perioperative prophylaxis longer than 24 h. Still 38.9% of all physicians asked did not know their regional antibiotic resistant data.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

168/PRP

Comparison of PCR systems for identification of Methicillin-susceptible and Methicillin-resistant Staphylococcus aureus carriers to prevent Surgical Site Infections

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Introduction: Even though surgical site infections (SSI) are the second most common hospital-acquired infections, there is growing evidence that the number of SSI with methicillin-resistant Staphylococcus aureus (MRSA) or methicillin-susceptible S. aureus (MSSA) may be reduced by preoperative screening and decolonization of nasal carriers.
Objective: Comparison of cobas ® MRSA/SA test, BD MAX StaphSR and RIDA ® GENE MRSA assay on nose/throat samples under routine surveillance conditions in order to select the most accurate as well as cost and time effective PCR test in a small scale laboratory.
Materials and Methods: Patients newly admitted to a university hospital in Cologne, Germany, were screened via MSwabs (Copan) consisting of a flocked swab and transport media. While processing the specimen a new version of the RIDA ® GENE MRSA assay was released. Residual eluates from the first passing were therefore used for a second testing with the new version. Consequently PCR testing was performed with a total four PCR tests and compared to a microbiological gold standard method.
For microbiological testing a fraction of the swab media was plated onto a half plate of blood agar, CHROMAgar MRSA II (BD), Mannitol salt agar and compounded into Tryptic soy broth (TSB) for enrichment. TSB was incubated for 24 hours and then plated onto agar plates. The assays were performed with the bigger amount of the media according to the instructions by the manufacturers.
Result: A total of 1016 patients were enrolled. Out of these 958 samples were analysed with the new RIDA ® GENE MRSA assay. According to the gold standard MSSA was detected in 293 (28.8 %) patients whereas MRSA was detected in 19 (1.87 %) patients.
The highest sensitivity for MRSA was obtained with the cobas ® MRSA/SA test (78.9%) and for MSSA with the BD MAX StaphSR assay (83.6%). In all assays the specificity as well as the negative predictive values was more accurate for MRSA than MSSA. However, while the positive predictive values (PPV) for MRSA varied between 22 - 66%, the BD MAX StaphSR demonstrated the highest PPV for MSSA with 83.2%. The processing time of the assays varied between 100 - 190 min for a minimal approach.
The cobas ® MRSA/SA test and BD MAX StaphSR assay performed similarly in regard to their accuracy values. However, when considering the turnaround time, the RIDA ® GENE MRSA assay was more efficient, despite its lower accuracy values.
Summary: PCR techniques are able to quickly and accurately determine the MRSA and MSSA carrier status of a patient contributing to epidemiologic and economic benefits. Surprisingly, the accuracy values of the evaluated multiplex PCR tests varied significantly. Yet one should take into consideration if the most accurate assay might always be the ideal choice especially when the turnaround time plays a vital role in reaching the overall best clinical outcome.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

169/PRP

Characterization of *P. aeruginosa* strains from tap water and patients in Tanzanian hospitals reveals only a single possible transmission, but a high rate of colistin resistance in water isolates.

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Introduction: Information regarding the role played by hospital water systems in transmission of *P. aeruginosa* (P.a.) causing healthcare-associated wound infections in low-income countries is limited.
Objective: To characterize the P. a. isolates from water and patients in order to establish the role of water systems in transmission.
Materials and Methods: Between Dec 2014 and Aug 2015, water samples were collected from regional and tertiary hospitals on monthly bases. In the same time period, rectal and wound swabs were taken from patients admitted for surgery. Malachite Green Broth enrichment was employed. VITEK®-MS and VITEK®-2 were used for identification and resistance testing, respectively. Typing of P. a. was done by MLST.
Result: Over an observation period of 9 months 930 patients underwent surgery, of whom 8 (0.9%) developed a P.a. wound infection. Three of the latter harbored the same strain in their intestines. None of the 8 wound infections were caused by P.a. strains with STs identified in tap water isolates. Carriage rate on admission and discharge was 1.2%. There was only one strain isolated upon admission sharing an ST with a strain from tap water. In regional and tertiary hospitals, 7 of 16 (44%) and 21 of 26 (81%) sampling points respectively, were positive for P.a. More than 50% of the 39 P.a. strains from water samples were ST 381. P.a. isolates from water systems were significantly more resistant to colistin (17.9% vs. 0.0%, p= 0.031) and fosfomycin (61.5% vs. 21.7%, p= 0.002), while isolates from patients were more resistant to piperacillin/tazobactam (34.7% vs. 2.6%, p= 0.001), ceftazidime (26.1% vs. 0.0%, p= 0.002) and meropenem (13.0% vs. 0.0%, p= 0.042).
Conclusion: The P.a. wound infection rate in surgical patients was less than 1%. There was no evidence for transmission from tap water. Although endogenous surgical wound infection was observed, the carriage rates in this setting are too low to demand pre-admission screening. Colistin resistance in environmental isolates is of concern. Carbapenem resistance rates in clinical isolates need to be followed in the future.

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170/PRP

Retrospective analysis addressing the emergence of the plasmid encoded colistin resistance gene *mcr-1* in German pig-fattening farms during the years 2011-2013

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Introduction: In November 2015 the emergence of the first plasmid-encoded colistin resistance gene *mcr-1* was detected in animals as well as human beings in China. Within the last couple of months, a multitude of further studies was performed, indicating a global spread of the plasmid encoded resistance gene, including Germany. As colistin is one of the last remaining substances for the treatment of serious infections caused by multidrug-resistant bacteria, a major concern for the public health was enunciated. To evaluate the *mcr-1* occurrence on farm level, bacterial cultures sampled and stored on pig-fattening farms during the years 2011 to 2013 in terms of the RESET project (www.reset-verbund.de), were systematically screened for the presence of this gene.
Materials and Methods: More than 400 primarily cultures deriving from pooled faces and boot swab samples, collected in a cross-sectional study including 58 pig-fattening farms throughout Germany were investigated by PCR. Subsequently single *mcr-1* positive *E. coli* colonies were isolated using MacConkey agar plates containing 2 µg/ml colistin. The received isolates are currently further investigated by MIC testing, determination of additional antibiotic resistance genes, phylo-typing, PFGE as well as plasmid transformation.
Results and Conclusions: Presence of the *mcr-1* gene was confirmed in 44 *E. coli* isolates deriving from 15 pig-fattening farms, indicating that the *mcr-1* gene was present in 26% of the investigated farms within the years 2011 to 2013. All of the tested isolates showed a colistin MIC value of 4 - 8 µg/ml and also provided resistance against several additional antibiotic substances, including cephalosporins and/or fluoroquinolones. With regard to the previously published findings, the here described results may support the already enunciated concern for the public health. Therefore, the current situation has to be assessed critically and ongoing and further monitoring programmes are highly recommended.

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171/PRP

Retrospective analysis of bacterial cultures sampled in chicken-fattening farms during the years 2011-2013 revealed two additional VIM-1 positive isolates

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Introduction: Carbapenems are considered as one of the last therapeutic options for the treatment of human infections caused by multidrug-resistant gram-negative bacteria. Therefore, the increasing spread of carbapenem resistance among Enterobacteriaceae has to be observed critically. Even if the last couple of years have shown, that increasing numbers of carbapenemases have been isolated from cases of human infections, their occurrence in livestock and livestock associated surroundings is still rarely reported. However, in 2013 the first VIM-1 producing *Salmonella* Infantis was isolated from dust, sampled in a German chicken-fattening farm. In the present study we investigated primarily stored bacterial cultures isolated in 45 chicken-fattening farms during the years 2011-2013.
Materials and Methods: More than 500 primarily stored bacterial cultures, originally deriving from pooled feces, boot swabs as well as dust samples, collected in a cross-sectional study including 45 chicken-fattening farms throughout Germany were selected on MacConkey agar plates containing 0.125 µg/ml meropenem. *E.*

coli as well as *Salmonella* isolates that were able to grow on these plates, were transferred to chromID™ CARBA plates (BioMerieux, France) and the presence of carbapenemase genes was tested by real-time PCR. The received isolates are currently further investigated by using different phenotypic- as well as genotypic approaches and whole genome sequencing.

Results and Conclusions: Beside the already by Fischer et al., 2013 described *Salmonella* Infantis strain (R3), one additional *Salmonella* subspecies I (rough phenotype; G-336-1a) as well as two *E. coli* isolates (G-336-2; G-268-2) were isolated. In the disc diffusion assay G-336-1a as well as G-336-2 possessed a resistant phenotype against imipenem, meropenem and ertapenem while G-268-2 showed resistance against ertapenem and an intermediate resistance level against meropenem. The subsequently performed real-time PCR indicated the presence of a $bla_{\text{VIM-1}}$ gene in G-336-1a as well as in G-336-2. Both isolates derived from a dust sampled taken on a different farm than the previously described isolate R3. In contrast to the findings described by Fischer et al., 2013, in *Salmonella* and *E. coli* the $bla_{\text{VIM-1}}$ was located on a plasmid of the same size (~ 300 kb). For G-268-2 no carbapenemase gene has been detected. Further analyses as well as comparing studies between the two VIM-1 producing Salmonella strains deriving from two different farms will certainly give more information about the serious problem of the occurrence of CPE within German livestock-farms.

Even if the detection rate of carbapenem-resistant *E. coli* and *Salmonella* was scarce, the topic is of utmost importance and a further spread of such isolates has to be avoided by all means. Therefore, ongoing and thorough investigations of the current situation in livestock farms are highly recommended.

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172/PRP

Notifications of severe cases of Clostridium difficile infections (sCDI) in North Rhine-Westphalia (NRW)

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In Germany in November 2007 sCDI has been classified as threatening disease with evidence of serious danger to the community. These cases are notifiable according to Infection Protection Act.

At federal state level, focused on sCDI-criteria, we analysed the sCDI notifications in order to characterize the sCDI epidemiology in NRW.

We used the sCDI-notifications of the 53 local health departments submitted to NRW Centre for Health from 2008 to 2015. The CDAD Reference data 2014 of German Reference Centre for Surveillance of nosocomial Infections (http://www.nrz-hygiene.de/en/surveillance/hospital-infection-surveillance-system/cdad-kiss/) were used to estimate possible underreporting. The number of hospitals and patient-days in NRW in 2014 were taken from NRW hospital statistics (https://www.destatis.de/DE/Publikationen/Thematisch/Gesundheit/Krankenhaeuser/GrunddatenKrankenhaeuser.html).

In NRW the number of notified sCDI cases was 61 in 2008 and 2009, 73 in 2010, 145 in 2011, 360 in 2012, 469 in 2013, 710 in 2014 and 1.094 in 2015. Extrapolating from data of 359 hospitals, which took part in the German CDI surveillance in 2014, we estimate for the 364 hospitals in NRW covering 33.418.000 patient-days in 2014 about 1.135 sCDI-cases. The 710 sCDI cases, submitted to the NRW Centre for Health in 2014, may indicate underreporting.

In 2015 in NRW in 30.6% of sCDI cases a detection of ribotype O27 is specified, in 26.0% a CDI-relapse, in 24.7% CDI-death, in 15.4% a transfer to ICU and in 3.3% surgery. Out of the 1.094 sCDI cases, notified in 2015 in NRW, 283 (25.9%) were notified because of detection of ribotype O27 only. In 153 notified sCDI cases (14.0%) additionally to detection of ribotype O27 just one more criteria is indicated: in 90 times a CDI-relapse (58.8%); 34 times death (22.2%); 25 times transfer to ICU (16.3%); 4 times surgery (2.6%).

The complete registration of sCDI is still a challenge. National surveillance data can be used to estimate regional underreporting. The increasing trend of sCDI notifications may indicate a decrease in underreporting. The high percentage of notified sCDI cases with detection of ribotype O27 only may indicate that O27 CDI not always implies a severe course. In 2016 the frequent notification criteria "CDI-relapse" of German Infection protection Act was eliminated and a new criteria "in-hospital-treatment of community acquired CDI" was introduced which may be useful to describe the sCDI epidemiology also in outpatients.

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173/PRP

Novel subclass B3 metallo-β-lactamase family LMB detected in *Enterobacter cloacae*

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Introduction: Metallo-β-lactamase are β-lactamases that require zinc as a cofactor. Metallo-β-lactamases confer resistance to almost all β-lactam antibiotics including carbapenems. No efficient inhibitors for metallo-β-lactamases are available for clinical application, expression of metallo-β-lactamase strongly diminishes treatment options for bacterial infections.

Method: Carbapenemase activity was detected by the modified Hodge test, combined disk test with boronic acid or EDTA and a bioassay based on cell-free extracts. Shotgun-cloning experiments were performed. The isolate was then subjected to whole-genome sequencing by MiSeq 2x300 bp paired-end sequencing. The SPAdes-assembler was used for draft-genome assembly. The draft-genome was then screened for sequences homologous to known metallo-β-lactamase sequences using tblastn. Homologous regions were screened for in-frame start-codons and likely promoter-regions. Suspicious sequences were amplified by PCR and cloned in the vector pBK-CMV. After transformation in *Escherichia coli* Top 10, comparative susceptiblity studies were performed by disk diffusion, Etest and microdilution with the strain carrying the putative metallo-β-lactamase-gene and *E. coli* Top 10 carrying the pBK-CMV vector without an insert.

Result: *E. cloacae* strain 10170 was isolated in 2013 from a rectal swab of an inpatient in an Austrian hospital. The strain was resistant to carbapenems, synergy with EDTA could be demonstrated and the modified Hodge test was positive. Detection of known metallo-β-lactamase-genes by PCR failed and the shotgun-cloning experiments did not yield any results.

In the draft-genome obtained by whole-genome sequencing, a sequence showing low homology to known subclass B3 metallo-β-lactamases was detected. After cloning the putative metallo-β-lactamase coding gene, synergy with EDTA could be demonstrated and comparative susceptibility analysis showed increased MICs for carbapenems. The novel metallo-β-lactamase was named Linz Metallo-β-lactamase (LMB-1).

Discussion: In this report, we describe the novel metallo-β-lactamase LMB-1. This study highlights the importance of an ongoing surveillance for new resistance mechanisms which will be overlooked by PCRs targeting only the most frequent resistance genes.

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174/PRP

TiO_2 and ZnO containing borosilicate glass - a novel thin glass with exceptional anti-biofilm performances to prevent microfouling

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Question: Biofilm formation, also known as microfouling, on indwelling medical devices such as catheters or prosthetic joints causes difficult to treat and recurrent infections. It is also the initial step for biocorrosion of surfaces in aquatic environments. An efficient prevention of microfouling is preferable but the development of anti-biofilm surfaces is enormously challenging. The aim of our study is to develop a surface that prevents biofilm formation of different pathogenic bacteria.
Method: Soda-lime-, alumino-silicate and three borosilicate glasses with different compositions were investigated for their feasibility to prevent biofilm formation by standardized in vitro biofilm assays using different pathogen bacteria. Furthermore, the biocompatibility of these glasses was evaluated using eukaryotic cell lines end erythrocytes.
Result: Only two borosilicate glasses, containing TiO_2 and ZnO, showed anti-biofilm properties inhibiting biofilm adhesion and formation. The biofilm thickness and area were significantly reduced by more than 90 %. All tested glass types showed neither cytotoxicity nor hemotoxicity.
Conclusion: The borosilicate-thin glasses might be suitable for surface coatings of rigid medical implants devices reducing to prevent biofilm associated infections.

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175/PRP

The carbapenemase New Delhi metallo-β-lactamase (NDM): emergence in pan-drug resistant isolates and impact on virulence and fitness

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Introduction: The emergence of pan drug-resistant, virulent Enterobacteriaceae represents a serious public health threat and can result in untreatable infections. Enterobacteriaceae carrying the carbapenemase New Delhi metallo-β-lactamase (NDM)-1 are multi-drug resistant. The gene encoding NDM-1, *bla*$_{NDM-1}$, is frequently located on plasmids and can thereby be transmitted via horizontal gene transfer. Due to the rapid dissemination it has been hypothesized that NDM-1 does not only confer antibiotic resistance but also increases bacterial virulence and/or fitness.
Aim: The objective of this study was to characterize a multidrug-resistant *Serratia marcescens* clinical isolate harbouring NDM-1 and to analyze if *bla*$_{NDM-1}$ acquisition increases fitness or virulence.
Materials and Methods: Horizontal gene transfer of *bla*$_{NDM-1}$ carrying plasmids from a pan drug-resistant *S. marcescens* clinical isolate was evaluated by liquid mating employing sodium azide-resistant *Escherichia coli* J53 and *Klebsiella pneumoniae* PRZ as recipients. Cytotoxicity to human cells was assessed *in vitro* using A549 lung epithelial cells via the lactate dehydrogenase (LDH) assay. *In vivo* virulence was investigated using larvae of the Greater wax moth (*Galleria mellonella*). Fitness of isogenic strains was analyzed in non-competitive and competitive growth kinetics.
Result: The *bla*$_{NDM-1}$ carrying *S. marcescens* clinical isolate was non-susceptible to all tested antibiotics and thus categorized as pan drug-resistant. *Bla*$_{NDM-1}$ was located on a 140 kb IncA/C type plasmid (pNDM-1) which was transferable to *E. coli* J53 and *K. pneumoniae* PRZ by conjugation with a high frequency of up to 50%. Investigation of the impact of NDM-1 on virulence did not reveal relevant differences since cytotoxicity in A549 cells as well as survival of *G. mellonella* larvae infected with NDM-1 expressing *E. coli* and *K. pneumoniae* strains and isogenic NDM-negative controls was similar. Likewise, non-competitive growth between NDM-1 expressing strains and isogenic controls did not reveal relevant differences in their growth kinetics. In contrast, a significant reduction of fitness in pNDM-1 carrying strains was observed for both *E. coli* J53 and *K. pneumoniae* PRZ. In addition, transformation of *E. coli* TOP10 with an expression vector carrying solely the *bla*$_{NDM-1}$ open reading frame resulted in a fitness loss.
Summary: Carriage of *bla*$_{NDM-1}$ did not increase virulence *in vitro* or *in vivo* but significantly decreased the fitness of isogenic *E.coli* or *K. pneumoniae* strains without *bla*$_{NDM-1}$. Thus, it is likely that efficient horizontal gene transfer has a bigger impact on dissemination of NDM-1 than fitness or virulence benefits.

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176/PRP

Expression of the *Acinetobacter baumannii* porins CarO, 33-36 kDa and OprD-like in response to different physiological conditions

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Introduction: A wide array of resistance mechanisms has been described in *Acinetobacter baumannii* including decreased antibiotic influx through the outer membrane of the bacterial cell. For example reduced expression of outer membrane proteins (OMPs) CarO, 33-36 kDa porin or OprD-like has been associated with carbapenem-resistance in *A. baumannii*.
Objective: The objective of this study was to investigate in *A. baumannii* ATCC 17978 the expression of the porins CarO, 33-36 kDa and OprD-like in response to different growth modes (planktonic, sessile, biofilm, motile) and different physiological conditions.
Materials and Methods: Expression of the porin-encoding genes was investigated using two different reporter systems. A GFP reporter plasmid was constructed for monitoring gene expression in *A. baumannii* ATCC 17978 biofilm bacterial cells under static and agitated conditions. Expression of CarO, 33-36 kDa and OprD-like porins in planktonic cells in response to different conditions (sodium salicylate, temperature (30ºC, 37ºC, 42ºC), pH, osmotic pressure), and in sessile and motile bacterial cells was determined by β-galactosidase reporter assay.

Result: CarO, 33-36 kDa and OprD-like were expressed in planktonic, sessile and biofilm cells, although expression levels were found to vary among porins; CarO and 33-36 kDa were expressed at high levels whereas OprD-like was expressed at low levels. OMP expression in *A. baumanniii* was unchanged by the physiological conditions tested here. In addition, it was found that motility inhibits expression of the OprD-like porin while CarO and 33-36 kDa porins are expressed in migrating cells.
Conclusion: The approach used in this study to investigate expression of porins under different conditions displays evidence that they are all constitutively expressed in *A. baumannii* ATCC 17978 when growing as planktonic, sessile and biofilm cells. We also found evidence for differential expression of OprD-like which was not expressed when cells were motile. Given that loss of these porins is associated with carbapenem-resistance it has to be determined if their incorporation in the outer membrane or secretion may be regulated under these conditions.

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177/PRP
Cold plasma kills multidrug resistant bacteria - Validation of a new wound healing patch
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Introduction: The emergence and global spread of multidrug resistant bacteria are of great concern to health services worldwide. Infections caused by these bacteria have limited treatment options. Cold atmospheric pressure plasma has become an interesting treatment option for chronic wounds, burn wounds, after surgery and wound care. In addition of wound healing effects cold plasma has antimicrobial activity which is associated to the direct oxidative effects of reactive oxygen (O^-, O2, O3, OH^-) and nitrogen (NO^-, NO_2) species on the outer surface of microbial cells. Physical treatment with tissue-tolerable plasma may be a promising alternative to antimicrobial therapy or chemical antiseptics.
Aim: Validation of the antimicrobial effect of a new development using cold atmospheric plasma which is under admission for medical devices.
Materials and Methods: The device is an active wound dressing (COLDPLASMATECH GmbH) based on the principle dielectric barrier discharge (DBD). We tested the cold atmospheric plasma susceptibility of clinical, multidrug resistant strains and other species that are also able to cause wound infections: *Escherichia coli* (KPC-2, MCR-1), *Klebsiella pneumoniae* (OXA-48), *Acinetobacter baumannii* (OXA-23), *Pseudomonas aeruginosa* (VIM-2), *Staphylococcus aureus* (PBP2a) *Staphylococcus lugdunensis* (DSM4804), *Streptococcus pyogenes*, *Enterococcus faecium* (VanA), *Candida albicans* (DSM 11948).
Cell suspensions of different cell counts were plated on CLED agar or Müller Hinton Blood agar and exposed to cold atmospheric plasma using the new plasma patch device for 10, 60 or 180 seconds. The plates were incubated for 24 h and the colony-forming units (cfu) that survived plasma exposition were counted. Plates that were not plasma treated were used to calculate the $\log_{10}$ level reduction.
Result: *S. lugdunensis* was most and *C. albicans* was least susceptible to cold plasma. A plasma exposition of 10 seconds reduced the microbial load by at least 4 $\log_{10}$ levels of *C. albicans* and up to 6 $\log_{10}$ levels in *S. lugdunensis*. A plasma exposition of 180 seconds expended the reduction of cfu by one $\log_{10}$ level in *C. albicans* (5 $\log_{10}$ levels) and two $\log_{10}$ levels in *S. lugdunensis* (8 $\log_{10}$ levels= sterile plates). All bacterial strains were reduced by around 6 to 7 $\log_{10}$ levels in 60 seconds of plasma treatment.
Conclusion: The antimicrobial activity of the new developed plasma patch was confirmed and the observed cell count reduction was both dosage dependent and species dependent. The vast bulk of cells were killed in the first 10 seconds of plasma exposition. The expansion of plasma exposition time to 60 and 180 seconds led to further reduction of viable cells up to sterilization of a plate that was inoculated with 10^8 cells. Cold plasma treatment using the new device has the potential to be a serious alternative to antimicrobial therapy and chemical antiseptics in wound sterilization, wound care and in therapy of burn and chronic wound infections in the near future.

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Eukaryotic Pathogens (FG EK)

178/EKP
A seven-helix protein is crucial for malaria transmission from the human to the mosquito
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Background: The complex life-cycle of the human malaria parasite *Plasmodium falciparum* requires a high degree of tight coordination allowing the parasite to adapt to changing environments. One of the major challenges is the rapid stage conversion of sexual precursor cells, the gametocytes, into gametes, which takes place immediately after parasite transmission from the human to the mosquito. Some components of the signaling pathway leading to the rapid onset of gametogenesis of *P. falciparum* have meanwhile been identified and hint to the involvement of seven-helix proteins.
Objective: In this study we aim to functionally characterize a seven-helix protein of *P. falciparum*, termed 7-Helix-1, and reveal its role for gametocytes in order to help deciphering the signaling pathway leading to gametogenesis.
Method: We performed an expression analysis via semi-quantitative reverse transcriptase PCR, Western blotting and immunofluorescence assays using polyclonal mouse antisera. We further generated a parasite line lacking 7-Helix-1 (7-Helix-1KO) via single-crossover homologous recombination and performed a microscopy-based phenotypical analysis with particular focus on the formation of gametes. Differences in transcript expression between wild-type and 7-Helix-1KO parasites at 30 min post-activation were studied using microarray analyses.
Result: We demonstrate that 7-Helix-1 is restricted to female gametocytes. In the developing macrogametocytes, 7-Helix-1 localizes in granular structures, while expression ceases after gametogenesis. 7-Helix-1KO parasites form significantly reduced numbers of emerged macrogametes *in vitro*, resulting in impaired transmission of 7-Helix-1KO parasites to mosquitoes. Microarray analyses revealed that the loss of 7-Helix-1 leads to an altered expression of genes related to translation in activated gametocytes, among these an up-regulation of elongation factor eEF1β.
Conclusion: Our combined data point to an important role of 7-Helix-1 in translational activation during the formation of macrogametes. This is one of the first reports on a putative seven-helix protein in *P. falciparum*.

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179/EKP

Candida albicans counteracts the Dectin-mediated antifungal host response

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Introduction: The human organism uses pattern-recognition-receptors (PRRs) as first sensors to recognize invading microbes and to develop a protective immune response. These PRRs recognize conserved microbial- and pathogen-associated-molecular-patterns (MAMPs and PAMPs), which are shared by a broad range of microbes. Human dendritic cells express different types of PRRs, including the C-type-lectin-Receptors Dectin-1 and Dectin-2. Dectin-1 and Dectin-2 are central for the recognition of fungal cell wall proteins, like β-glucans, mannoproteins and chitin. Dectin-1 binds fungal β-glucans and Dectin-2 binds mannan on the fungal hyphae. *C. albicans*, as the most abundant candida species in humans, can colonize skin and mucosa as a harmless commensal, but can also cause severe systemic infections. The development of antifungal drugs or therapies is so far only partially successful. Therefore it is important to study the interaction between *C. albicans* and the human innate immune system. In order to identify and characterize central fungal targets relevant for immune interference and to understand the molecular mechanisms of fungal counterstrike.
Aim: Our aim is to understand how *C. albicans* evades the recognition via Dectin-2 and to define the general features of microbial counter response to Dectin-2 mediated pattern recognition. We also aim to characterize how the candida immune evasion protein CRASP11, which binds to human dendritic cells via Dectin-2, influences dendritic cell function.
Method: ELISA, Immunoprecipitation, Western Blot, Biolayer Interferometry, Flow Cytometry, Confocal Microscopy
Result: We have identified a new candida protein, i.e. CRASP11, which binds to human Dectin-2 and modulates dendritic cell function. CRASP11 upon binding to Dectin-2 on dendritic cells induces production of anti-inflammatory IL-10 and blocks the secretion of pro-inflammatory TNF-α and IFN-γ. In addition CRASP11 blocks LPS induced activation and maturation of human dendritic cells and down regulates the expression of the maturation markers CD80, CD83 and CD86.
Conclusion: *Candida albicans* counteracts dendritic cell pattern recognition by secretion of CRASP11. CRASP11 modulates the inflammatory response, down-regulates the expression of pro-inflammatory cytokines and induces anti-inflammatory IL-10 and an immunosuppressive phenotype in dendritic cells.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

180/EKP

Interactions between *Candida albicans* and intestinal bacteria affect damage of enterocytes

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The opportunistic fungal pathogen *Candida albicans* colonizes the gastro-intestinal tract of most humans asymptomatically. However, if the intestinal equilibrium of host, fungus and bacterial microbiota is disturbed, *C. albicans* can translocate into deeper tissue and cause disseminated infection. These infections are life-threatening and lead to high mortality rates. Disseminated candidiasis can also be accompanied by bacterial blood stream infections. Mixed infections are associated with altered morbidity and mortality, indicating differences in damage potential and immune response compared to the respective single-species infections.
Since the understanding of bacterial-fungal interactions in the human gut and its impact on dissemination and disease development are very limited, we investigated the interplay of *C. albicans* with gut-associated bacteria.
For this, we selected 25 gram-positive and gram-negative bacteria from different sepsis-relevant species, including *Escherichia coli* and *Pseudomonas aeruginosa*. Culture supernatants retrieved under different growth conditions were tested for their potential to inhibit *Candida* growth and filamentation. Simultaneously, the damage potential of *Candida*-bacteria coinfections was assessed using an epithelial layer composed of HT29-MTX and C2BBe1 cells.
We found that several of the bacterial species tested affected growth and filamentation of *C. albicans*. Importantly, the bacterial inhibitory potential towards *C. albicans* was not only species-, but also highly strain-dependent. For instance among spent media derived from 6 *E. coli* strains tested in an agar diffusion assay, two inhibited *Candida* growth (ATCC 25922, ATCC 11775) and only one reduced *Candida* filamentation (Nissle). In contrast, spent media of all 4 *P. aeruginosa* strains tested affected *Candida* growth, while only the supernatant of *P. aeruginosa* PAO1 interfered with hyphae formation.
Similarly, host cell damage during bacterial-fungal coinfections depended not only on the bacterial species, but also on the strain used. For both, *E. coli* and *P. aeruginosa*, we identified strains with synergistic effects on host damage and strains causing unaltered or even reduced LDH release in mixed infections. The strongest synergism was detected for the clinical isolate of *P. aeruginosa* (ATCC 27853).
Thus, our data indicates that fungal-bacterial interactions are complex and strain-dependent. In future experiments, we aim to dissect the mechanism underlying synergism or antagonism during mixed infections with *C. albicans* and develop a co-colonization model in mice to investigate the impact of the mammalian immune system to the interplay.

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181/EKP

New methods to measure killing of *Aspergillus* hyphae by neutrophil granulocytes

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Aspergillus fumigatus is an opportunistic fungal pathogen and the most frequent cause of invasive aspergillosis. This systemic and often fatal infection occurs in immunocompromised patients and typically originates from inhaled airborne *Aspergillus* spores. The spores subsequently germinate and produce an infiltrating fungal mycelium which causes local destruction and spreads within the human body. Studying the antimicrobial activity of innate immune cells often relies on killing assays that are optimized for single cell organisms such as yeasts and bacteria. However, *Aspergillus* produces hyphae which branch, form a mycelium and stick to surfaces. Assays to quantify fungal killing by plating a suspension of detached hyphae on agar plates and count the colony forming units are inadequate. We established two independent killing assays that allow the measurement of fungal killing based on microscopic evaluation of single hyphae and metabolic activity. The assays were subsequently validated and evaluated by screening a collection of *A. fumigatus* mutant strains. We identified several mutants which are more susceptible to killing by neutrophil granulocytes. Our results indicate the suitability of the killing assays and suggest a possible role of the identified genes in the virulence *A. fumigatus*.

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182/EKP

Candida albicans colonization of the murine gut alters the host immune response

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Candida albicans is an opportunistic fungal pathogen, which is found as a commensal on mucosal epithelia, especially the human gut. Under predisposing conditions *C. albicans* can translocate through the intestinal barrier into the bloodstream. Entering blood vessels, the fungus can disseminate into a variety of organs, leading to life-threatening disseminated infections.
Both colonization and dissemination are poorly understood to date. Therefore, the aim of this project is to characterize colonization and translocation with regard to the involved anatomical sites, cell populations and immune response and to analyze the role of selected *C. albicans* genes for these processes.
In order to determine at which anatomical sites translocation of *C. albicans* occurs and which host cells are involved, a murine *in vivo* colonization/dissemination model (according to Koh et al.; 2008) was established. Following antibiosis, mice were infected orally by gavage with selected mutant strains of *C. albicans.* Fungal burden and *C. albicans* morphology were analyzed in feces, different parts of the gut, mesenteric lymph nodes, liver, spleen, kidney and the brain. Flow cytometry was employed to determine the host response. *In vivo* or *ex vivo* bioluminescence imaging was performed to visualize sites of colonization.
Animals remained clinically healthy throughout the experiments. All fungal morphologies were found in the content and in the homogenates of the stomach, small intestine, cecum and colon at different time points after infection. While fecal burden remained relatively stable over the course of colonization, *in vivo* imaging revealed a rather dynamic behavior with changing intensity and localization of signals. *Ex vivo* imaging of the gastrointestinal tract revealed distinct foci with bioluminescent signals. By extracting these foci for flow cytometry analysis and comparison to control animals, a distinct immune response of the host was observed upon colonization. Neither deletion of *C. albicans EED1* (essential for hyphal maintenance) nor *ECE1* (important for cell damage) affected colonization levels.
Thus, *C. albicans* persistently colonized all parts of the murine gut in stable numbers during antibiosis, independent of its ability to form elongated hyphae and damage potential. Colonization alone was sufficient to affect the immune cell populations within the gut. Bioluminescence revealed foci with increased signal intensity. Whether these foci indicate sites of increased fungal density and/or focal invasion is currently under investigation.

Reference

[1] Koh, A.Y., Köhler, J.R., Coggshall, K.T., Van Rooijen, N., and Pier, G.B. (2008). Mucosal Damage and Neutropenia Are Required for Candida albicans Dissemination. PLoS Pathogens *4*, e35.

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183/EKP

Impact of host cell carbohydrate metabolism on *Toxoplasma gondii* bradyzoite formation in skeletal muscle cells

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Toxoplasma gondii is an obligate intracellular protozoan parasite that establishes chronic infection in warm-blooded animals including humans. Parasite persistence during chronic infection requires conversion of fast replicating tachyzoites to slowly replicating bradyzoites preferentially in neurons or skeletal muscle cells (SkMCs). The factors that trigger this stage conversion are only partially known. We have shown previously that terminally differentiated SkMCs, i.e. myotubes but not their proliferating precursors, i.e. myoblasts promote *T. gondii* bradyzoite formation. In this study, we have established that myoblasts and myotubes clearly differ in expression of carbohydrate metabolic enzymes and their activities. RNAseq revealed that infected and non-infected myotubes express significantly more pyruvate carboxylase (PC) and glycogen synthase 1 mRNAs than myoblasts. Conversely, myoblasts expressed significantly more mRNAs of glucose-6P-dehydrogenase (G6PDH) X-linked and more strongly up-regulated G6PDH2 after infection with *T. gondii* than myotubes. After labeling with ^{13}C-glucose, GC-MS analysis showed an increased pentose phosphate pathway (PPP) activity in infected and non-infected myoblasts than in myotubes. In contrast, myotubes showed an increased fueling of the TCA cycle via an anaplerotic reaction as compared to myoblasts irrespective of infection. In order to functionally analyze these metabolic differences on *T. gondii* stage conversion, pharmacological inhibitors or metabolic analogues were employed. Inhibition of the PPP enzymes G6PDH and 6P-gluconate dehydrogenase accelerated *T. gondii* bradyzoite antigen (BAG) 1 expression particularly in myoblasts and in myotubes to a lesser extent. Furthermore, fueling of the TCA cycle using dimethyl-α-ketogluterate also increased bradyzoite formation in myoblasts whereas inhibition of anaplerosis of the TCA cycle slightly increased BAG1 expression in infected myotubes. Together, these data suggest that metabolomic characteristics of mature myotubes may trigger *T. gondii* bradyzoite formation and that inhibition of the PPP pathway or fueling of the TCA cycle can accelerate bradyzoite formation particularly in myoblasts.

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184/EKP

Functional characterization of the Lah protein of Aspergillus fumigatus.

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In Ascomycetes Woronin bodies are anchored at the septal pore to protect injured hyphae from excessive cytoplasmic bleeding. This organelle positioning is mediated by the giant protein Lah that connects Woronin bodies to an unknown receptor at the septal pore. We have recently shown that the C-terminal 1000 amino acids of Lah contain all information that is required for this positioning. We have used this as a starting point to define the minimal domain that is still correctly targeted. We found evidence that this domain comprises a transmembrane region and that the corresponding extracellular domain is required for the interaction

with the receptor protein at the septal pore. Live cell imaging revealed that the recruitment of this receptor is an early event during septation. In a Δrho4 mutant, which is deficient in septum formation, LahC is not recruited to the septum, but instead targeted to protein complexes present in the cytoplasmic membrane. We assume that these complexes are formed during an early stage of septation. Due to the lack of Rho4, septation is abrogated at a certain step resulting in the observed protein complexes. We have furthermore identified a protein that is required for the correct targeting of LahC and may directly interact with the LahC domain.

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Gastrointestinal Infections (FG GI)

185/GIP

Development of neutralizing human antibodies against Clostridium difficile toxins

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Question: The nosocomial pathogen Clostridium difficile is causing the C. difficile associated diarrhea (CDAD), a life-threatening disease. A key factor for the disease is the secretion of the toxins TcdA and TcdB. The neutralization of these toxins is a strategy for the treatment of CDAD.
Method: Using antibody phage - an in vitro technology - human antibodies were generated against different domains of TcdA and TcdB.
Result: These antibodies are neutralizing in vitro as single antibodies and show a synergistic effect when used in combination. The antibodies will be further characterized in a mouse model for potential future clinical development. The approach to combine antibodies against different domains may improve the clinical efficiency compared to bezlotoxumab which is currently in phase 3 studies.
Conclusion: Fully human antibodies neutralizing TcdA or TcdB were developed by phage display. These antibodies are potential lead candidates for future clinical development.

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186/GIP

Antibodies against *Clostridium difficile* - Differences between antibodies against recombinant antigens and neutralizing antibodies against conditioned media from ribotype 014 and 027

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Clostridium difficile infection (CDI) is caused by the presence of two major toxins, toxin A (TcdA, enterotoxin) and toxin B (TcdB, cytotoxin); however, pathogenesis of disease is more complex due to the presence of the binary toxin and other virulence factors (e.g. cell-wall protein, Cwp-84). Neutralizing antibodies against toxins may protect from disease; however, routine testing of antitoxin antibodies have not been established. In the present prospective observational study, we investigated antibody response of patient with CDI and healthy volunteers. Enzyme Immunoassay (EIA) to GDH, TcdA, TcdB and Cwp-84 and also with cytotoxicity neutralisation assay against conditioned media from two toxigenic isolates (ribotype 014 and 027) was performed. For a subgroup of patients also specific antibodies were investigated in stools (EIA).
Analysis of 147 samples detected higher anti-GDH antibodies rated in patients (72%) as compared in controls (30%) (p
Antibodies in stools (n=68) were detected against GDH (6%), Toxin A (18%), Toxin B (18%) and Cwp-84 (40%) by EIA. This antibody pattern was different to plasma antibodies which suggests a local antibody response and not a passive transmission. Again, the presence of specific antibodies could not differentiate between patients with mild or severe CDI.
In conclusion, the clinical role of *C. difficile* specific antibodies in human remains complex. We demonstrate that neutralizing antibodies could be strain specific and none of the various assays, including early follow-up analysis, could be used to predict patients with severe CDI.

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187/GIP

***In vivo* genome and methylome adaptation of *Helicobacter pylori* BCS 100 during an experimental human infection study**

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Helicobacter pylori has been shown to undergo rapid *in vivo* evolution. Very little is known about methylome and genome variation during the first stages of the infection, and particularly during the *in vivo* adaptation to the antrum and corpus of the stomach.
The aim of the present study is to investigate the degree of genome and methylome evolution of *H. pylori* isolated from the antrum and corpus of different infected volunteers during an experimental human infection study (Aebischer *et al*., Gut 2008).
We used the SMRT® sequencing technology to determine the genome sequences and methylomes of the *H. pylori* challenge strain BCS 100 and 20 reisolates from the stomach antrum and corpus of 10 different human volunteers infected with this strain.
The analysis yielded finished (circular, 1 contig) genome sequences for all strains. We observed 25 unique SNPs (which occurred in only one isolate), 3 pair-specific SNPs (affecting both isolates from the same individual) and a single recombination event. The average mutation rate was 5.17×10^{-6} mutations per site per year. Mutation rates were comparable in antrum and corpus isolates. Genes of interest will be investigated for evidence of niche-specific selection. Methylome analysis of the challenge strain and volunteer reisolates identified a total of 24 methylated motifs. Inter-strain variability in the methylomes was observed, which resulted from phase variation of two methyltransferase (MTase) genes. Four of the methylated motifs are described for the first time in the present study. Insertion mutagenesis enabled the assignment of two of these sites to novel MTase genes. The identification of the MTase genes responsible for the methylation of the two remaining novel motifs is currently under way.
We conclude that adaptation of *H. pylori* to different human individuals included substantial changes of the methylome whose functional relevance will need to be further elucidated.

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188/GIP

Multiplex Serology for the specific detection of antibodies to *Helicobacter bilis* and *hepaticus*

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Background: Seropositivity to *Helicobacter (H.*) *pylori* proteins Urease A, HP0305, HP0231 and OMP, but not to *H. pylori*-specific virulence factors CagA and VacA, was found to be associated with biliary tract cancers in humans. Infection with *H. bilis* and *H. hepaticus* was previously shown to be serologically associated with biliary tract cancer as well, however, cross-reacting antibodies might be the underlying cause of this observation.
Aim: Our aim is to develop multiplex serology for *H. bilis* and *H. hepaticus* to specifically assess whether these non-pylori *Helicobacter* species may also infect humans and are associated with biliary tract disease.
Method: Each seven *H. hepaticus* and *H. bilis* proteins were recombinantly expressed in fusion with Glutathione S-Transferase (GST) as antigens for fluorescent bead-based multiplex serology and applied in addition to 15 previously established *H. pylori* proteins. Sera of 307 laboratory mice which were tested for the presence of *Helicobacter spp.* DNA in feces by a new *Helicobacter* species-specific PCR were used for assay validation.
Result: Antibody responses to two of the seven *H. bilis* proteins identified *H. bilis*-DNA positive animals with high specificity (P167C: 93%, P167D: 87%) but rather low sensitivity (45% and 64%, respectively). Similarly, antibody responses to HH0713 identified *H. hepaticus*-DNA positive mice with high specificity (96%) but limited sensitivity (45%).
Conclusion: We identified three proteins that specifically detect antibody responses in *H. bilis* and *H. hepaticus* feces-positive mice. Adding these proteins to the *H. pylori* antigens in serological analyses enables exploration of the presence of different *Helicobacter spp.* in human biliary tract disease.

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189/GIP

Bacterial-derived cell-penetrating peptides deliver gentamicin to kill intracellular pathogens

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Commonly used antimicrobials show poor cellular uptake and often have limited access to intracellular targets, resulting in low antimicrobial activity against intracellular pathogens. An efficient delivery system to transport these drugs to the intracellular site of action is needed. Cell-penetrating peptides (CPPs) mediate the internalization of biologically active molecules into the cytoplasm. Here we characterized two CPPs, α1H and α2H, derived from the *Y. enterocolitica* YopM effector protein. These CPPs, as well as Tat, were used to deliver the antibiotic gentamicin to target intracellular bacteria. The YopM-derived CPPs penetrated different endothelial and epithelial cells to the same extent as Tat. CPPs were covalently conjugated to gentamicin and CPP-gentamicin conjugates were used to target infected cells to kill multiple intracellular Gram-negative pathogenic bacteria, such as *E. coli* K1 RS218, *Salmonella enterica* serovar Thyphimurium, and *Shigella flexneri*. Taken together, CPPs show great potential as delivery vehicles for antimicrobial agents and could contribute to the generation of new therapeutic tools to treat infectious diseases caused by intracellular pathogens.

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190/GIP

Identification of *Listeria monocytogenes* pathogenicity determinants by genome sequencing of outbreak isolates

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A cluster of 44 human *L. monocytogenes* serotype IVb isolates with identical PFGE profiles was detected by the binational German-Austrian consiliary laboratory for *L. monocytogenes* in spring 2015. All but one isolates originated from stool samples of young children (2-5 years) with non-invasive gastroenteritis. A notable exception was one clone isolated from a 72 year old patient suffering from invasive listeriosis. Epidemiological investigations identified rice pudding contaminated with high loads of *L. monocytogenes* as the most probable source of infection and isolates from this source had indeed the same PFGE type. Genome sequencing and core genome MLST using a scheme containing 1701 alleles revealed that the non-invasive isolates from young children formed a cluster together with all food isolates, whereas the isolate from the invasive listeriosis patient differed in 51 alleles. More detailed genome comparisons between one non-invasive and the invasive clone identified 180 sequence variations which included 36 non-silent changes. The contribution of selected genomic differences to listerial virulence, specifically invasion, intracellular replication, hemolytic potential, and secretion of virulence factors was further analyzed. This approach will be helpful to identify new genetic determinants required for *L. monocytogenes* virulence in the course of human infections.

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191/GIP

Diversity of clinical *Clostridium difficile* isolates in sporulation and growth capacities

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The spread of *Clostridium difficile* infections (CDI) in healthcare systems has become a major public health threat over the last years. Not only patient hospitalization time increased, an infection can also lead to severe health issues such as toxic megacolon or colectomy. Additionally, the treatment costs have become an increasing burden by adding costs of 464 million Euro per year (1). Transmission of *C. difficile* is primarily contributed by the formation of highly resistant spores that survive environmental conditions like desiccation, heat or chemicals. The aim of the study is to investigate 30 different *C. difficile* isolates for growth and sporulation, as well as the susceptibility to several antibiotics to

disclose factors that might contribute to the transmission and persistence of strains. Isolates with significant reduced or increased sporulation will be further investigated for adhesion and motility and the respective stool samples of CDAD patients will be determined for total spore titers.
Clinical *C. difficile* strains isolated from diarrheal stool samples of CDAD patients in Indonesia, Ghana and Germany were selected regarding the ribo- and toxinotype, origin and the respective phylogenetic clade. Growth and sporulation assays were performed in BHIS medium and colony forming units were determined on chromID™ *C. difficile* agar after 24 h of incubation. The susceptibility to antibiotics was performed using Etest® strips with standard methods. To investigate the motility of strains, a respective single colony was stab-inoculated in 0.3% BHIS agar and analyzed after 48 h of incubation. For analysis of cell adhesion, the OD_{595} was measured at different time points of incubation in BHIS (2).
All tested isolates were susceptible to vancomycin and metronidazole that are used in CDI therapy. The resistance of strains to erythromycin and moxifloxacin accounted for 45% and 26%, respectively, with a high rate of resistant strains especially in Germany. The ribotype 126 displayed a significant elongated cell-phenotype as determined by phase-contrast microscopy and fluorescence microscopy using a FM 4-64 membrane dye. Additionally, this ribotype lost its ability of motility, as described previously (3). One isolate does not belong to any published ribotype so far and revealed a significant delayed sporulation in comparison to the other investigated strains.
In conclusion, clinical isolates of *C. difficile* exhibit a surprising diversity of phenotypes concerning colony shape and colony forming units, as well as growth and pathogenicity.

[1] Grube et al., 2015. *Z. Gastroenterol.* 53, 391-397.
[2] Faulds-Pain et al., 2014. *Mol. Microbiol.* 94, 272-289.
[3] Stevenson et al., 2015. *Trends Microbiol.* 23, 275-282.

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192/GIP

Accessory genes of the *Helicobacter pylori* Cag type IV secretion system influence levels of the pilus assembly factors CagH, CagI and CagL

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Most strains of the human gastric pathogen *Helicobacter pylori*, which infects approximately 50% of the world population, harbour the cytotoxin-associated gene (*cag*) pathogenicity island, a major virulence determinant and risk factor for development of gastric adenocarcinoma. The *cag* pathogenicity island encodes 19 functional components of a type IV secretion system, as well as the translocated effector protein CagA. Apart from these essential proteins, several accessory genes have been found to support type IV secretion-associated functions to different degrees, although their functional role has not been elucidated so far. Furthermore, the *cag* pathogenicity island contains six non-essential short open reading frames with as yet unknown functions. Here, we show that one of these short open reading frames, *cagP*, and one accessory gene, *cagG*, influence type IV secretion function in a similar manner.
Using an isogenic deletion mutant of *H. pylori* strain P12, we found that the *cagP* locus is not essential, but nevertheless required for full function of the secretion apparatus in this strain. Analysis of the *cagP* locus is complicated by the fact that a small regulatory RNA (HPnc2630/CncR1) is encoded upstream of *cagP*, and under the control of the *cagP* promoter. However, thorough analysis of corresponding deletion mutants and complemented strains demonstrated that the *cagP* gene itself, rather than the small RNA, contributes to full-level type IV secretion function. Protein profile analysis of *cagP* deletion and complementation strains showed that protein levels of CagH, CagI and CagL, components which are considered as assembly factors for the secretion apparatus-derived pilus structures, are influenced by the *cagP* locus. These proteins are encoded by a putative operon which comprises the *cagG* gene as well, indicating a possible link between *cagP*-dependent effects and the *cagHIL* locus. Taken together, our data suggest that *cagP* expression might have a novel regulatory function in modulating Cag type IV secretion activity.

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193/GIP

Influence of the cytotoxin associated gene A of *Helicobacter pylori* on the in vitro migration behavior of human leukocytes

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Helicobacter pylori is a well known human pathogen that exclusively colonizes the human gastric mucosa and establishes a persistent chronic infection. Thereby *H. pylori* utilizes various strategies to evade proper recognition by the human immune system. These range from passive defense strategies (e.g. altered LPS- or flagellin structures) that result in avoiding detection by pattern recognition receptors (PRR) to subversion of adaptive immunity e.g. inhibition of IL-2 secretion and proliferation of T-cells via VacA.[1] Previously, we could show that *H. pylori* is also able to interfere with the migratory behavior of immune cells (e.g. neutrophils or neutrophil-like cells) in a cytotoxin associated gene A (cagA) phosphorylation-depended manner hence adding another layer of immune modulation.[2] In this study we aimed to further understand this inhibitory effect on migration by generating defined mutants of CagA in the so called EPIYA- and the mark1 kinase inhibitory (MKI) region. In addition, we investigated the impact of *H. pylori* infection on the microtubule cytoskeleton organization of migrating leukocytes. First results indicate a more prominent role for the EPIYA-AB motives than for the EPIYA-C motives in the inhibition of leukocyte migration. Furthermore, the MKI region seem to be involved in the modulation of leukocyte migration.

References

[1] N. R. Salama, M. L. Hartung, A. Muller, *Nature reviews. Microbiology* **2013**, *11*, 385-399.
[2] B. Busch, R. Weimer, C. Woischke, W. Fischer, R. Haas, *International journal of medical microbiology: IJMM* **2015**, *305*, 355-364.

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194/GIP

Analysis of Salmonella pathogenicity island (SPI)1 mediated enterocyte invasion in vivo

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The coordinated action of a variety of virulence factors allows *Salmonella enterica* to invade epithelial cells and penetrate the mucosal barrier. Due to the lack of a suitable animal model, the molecular mechanisms of this process have been primarily studied *in vitro* using immortalized cell culture models. We have recently

established a neonatal murine infection model that allows the analysis of both bacterial and host factors during the host-microbial interplay *in vivo*. Using this model, we demonstrate spontaneous intestinal colonization and Salmonella pathogenicity island (SPI)1-dependent mucosal translocation and spread to systemic organs following oral administration of *Salmonella*. Following invasion of enterocytes, intraepithelial proliferation and the formation of large intraepithelial microcolonies accompanied by Toll-like receptor (Tlr) 4 and 9-mediated immune stimulation is observed. Using quadruple, triple and single mutant bacteria in combination with complementation of individual SPI1 effector molecules we analyzed their contribution to enterocyte invasion, innate immune stimulation, microcolony formation and mucosal translocation *in vivo*. Together, our results characterize the critical role of individual bacterial effector molecules for the bacteria-enterocyte interaction during early infection *in vivo*.

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195/GIP

Oral enteropathogenic *E.coli* (EPEC) infection of the newborn mouse

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Enteropathogenic *Escherichia coli* (EPEC) represent an important causative agent of gastroenteritis worldwide affecting mainly infants below the age of two and causing up to 80.000 deaths per year. Despite extensive analysis using *in vitro* culture models, the investigation of the host-pathogen interaction *in vivo* has been hampered by the lack of a suitable small animal model. Here, we present here the first murine infection model to investigate the pathogenesis of EPEC infection *in vivo* using neonate mice. Infection of newborn but not adult mice led to the hallmark of EPEC pathogenesis, the formation of epithelium-attached microcolonies. Microcolony formation was dependent on the expression of bundle forming pili (BFP) and an intact type three secretion system (T3SS) and associated with Toll-like receptor (Tlr)5 and Tlr9-mediated epithelial innate immune stimulation and impaired enterocyte survival. Together, we demonstrate an age-dependent susceptibility to EPEC infection in mice and introduce the first suitable small animal model amenable to genetic modifications to study the pathogenesis of EPEC.

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Infection Immunology (FG II)

196/IIP

***In vitro* detection of *Candida albicans* factor H binding molecule Hgt1p by monoclonal antibody - an approach for *in-vivo* immunotherapy?**

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Introduction: The complement system is tightly controlled by several regulators. In particular Factor H (FH) is preferentially acquired by pathogens conveying resistance to complement attack.

Objective: The aim of the study was to determine by an *in vitro* assay whether the FH binding molecule „high affinity glucose transporter 1" (CaHgt1p) of *Candida albicans* is a significant virulence factor. Another aim was to determine whether the binding and thus inhibition by monoclonal antibody (Hgt1p-mAb) is a possible approach for an *in vivo* immunotherapy of *C. albicans.*
Method: An *in vitro* phagocytosis study was performed to demonstrate the ability of Hgt1p-mAb to increase the phagocytosis of *C. albicans* wild type (SN-152) by human polymorphonuclear cells (PMNs). Both wild type (SN-152) and knock-out strain (*hgt1Δ/Δ)* were treated with Hgt1p-mAb, opsonized with human serum (HS) and then stained with fluorescein isothiocyanate (FITC). Fresh human PMNs cells were co-cultured with these strains for 30´ at 30°C. Positive PMNs, with internalized *C .albicans*, were detected using FACS analyses.
Result: Phagocytosis experiments showed a significant ($p<0.05$) lower phagocytosis of the wild type strain in contrast to *hgt1Δ/Δ* knock-out strain, unable to bind FH. The wild type treated with Hgt1p-mAb also showed a significant ($p<0.05$), albeit small, increase in phagocytosis in comparison to untreated wild type.
At the same time our data showed a similar phagocytosis of SN-152 wild type treated with Hgt1p-mAb in comparison with *hgt1Δ/Δ* knock-out strain.
Conclusion: CaHgt1p is not only a complement inhibitor, but also a virulence factor, as corroborated by *in vitro* data. The "restored" phagocytosis of SN-152 wild type treated with Hgt1p-mAb, then comparable with *hgt1Δ/Δ* knock-out strain, represents a starting point for a possible *in vivo* immunotherapy of *C. albicans*.

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197/IIP

Differential activation of formyl-peptide receptors by *Staphylococcus aureus* and consequences for inflammation

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Formyl peptide receptors (FPR1-3) are crucial pattern recognition receptors governing leukocyte chemotaxis and cytokine release in response to microbe-associated molecular patterns (MAMPs). FPR1 senses formylated peptides produced by all kinds of bacteria, while FPR2 and 3 respond to certain endogenous peptides. In addition, we have recently demonstrated that phenol-soluble modulin (PSM) peptides from highly pathogenic *Staphylococcus aureus* are not only important cytolytic toxins but also highly efficient ligands for the human FPR2. Mouse neutrophils also respond to PSMs, but it has remained unclear, which of the mouse FPR paralogs senses staphylococcal PSMs.
To analyze the role of mouse FPRs, stably transfected RBL cells were generated, which either express mFpr1 or mFpr2. After stimulation with PSMs or culture filtrates of PSMs-secreting *S. aureus* strain USA300, we noticed strong calcium influx and degranulation in mFpr2-transfected cells, but no response in mFpr1-transfected cells and control cells. Moreover, by using HoxB8 neutrophils, a primary neutrophil cell line prepared from wild-type (WT) and mFpr2 knockout mice (Fpr2-/-), we observed strong calcium influx, chemotaxis, MIP2 release and CD11b upregulation in wild-type HoxB8 but not in Fpr2-/- HoxB8 after stimulation with PSMs or culture filtrates of USA300.

These data indicate that the mouse Fpr2 is specifically activated by PSMs. Therefore, PSMs represent the first secreted MAMPs for the mouse Fpr2. Our data support the hypothesis that the mouse Fpr2 is the functional orthologue of the human FPR2 and that a mouse infection model may be a suitable model for analyzing the role of PSMs and FPRs during infection.

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198/IIP

***Mycobacterium tuberculosis*-specific T-cell activity is impaired by Abl tyrosine kinase inhibitors**

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Question: Tuberculosis remains the major killer among infectious diseases, even exceeding the HIV-mediated mortality. Due to the emergence of multidrug-resistant strains, new strategies for the treatment of tuberculosis are urgently needed. For example host-directed therapies interfering with checkpoints of cellular signal transduction such as the Abl tyrosine kinase have been shown to support antimicrobial activity of human macrophages. Here, we investigated the influence of Abl tyrosine kinase inhibitors (TKI) on central effector functions of human T-cells required for protection against *Mycobacterium tuberculosis (Mtb).*

Method: Peripheral blood mononuclear cells were stimulated with mycobacterial antigens in the presence of TKI (Nilotinib, Imatinib, Dasatinib) which have recently been introduced in the standard treatment of hematologic malignancies. T-cell function was evaluated by measuring cytokine release (ELISA), proliferation (CFSE dilution) and degranulation as a surrogate marker of cytotoxicity.

Result: Nilotinib, in concentrations required for the inhibition of Abl tyrosine kinase *in vivo*, inhibited the *Mtb*-specific proliferation (- 80%) and cytokine release of IFNg, TNFa and GM-CSF. In addition degranulation of cytotoxic granules, a critical effector mechanism in T-cell mediated killing of *Mtb*, was reduced in the presence of Nilotinib (- 40%). These findings are not specific for Nilotinib but were also found for the two other clinically relevant TKI Imatinib and Dasatinib. Therefore TKI impair three major T-cell mediated effector mechanisms required for control of *Mtb*-infection: proliferation, Th1 cytokine release and degranulation of cytotoxic granules.

Conclusion: These results suggest that the effect of TKI on T-cell function is opposed to the effect on macrophages. Given the essential role of T-cells in the immune response against tuberculosis the pharmacological inhibition of Abl tyrosine kinase for the treatment of malignancies should be accompanied by careful monitoring of patients for the reactivation of infections with intracellular bacteria such as *Mtb*.

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199/IIP

A nutrient sensor confers tolerance to α-toxin by modulating ADAM10

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Introduction: In the context of an infection, the term "tolerance" denotes the ability of an organism to maintain health despite persisting pathogen load (1). Unlike resistance (the ability of an organism to eliminate the pathogen), the mechanisms which confer tolerance are not well understood. When analyzing cellular tolerance to pore forming toxins, we found that basal activity of a nutrient sensor in cells enhances tolerance to the small pore forming *S. aureus* α-toxin.

Aim: The aim of the present study was to elucidate the mechanisms of cellular tolerance to α-toxin.

Materials and Methods: We exploited small molecular weight inhibitors, previously shown to mediate repair and survival in HaCaT cells and variants of murine embryonal fibroblast (MEF) that display defects in the regulation of eIF2α de-/phosphorylation to uncover the mechanisms of MEF tolerance to α-toxin.

Result: Our data show that basal activity of amino acid deprivation sensitive EIF2AK4/GCN2 confers tolerance to α-toxin in MEF: Defects in eIF2α de-/phosphorylation render MEF susceptible to α-toxin. Although the molecules involved are highly conserved in eukaryotes, the protective effect towards α-toxin proved to be surprisingly selective with regard to toxin and cell type. An explanation for the unexpected toxin-specificity emerged when we found that basal activity of amino acid sensor EIF2AK4/GCN2 modulates surface expression of ADAM10 (2), the putative receptor for α-toxin. Because lack of EIF2aK4/GCN2 resembles deficiency of ATG16L, EIF2aK4/GCN2 could act by maintaining basal autophagic flux (3), but our data indicate that yet another mode of action might be involved**.**

Summary: Basal activity of nutrient sensor EIF2aK4/GCN2 enhances cellular tolerance to pore-forming *S. aureus* α-toxin via modulation of surface expression of its receptor ADAM10.

References

[1] Schneider DS, Ayres JS. Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. Nature reviews Immunology. 2008;8(11):889-95.
[2] von Hoven G, Neukirch C, Meyenburg M, Füser S, Bidna Petrivna M, Rivas AJ, Ryazanov A, Kaufman RJ, Aroian RV, Husmann M. eIF2alpha Confers Cellular Tolerance to S. aureus alpha-Toxin. Frontiers in immunology. 2015;6:383.
[3] Maurer K, Reyes-Robles T, Alonzo F, Durbin J, Torres VJ, Cadwell K. Autophagy Mediates Tolerance to Staphylococcus aureus Alpha-Toxin. Cell Host Microbe. 2015;17(4):429-40.

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200/IIP

The phosphoproteome of cord factor-activated primary macrophages

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Mycobacterium tuberculosis, the causative agent of tuberculosis, reprograms macrophages to create its intracellular niche. The most abundant mycobacterial cell wall glycolipid Trehalose-6,6-dimycolate (TDM), also known as cord factor, is recognized by the C-type lectin receptor Mincle leading to transcriptional induction of inflammatory cytokines and chemokines depending on FcRg-Syk-Card9 signaling. However, the pathways linking TDM-Mincle to gene expression in the nucleus are surprisingly little understood. To investigate kinase cascades triggered by TDM we performed quantitative analysis of the phosphoproteome of primary macrophages of wildtype and Mincle-deficient mice using dimethyl-labeling of the phosphopeptides, phosphopeptide enrichment and high-resolution mass spectrometry. We identified around 4.000 phosphoproteins with around 12.000 phosphorylation sites; 4 % of these sites were upregulated by TDM and 1 % was downregulated. Interestingly, Mincle-dependence of phosphorylation was less complete than expected considering the nearly complete reduction in Syk-activation in Mincle-/- macrophages. In addition to several canonical players of Mincle-Syk signaling, a number of kinases and adapter proteins were detected which may link Mincle to the Card9-Bcl10-Malt1-complex and MAPK signaling, like the Src kinase FYN and the adapters FYB and SKAP. Bioinformatic analysis revealed that TDM-induced phosphorylation was particularly enriched among endosomal, cytoskeletal and nuclear membrane proteins, and especially targets proteins involved in lipid binding, transport processes and control of gene expression and translation. Collectively, our findings show substantial, only partially Mincle-dependent, changes in the phosphoproteme of macrophages stimulated with mycobacterial cord factor. These findings provide new perspectives on the signaling cascades, cellular compartments and biological processes targeted by the mycobacterial cord factor in macrophages.

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201/IIP

Effects of Listeria monocytogenes infection on different subsets of primary human macrophages

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Listeria monocytogenes (*Lm*) is an important human, food-borne pathogen and a model organism for intracellular pathogenesis. Macrophages are a major phagocyte population of the human innate immune system with an important role in mounting appropriate immune responses against pathogens. The aim of the presented study was to characteriz the response of different subsets of human primary macrophages to infection with *Lm* in detail.
Pro-inflammatory M1 and anti-inflammatory M2 macrophage subsets were generated from human primary blood monocytes by *ex vivo* differentiation in the presence of GM-CSF or M-CSF, respectively. The effects of *Lm* infection on these macrophage subsets was analyzed using RNASeq transcriptomics, a high content siRNA knockdown screen as well as various functional assays measuring chemotaxis, phagocytic activity, bacterial killing, cytokine secretion, programmed cell death, T cell polarization.
M-CSF-derived M2 macrophages (M-Mq) showed higher phagocytic activity than GM-CSF-derived macrophages (GM-Mq). Surprisingly, TLR2 does not seem to play a role in phagocytosis of *Lm* by human macrophages as shown by siRNA knowdown experiments. RNASeq transcriptome analysis revealed that a large number of immune related pathways were triggered by *Lm* infection in M-Mq. By contrast only a few select pathways were significantly modulated in GM-Mq including apoptosis. In line with this observation, GM-Mq infected with *Lm* rapidly stained positive in TUNEL assays indicating cell death. High levels of IL-1ß in supernatants as well as Caspase 1 activity of these cells suggest that infected GM-Mq undergo pyroptosis. By contrast, M-Mq survived infection for prolonged periods of time without any signs of cell death. *Lm*-primed GM-Mq obtained the ability to activate T- cells and induce proliferation of $CD8^+$ T cell. By contrast, no significant T- cell response was observed with *Lm*-infected M-Mq. Also, M-Mq showed no chemotaxis towards *L. monocytogenes*, MCP-1, fMLP whereas uninfected GM-Mq strongly migrated towards these stimuli. The chemotactic activty of GM-Mq was lost upon infection with *L. monocytogenes*. Cytokine profiles of infected cells confirmed the pro- and anti-inflammatory phenotype of GM-Mq and M-Mq.

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202/IIP

Myeloid cell-derived HIF1α promotes control of *Leishmania major*

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Hypoxia-inducible factor 1α (HIF1α), which accumulates in mammalian host organisms during infections, supports the defense against microbial pathogens. However, whether and to which degree HIF1α expressed by myeloid cells contributes to the innate immune response against *Leishmania* (*L.*) *major* parasites is unknown. We observed that *L. major*-infected C57BL/6 mice displayed substantial amounts of HIF1α in acute cutaneous lesions. *In vitro*, HIF1α was required for leishmanicidal activity and high level nitric oxide (NO) production by IFNg/LPS-activated macrophages. Mice deficient for HIF1α in their myeloid cell compartment showed a more severe clinical course of infection and increased parasite burden in the skin lesions as compared to wildtype controls. These findings were paralleled by reduced expression of type 2 nitric oxide synthase by lesional $CD11b^+$ cells. Together, these data illustrate that HIF1α is required for optimal innate leishmanicidal immune responses and thereby helps to cure cutaneous leishmaniasis.

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203/IIP

***Yersinia enterocolitica* targets several key kinase checkpoints to trigger macrophage apoptosis**

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Death of host cells is a common outcome in the interaction of pathogenic microbes with the host immune response that may have substantial consequences for the development of inflammation and disease. Enteropathogenic *Yersinia enterocolitica* triggers apoptosis in macrophages by injecting the YopP effector protein through type III secretion into host cells. YopP deactivates several TLR-induced signaling pathways which impedes the development of a coordinated immune response and finally affects cell survival, leading to macrophage apoptosis. The mechanisms involved in *Yersinia*-induced apoptosis are, however, less clear. We use *Yersinia* as tool to gain more insights into the regulation of cellular life and death in bacteria-infected macrophages. Our results showed that the RIP-1 kinase is centrally implicated in *Y. enterocolitica*-induced apoptosis. The pathways that keep RIP-1-related cell death in infected macrophages in check are, however, yet poorly defined. Our data indicate that YopP impairs several kinases that control the transition of RIP-1 from a pro-survival factor to a mediator of macrophage apoptosis. Accordingly, the targeting of TAK-1 by YopP was sufficient to release the cytotoxic potential of RIP-1 and to activate RIP-1 kinase-dependent apoptosis in *Yersinia*-infected and LPS-stimulated macrophages independently from TNF receptor-1 signaling. Downstream from TAK-1, the deactivation of IKK and of MAPK pathways contributed to initiate macrophage apoptosis through RIP-1. It appears that there are several inhibitory feedback signaling loops to RIP-1 in TLR-stimulated macrophages that are targeted by YopP and that convert RIP-1 from an inhibitor to an inducer of macrophage apoptosis.

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204/IIP

Cell-surface localized pneumococcal proteins - new insights into the abundance and immunogenicity

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Introduction: *Streptococcus pneumoniae* is a commensal of the human upper respiratory tract and despite the availability of effective vaccines the incidence mortality rates of pneumococcal pneumonia, sepsis or meningitis is high. The pneumococcal cell surface is decorated with several clusters of proteins. Namely these are choline-binding proteins (CBPs), sortase anchored proteins, lipoproteins, (trans)membrane proteins and so called non-classical surface proteins (NCSP). Especially lipoproteins might be promising candidates for a next-generation, serotype-independent and protein-based vaccine as they are highly conserved among pneumococci.
Objective: Several proteins from these different protein clusters were analyzed regarding their abundance on the pneumococcal surface and immunogenicity.
Materials and Methods: The cell-surface abundance of proteins was examined by flow cytometry using protein specific polyclonal IgGs generated in mice. The relative antibody titers from these mouse sera were determined by ELISA and antibody titers were also analyzed using the Luminex® FlexMap3D® technique. The immunogenicity of the selected proteins was assessed with convalescent sera from patients infected with different pneumococcal serotypes showing different clinical outcomes.
Result: The Luminex-based immunoproteomics approach showed that nearly all proteins are immunogenic when administered to mice. The polyclonal mouse antibodies suggested differences in the abundance of the candidates on the pneumococcal cell surface while exhibiting a high specificity. The most abundant proteins were PspC, PnrA, PpmA and PsaA. Importantly, these antibodies also bound efficiently to encapsulated pneumococci. The analysis of convalescent patient sera revealed a high immunogenicity of 5 candidate proteins from different protein clusters (PsaA, PnrA, PspA, PspC, PavB) during pneumococcal infections. The fact that immunogenicity of some proteins correlates with their cell-surface abundance (PspC, PsaA, PnrA) turns them into promising candidates for a protein-based multicomponent vaccine. Further experiments will indicate the efficacy of the specific antibodies to opsonize pneumococci and therefore trigger recognition by the immune system. Moreover, we are investigating the humoral immune response profiles induced by immunization of mice with the different surface proteins.
Conclusion: The analyzed pneumococcal surface proteins are in parts highly conserved and immunogenic. Besides, the surface abundance of some proteins is high and partially correlates with the observed immunogenicity. Therefore, these proteins represent promising candidates for a protein-based conjugate or subunit vaccine, which are urgently needed to combat pneumococcal infections in a serotype-independent manner.

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205/IIP

NLRP3 protects alveolar barrier integrity by an inflammasome-independent increase of epithelial cell adherence

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Bacterial pneumonia is a major cause of acute lung injury and acute respiratory distress syndrome, characterized by alveolar barrier disruption. NLRP3 is best known for its ability to form inflammasomes and to regulate IL-1b and IL-18 production in myeloid cells. Here we show that NLRP3 protects the integrity of the alveolar barrier during *Streptococcus pneumoniae*-induced pneumonia *in vivo*, and *ex vivo* upon treatment of isolated perfused and ventilated lungs with the purified bacterial toxin, pneumolysin. We reveal that the preserving effect of NLRP3 on the lung barrier is independent of inflammasomes, IL-1b and IL-18. NLRP3 improves the integrity of alveolar epithelial cell monolayers by enhancing cellular adherence. Collectively, our study uncovers a novel function of NLRP3 by demonstrating that it protects epithelial barrier function independently of inflammasomes.

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206/IIP

Modulation of dendritic cell maturation by the fungal quorum sensing molecule farnesol

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Introduction: Farnesol, the first quorum sensing molecule described in eukaryotes, is produced by *Candida albicans* and controls filamentation in these fungi. The mechanisms of how farnesol blocks the *C. albicans* yeast to hyphae form are well known; nevertheless, the impact of farnesol on host cells is poorly understood, especially on dendritic cells (DCs) which are key regulators of immunity and promote an anti-fungal Th1 immune response. For this work, we evaluated the effect of farnesol in dendritic cell maturation by analyzing the phenotype and transcriptional response.
Method: Human monocytes were isolated from buffy coats of healthy volunteers and differentiated into monocyte-derived dendritic cells (DCs) in the presence of GM-CSF and IL-4. The immunophenotype of DCs was addressed by FACS and the transcriptome analysis of DCs in response to farnesol was performed by a whole-genome expression direct hybridization assay on a bead chip array.
Result: Transcriptional analysis of farnesol-mediated DC maturation revealed a high number of differentially regulated genes involved in cytokine-cytokine receptor interaction, cell adhesion molecules and antigen processing and presentation. Farnesol significantly interfered with the differentiation process from monocytes to DCs, by impairing surface expression of key markers for maturation and antigen presentation (HLA-DR CD83, CD86 and CD80). Furthermore, farnesol modulates the displacement of CD1 molecules. While CD1a showed reduced expression; CD1d, a molecule involved in invariant NKT (iNKT) cell activation, was increased on DCs generated in the presence of farnesol. Interestingly, we found increased expression of PPARγ-associated pathway genes. This nuclear receptor regulates CD1 expression and might be a potential target addressed by farnesol.
Conclusion: Farnesol regulates DCs phenotype and is able to induce transcriptional rewiring in these cells. Further experiments will be performed to evaluate the possible role of PPARγ activation and signaling in farnesol-treated moDCs.

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207/IIP

YopM as a Bacteria-Derived Anti-Inflammatory Cell-Penetrating Peptide (CPP) for the Topical Treatment of Immune-Mediated Inflammatory Skin Diseases

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Psoriasis is one of the most common immune-mediated chronic inflammatory skin disorders affecting approximately 2-3% of the population. It is considered as an incurable, life-long skin condition that affects all races, age groups and both sexes. A common disadvantage of all currently available drugs for treating this primarily cutaneous disease is that they need to be applied systemically. Previously, we identified and characterized the bacterial effector protein YopM of pathogenic *Yersinia* as a novel cell-penetrating peptide. Furthermore, once inside the cells, YopM is able to down-regulate the transcription of several pro-inflammatory cytokines such as TNF-α. These novel findings suggest a potential immunotherapeutic application.
To investigate whether YopM might be applicable as a topical therapeutic agent for the treatment of psoriasis, we utilized the murine imiquimod (IMQ)-induced psoriasis model. In this model, daily topical application of imiquimod (IMQ), a TLR7/8 ligand and potent immune activator, on mouse back skin induces and exacerbates psoriasis. Here, we applied rYopM either topically or subcutaneously for 5 consecutive days. Our results confirmed the 'self-delivering' abilities of YopM across the cutaneous barrier for topically applied rYopM, and indicated a remarkable dampening of overt inflammatory reactions. Furthermore, truncated rYopM variants were generated via site-directed mutagenesis to determine domains required for the penetration of epithelial barriers and for its anti-inflammatory activity. We found out that a single nuclear localization signal might be sufficient for the translocation of YopM into the nucleus and fulfilling the anti-inflammatory properties, which would facilitate the design of a minimal costruct. Candidates will then be tested in the IMQ-induced psoriasis model. Taken together, our data indicate that epicutaneously applied YopM can penetrate across the cutaneous barrier in an IMQ-induced psoriasis mouse model and triggers remarkable anti-inflammatory effect. Therefore topical YopM treatment might be suitable for targeted therapy of immune-mediated inflammatory skin disorders.

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208/IIP

Hypoxia-inducible factor-1α controls granulysin expression in human *Mycobacterium tuberculosis*-specific T cells

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Introduction: Cytotoxic T lymphocytes (CTL) are a major component of the adaptive immune response to restrict the growth of intracellular pathogens. In response to antigen-specific activation CTL release lytic granules containing perforin, granulysin and granzymes.
Aim: We have recently shown that hypoxia mediates upregulation of granulysin in human T cells. Since Hypoxia-inducible factor-1α (HIF-1α) is a key metabolic sensor that controls T cell biology, we asked if HIF-1α provides a link between antigen-specific and hypoxia-mediated upregulation of granulysin.
Materials and Methods: Granulysin expression was analyzed in *Mycobacterium tuberculosis* (*Mtb*)-antigen activated *Mtb*-specific short term T cell lines as compared to unspecific bacterial antigens and under microaerophilic oxygen conditions. Therefore granulysin expression was tested on mRNA (qPCR) and protein level (flow cytometry, immunoblot). In parallel HIF-1α expression was investigated by western blot analysis. Furthermore Chetomin- and BAY 87-2243-mediated inhibition of HIF-1α and granulysin expression was evaluated. Inhibition of granulysin expression by BAY 87-2243 in different T cell subsets was quantified by flow cytometry. K562-mediated degranulation of NK cells was shown by measuring CD107.
Result: Our results demonstrate that *Mtb*-antigens upregulate granulysin in human T cells. Antigen specific granulysin expression was further enhanced by microaerophilic oxygen conditions. In parallel oxygen restriction as well as *Mtb*-antigens increased HIF-1α levels. This observation indicated that HIF-1α is a shared molecular target of antigen-specific and oxygen-dependent T cell activation. In order to proof our hypothesis, that there is a link between the upregulation of HIF-1α and granulysin, we inhibited the expression of HIF-1α on a nuclear (Chetomin, CTM) and on a cytoplasmic level (BAY 87-2243). Both inhibitors antagonized the *Mtb*-specific upregulation of granulysin (750 nM CTM by 37±21%; 10 nM BAY 87-2243 by 64±11%). This

regulatory pathway was active in T cells as well as NK cells. In NK cells the upregulation of granulysin correlated with a 28% increased degranulation of cytotoxic granules, which was dependent on HIF-1α.
Summary and Conclusion: These results provide evidence that HIF-1α functionally links antigen-specific and oxygen-dependent T cell activation. Therefore the pharmacological modulation of HIF-1α might promote the expression of granulysin and could therefore provide a new strategy for a novel host-directed therapy to treat drug-resistant tuberculosis.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

209/IIP
Gene-expression profiling of human monocytes after stimulation with pathogens of systemic infections
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Bloodstream infections are caused by fungi, Gram negative and Gram positive bacteria. Human monocytes are of particular importance to defend microbial pathogens because they are able to phagocytose, to release cytokines and chemokines and to trigger adaptive immune responses. Genome-wide gene expression analysis can help to distinguish the response of monocytes towards different pathogens and to identify pathogen specific patterns.
Monocytes of five healthy male donors were isolated from venous blood via Ficoll density centrifugation and a subsequent CD14+ separation. The cells were stimulated with *A. fumigatus*, *N. meningitidis* or *S. aureus* for 3 h and 6 h. RNA of monocytes was used for RNAseq and microarray analysis. Differentially expressed genes (FDR < 0.05, Fold Change > 1.5) were determined for each pathogen.
Transcriptomic analysis of human monocytes after confrontation with different pathogens of systemic infections revealed a strong immunological core response for all pathogens. Within this group mainly genes encoding chemokines, pro-inflammatory cytokines or surface marker for activation or adhesion were found. *A. fumigatus* induced a strong specific response by upregulating genes of the HIF- or the MAPK-signaling. In contrast, the bacterial pathogens shared a large number of differentially expressed genes encoding chemokines, cytokines or transcription factors of the Jak-STAT signaling pathway. Only for bacteria we found next to the common core response many genes encoding cytokines that activate the adaptive immune response.
These characteristic transcriptional differences between the pathogens can be used as a fundament for future studies to aid the diagnosis of sepsis and to characterize a pathogen specific immune response.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

210/IIP
Dominant low avidity clonotypes arise from reverse TCR repertoire evolution during chronic CMV infection
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During repetitive acute infections, antigen-specific T cell populations can undergo some degree of avidity maturation by preferential expansion of high avidity T cell receptors (TCRs). For chronic infections, such as infection with cytomegalovirus (CMV), dominant T cell clones have been reported to occur at later time points, but it is controversial whether the TCRs of these oligoclonal T cell populations are selected for specific avidity characteristics.
CMV transcripts expressed during latency elicit a large-sized T cell response, also known as 'memory inflation'. We here report that during chronic human (HCMV) as well as murine CMV (mCMV) infection, inflationary T cell populations are characterized by low TCR avidity. This holds true for both structural and functional avidity.
It has previously been hypothesized by us and others that during chronic antigen exposure, preferentially high avidity T cell clones might become senescent, whereas the overall epitope-specific population could still be maintained at a large size by the domination of low avidity T cells.
We now experimentally show for mCMV infection that this is indeed true: Whereas early during primary infection the TCR repertoire is both diverse and of variable avidity, at later time points during chronic infection time points the population is continuously driven towards oligoclonality and low avidity TCRs. In thymectomized mice, this reverse TCR repertoire evolution was accelerated, pointing towards the importance of precursor frequencies within the naïve repertoire and/or replenishment of the T cell pool by thymic emigrants.
The observation that dominant clonotypes at late time points of chronic infection can get strongly selected towards low avidity not only adds to our understanding of immune control during chronic infections, but also has profound translational implications, as the presence or absence of high avidity TCRs markedly influences the choice of immunotherapeutic strategies.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

Food Microbiology and Food Hygiene (FG LM)

211/LMP
Monitoring of Salmonella Infections in Turkey Flocks in Germany 2005-2015
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Despite significant improvements in hygienic practice, food technology and public sanitation salmonellosis remains a persistent threat to human and animal health. The European Parliament Council Regulation 2160/2003/EC was passed in November 2003 and regulates the control of *Salmonella* and other food-borne zoonotic agents. This regulation sets targets for the reduction of the prevalence of specific zoonotic pathogens in animal populations at the level of primary production, including meat turkey. Consequently, the European Commission regulation (EC) No 584/2008 was implemented and sets Community targets for the reduction of the prevalences of S. Enteritidis and S. Typhimurium in turkeys. The Community target is to reduce both *Salmonella* serovars to a maximum prevalence of 1 % or less in fattening and breeder flocks since December 31st, 2012.
The present study was carried out to investigate the prevalence of *Salmonella* infections in fattening turkeys in Germany as well as from some flocks reared in Austria, Czech Republic and Poland prior slaughter from 2005 to 2015. In addition, the isolates were monitored for antibiotic resistance profiles and examined for ESBL properties.
Over 15 000 samples from fattening turkey flocks were monitored for *Salmonella*. Two boot swab samples from each flock three weeks prior to slaughtering were examined in accordance to the regulation (EC) No 584/2008. *Salmonella* isolates were submitted for serotyping and micro-broth antibiotic resistance test against up

to 19 antibiotic substances to the Federal Institute for Risk Assessment (BfR).
The obtained results showed a continuous overall reduction of positive *Salmonella* samples varying between 9,8% (2005) and 1,4% (2015). The prevalence of S. Typhimurium and S. Enteridis was fluctuating, increasing in some year and disappearing again.
In the years 2005-2015 resistances against ampicillin, sulfamethoxazole and tetracycline occur most frequently in the isolated *Salmonella*. Peaks have been observed in 2008 for ampicillin and sulfamethoxazole both in approx. 80% of isolates, while Tetracycline resistance was observed most frequently in 2006 (approx. 80%). Concerning ciprofloxacin we observed an increase in resistance from 2005 (2%) to 2013 (45%), however it occurred less frequently in the most recent years.
According to our results, isolated Salmonella serovars differ over the years and seem to undergo substitution and replacement processes. Since the implementation of regulation (EC) No 584/2008 we did not observe prevalences above 1% for S. Enteritidis and S. Typhimurium. The resistance pattern of isolated *Salmonella* is very diverse. Resistances against ampicillin, sulfamethoxazole still occur in roughly half of the isolates. Interestingly, resistances against cefotaxime or ceftazidime were not found. Along with our investigation of ESBL properties this indicates that ESBL-Salmonella are not prevalent in examined turkey flocks.

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212/LMP
Phenotypic antimicrobial resistance and biofilm profile of mastitis-associated bacteria
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Introduction: Mastitis is a worldwide cause of enormous economic losses in dairy industry, caused by a variety of grampositive and gramnegative bacteria. The general therapeutical approach is the use of antibiotics, which is partly ineffective if the inflammation is caused by a biofilm forming pathogen. The at worst nearly permanent selective pressure by the antimicrobial substances can lead to enhanced antimicrobial resistances.
Aim: The characterization of mastitis associated isolates in this study grants an overview about the resistance pattern and the biofilm forming ability. The new biofilm assay allows a concrete classification of the biofilm and comparison of different isolates.
Materials and Methods: Seven mastitis-relevant bacterial species were defined and 50 isolates per bacteria are included in the strain collection. Species confirmation was implemented by MALDI-TOF (Ripac-Labor GmbH, Potsdam) and MICs were determined using MICRONAUT-S Mastitis 3 plates (MERLIN diagnostics, Bornheim-Hersel).
Per species, a subset of 8 isolates was chosen for biofilm experiment. The ability of the isolates to form biofilms was characterized by determination of the CFU grown on a silicone coupon.
Result: To date, this study includes 438 isolates of cows suffering from clinical and subclinical mastitis.
MIC testing revealed a rather low variability between the strains and a low rate of multidrug resistant (MDR) bacteria. We were able to identify one MDR *E. coli* and one Methicillin-resistant *S. aureus* (MRSA).
Most of the isolates were identified as good biofilm formers (CFU $1{,}0x10^5$-$1{,}0x10^7$/5 mm^2) compared to an EAEC known to be an excellent biofilm former (CFU $1{,}0x10^6$/5 mm^2). Lower CFU were determined within the tested streptococci (CFU 0-$1{,}0x10^5$/5 mm^2).
Summary: The heterogenic species collection showed quite sensitive resistance patterns consistent with the literature and routine diagnostic. The majority of the mastitis associated isolates were able to form a stable biofilm, which has to be considered for a successful treatment and prophylactic approaches.

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213/LMP
Antimicrobial activities of mushroom species and bioburden germs from medicinal mushroom products
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Medicinal mushroom products are usually imported from Asia. These alternative drugs are promised to have positive healthy and antimicrobial effects, but many products contain bioburden germs, what is not mentioned. Some of these germs are of interest because antimicrobial effects, which are not investigated so far.
Primary objective was to determine the bioburden (bacteria and fungi) of medicinal mushroom products and to analyse antimicrobial activities of both bioburden germs and lab strains from medicinal mushroom species.
Foreign germs were isolated as strains from each 10 medicinal mushroom products from 4 retailers and identified by conventional and biomolecular methods. Antimicrobial activities of isolated bioburden strains and of 8 mushroom *(Agaricus subrufescens*, *Auricularia auricula-judae*, *Hericium erinaceus*, *Ganoderma lucidum*, *Trametes versicolor*, *Lentinula edodes*, *Coprinus comatus*, *Grifola frondosa*) lab strains were evaluated by parallel streak method.
As result, 32 fungus and 22 bacteria species (130 strains) were identified as bioburden. Most frequent bacteria (apathogenic and atoxinogenic type) were *Bacillus subtilis*, *B. megaterium*, *B. amyloliquefaciens*, *Micrococcus luteus*, and *Paenibacillus provencensis*; most frequent fungi were *Paecilomyces variotii*, *Aspergillus niger*, *Mucor racemosus*, and *Trichoderma longibrachiatum.*
As result of a primary screening with 22 bacteria isolates, strains with the highest antimicrobial activities were *Bacillus amyloliquefaciens* and *B. pumilus* and *Streptomyces album.* The highest activities against *Aspergillus flumigatus*, *Alternaria alternata*, *Penicillium chrysogenum*, and other fungi (Figure 1) showed *B. amyloliquefaciens*, which had the strongest inhibitory effect on bacterial growth. But, highest antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* showed *S. album.* Medicinal mushrooms strains, especially *L. edodes*, *G. frondosa*, *T. versicolor*, and *A. subrufescens*, had inhibiting effects against the above mentioned bacteria and fungi. But, their antimicrobial effects were low, compared to *B. amyloliquefaciens*, which was often detected in some mushroom products (up to 10^6 CFU/g). *B. amyloliquefaciens* contains barnase, a ribonuclease and known as a natural antibiotic protein.
The bioburden germs of medicinal mushroom products and their effects to human health are largely unexplored yet. The study proved a considerable potential for further research; a corresponding project is prepared at Mykolabor Dresden.

Figure 1

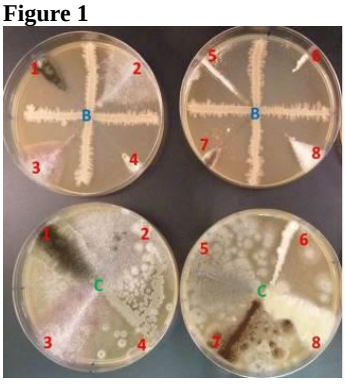

Figure 1: Screening by parallel streak method with an isolated strain of *Bacillus amyloliquefaciens* (B) against strains of
1) *Alternaria alternata*,
2) *Rhizopus microsporus*,
3) *Fusarium oxysporum*,
4) *Penicillium chrysogenum*,
5) *Aspergillus fumigatus*,
6) *Aspergillus versicolor*,
7) *Aspergillus niger*,
8) *Aspergillus flavus*;
C – Control plates only with fungi 1- 8

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

214/LMP

Discrimination between *Staphylococcus aureus* of human and bovine origin which are attributed to clonal complex 8

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Objective: *S.aureus* attributed to clonal complex 8 (CC8) is widely disseminated as nasal colonizer and infectious agent among humans. Recently it was also reported from Switzerland for isolates from mastitis in cattle, and we also detected it among mastitis isolates. For further epidemiological analysis with respect to colonization and infections in humans with occupational exposure to cattle and food products and for tracing the origin of contamination in cases of food intoxication the discrimination from human isolates attributed to CC8 is important.
Method: The Isolates, Methicillin susceptible *S.aureus* attributed to CC8 of human origin (n=36) and from milk samples from cows suffering from subclinical mastitis (n=12). The isolates were collected in several areas of Germany. They were characterized by typing (*spa*, MLST) and by PCR for (i) the presence of the immune evasion gene cluster (IEC) and *int*3 prophages (ii), and also for *luk*M, (iii) for an LPXTG motif coding gene associated with a non SCC*mec*, and (iv) for enterotoxin genes.
Result: Among the 12 isolates from cattle 9 exhibited t2953, 8 of them contained *luk*M, and 4 of them contained the IEC including *sea*. Three isolates contained *sea* independent from *int*3 prophages (also no truncated *hlb*).Two isolates exhibited t008 and contained *luk*M but were negative for the IEC and enterotoxin genes. One isolate exhibited t118, it contained *luk*M and the IEC and also *sea*. Only 7 among the 12 isolates contained the LPXTG motif coding gene which was reported for MSSA CCC8 of bovine origin from Switzerland[1].
All of the 36 MSSA of human origin were negative for *luk*M and contained the IEC.
Conclusion: It is known that "classical" mastitis isolates attributed to ST133 and ST522 contain *luk*M. PCR for *luk*M is also useful for discrimination between isolates of human and bovine origin which are attributed to CC8. We could not confirm the absence of IEC and possession of the LPXTG motiv coding gene for all of our bovine CC8 isolates as reported for CC8 isolates of bovine origin from Switzerland[1].

Reference

[1] Resch, G. et al., PLOsONE, 2013, e58187.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

215/LMP

***Staphylococcus aureus* in raw milk cheese of small ruminants and raw prawns: consumer health risk characterization**

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Introduction: Coagulase positive staphylococci (CPS), mainly *S. aureus* are among the leading causes of foodborne illnesses due to its ability to produce heat-stable staphylococcal enterotoxins (SE) in food. Most often, enterotoxigenic *S. aureus* are introduced into the food chain by human carriers during harvest, processing or further preparation of food. Knowing about improper hygienic conditions being a favourable growth factor of bacteria and subsequent SE production the European Union has put in place EU legislation No 2073/2005 applying food process hygiene criteria on CPS in milk/milk products and products of cooked crustaceans and molluscan shellfish.
Objective: To characterize the consumer health risk due to enterotoxigenic CPS in cheese made from raw milk from sheep and goat, and raw prawns in Germany.
Materials and Methods: In the course of the National Zoonosis Monitoring program in 2015 a representative number of raw milk cheese from sheep or goat as well as raw prawns were sampled at retail. Enumeration of CPS was conducted by the Federal laboratories (ISO 6888). In case that CPS count exceeded >log4 (cheese) and >log3 (prawns) CFU/g, isolates were sent to the BfR for further investigation. Isolates were confirmed as *S. aureus* by mReal-time PCR targeting *tuf*, *nuc* and *mecA* genes. *Spa* typing and MLST analysis was performed as well as a microarray (Alere Technologies) for the detection of SE-encoding genes and other virulence and resistance determinants. In cases where genes *sea* - *see* were found, the VIDAS SET 2 immunoassay (bioMérieux) was used to test for staphylococcal enterotoxin production.
Result: Overall, 671 food samples were investigated for the presence of CPS. 17 isolates were sent to the BfR: 16 were confirmed as *S. aureus* and one as *S. argenteus*. They were assigned to nine different clonal complexes and 13 different *spa* types. 14 strains harboured at least one gene for SE or staphylococcal *like*-enterotoxins with *sec* and *sel* being the most common. All of the 12 isolates harbouring a gene encoding for SE types A-E were positively tested for in-vitro SE production. *LukF-PV*/*lukM* related to mastitis of ruminants was present in nine strains. On the other hand, virulence genes associated to the human host like *tst* and components of the immune evasion cluster (*sak*, *chp* and *scn*) were found in eight strains. The content of resistance genes was low, but *mupA* (encoding mupirocin resistance) was detected in the *S. argenteus* isolate.
Conclusion: The majority of strains isolated from sheep or goat raw milk cheese and from raw prawns harboured a variety of SE genes as well as some virulence factors but only very few resistance determinants. Despite the low number of samples that exceeded CFU counts mirroring improper hygienic conditions during processing findings of enterotoxigenic strains in both food matrices tested proof the necessity of applying process hygiene criteria to ensure consumer safety.

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216/LMP

Studies on degradation of the Maillard reaction product N-ε-carboxymethyllysine by the host's intestinal microbiota

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Introduction: During heat treatment of food, Maillard reaction products such as N-ε-carboxymethyllysine (CML) are formed. CML is suspected - among others - to promote aging and diabetes with long-term consequences (1). In the human gut CML is degraded under anaerobic conditions by the intestinal microbiota. Since the human microbiota is individualistic, depending on lifestyle and nutrition, degradation rates of CML may potentially show differences (2).

Aim: In this study we wanted to identify potential individual differences in CML metabolism under aerobic conditions between humans following a western diet and vegetarians. In addition, we were investigating the capability of the colonic microbiota - especially for the genera *Klebsiella* and *Enterobacter* - to use CML as an alternative nitrogen source.

Materials and Methods: Three stool samples were collected over three weeks from six healthy non-smoking donors, which had not taken any antibiotics in the previous three months. Three of them consumed a western diet and three were vegetarians. Fecal samples were suspended both in brain heart infusion broth (BHI) and in a nitrogen-deficient medium to a concentration of 25 % (w/v). CML was applied to the suspension and samples were incubated aerobically at 37 °C. Samples were taken immediately after CML addition and after 2, 4, 24 and 48 h and prepared for analysis via liquid chromatography-mass spectrometry (LC/MS) to measure final CML concentrations.

Result: A significant degradation of CML was observed for both media. CML decreased to "not detectable" within 48 h for BHI medium and approx. at about 25 % in the nitrogen deficient medium, in relation to the starting concentration. As no degradation of CML in nitrogen-deficient medium was found in preliminary studies with monocultures of different probiotic *Escherichia coli* strains (Symbioflor2 from SymbioPharm), we conclude that CML might be metabolized more efficient by genera *Klebsiella* and *Enterobacter.*

Discussion: Due to the individual composition of the intestinal microbiota differences of CML degradation between donors can be expected under aerobic conditions. Further studies are necessary to show if differences in the CML-related metabolic capacities of the intestinal microbiota are also associated with different types of nutrition (ovo-lacto vegetarian and non-specific western diet). Possibly, a specific diet is able to promote the degradation of CML by establishing a specific intestinal microbiota

References

[1] Hellwig, M., & Henle, T. (2014). Baking, Ageing, Diabetes: A Short History of the Maillard Reaction. Angewandte Chemie, S. 10482-10496.
[2] Hellwig, M., Bunzel, D., Huch, M., Franz, C. M., Kulling, S. E., & Henle, T. (2015). Stability of Individual Maillard Reaction Products in the Presence of the Human Colonic Microbiota. *Journal of Agricultural and Food Chemistry*, S. 6723-6730.

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217/LMP

***Staphylococcus aureus* Isolates from Meat Products Legally and Illegally Introduced to Germany from non-EU countries harbor a variety of antimicrobial resistances and virulence factors**

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Introduction: Foodstuffs, particularly products of animal origin, can serve as a vector for *Staphylococcus aureus*, a pathogen which can cause a wide variety of diseases ranging from mild skin infections to severe conditions such as toxic shock syndrome and necrotizing pneumonia. Furthermore, *S. aureus* can produce heat-stable enterotoxins, which can lead to symptoms such as nausea, diarrhea or abdominal cramping even after the respective bacteria have been destroyed by cooking.

Objective: In this study, we investigated *S. aureus* isolates recovered from meat and meat products confiscated from passengers returning from non-EU countries at two German airports and from samples of legally imported meats from non-EU countries. The aim was to characterize isolates in regard to their genetic relatedness as well as their antimicrobial resistance profiles and major virulence factors in order to assess the potential risks associated with these products.

Materials and Methods: A total of 297 samples were taken from confiscates seized during routine passenger controls at two German airports and, 151 samples were included from legally imported batches taken at the border inspection post at the Port of Hamburg and at a major German cash-and-carry wholesaler. Isolation of *S. aureus* was performed according to ISO 6887-2:2003 and DIN EN ISO 6888-1. Isolates were characterized by molecular methods (*spa* typing, MLST, macrorestriction analysis, microarray analysis) and antimicrobial susceptibility testing. MRSA isolates were further characterized by *dru* typing.

Result: Nine MRSA and 65 MSSA isolates were detected among the samples, all MRSA originating from illegally introduced poultry meat from Egypt. The MRSA isolates as well as 14 MSSA from legally and illegally imported samples from 8 different non-EU countries were included in further investigations. *Spa* typing revealed 10 different *spa* types, most prevalent was t127. Overall, 9 different MLST sequence types were detected. Three of these were novel sequence types and were assigned to ST3216 (CC15), ST3217 (CC80) and ST3218 (CC5). A variety of antimicrobial resistances was detected and various antimicrobial resistance genes were identified. Microarray analysis revealed a number of different virulence genes. The Panton-Valentine leukocidin (PVL) encoding genes were detected in 6 isolates, all MRSA and 15 isolates were positive for at least one staphylococcal enterotoxin (SE) gene. The toxic shock syndrome toxin gene (*tst1*) was detected in 2 CC5 MSSA isolates.

Conclusion: Despite the common origin of MRSA isolates, a considerable heterogeneity was detected among the isolates. Furthermore, a plenitude of major virulence factors and antimicrobial resistances was detected in the *S. aureus*. This highlights the risks associated with contaminated food of animal origin and the transportation of such products among different countries.

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218/LMP

Psychrotolerant spores in microfiltered and pasteurized ESL milk - spoilage potential and route of transmission

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Extended shelf life (ESL) milk that is produced by combined microfiltration and pasteurization has a given shelf life of 24 days. The main factor limiting this durability is the end product's microbiota. In order to adapt the production process in a way that reduces spoilage organisms this project was aimed to identify those bacteria and to investigate their origin and routes of transmission.
For this, 291 packages of microfiltered and pasteurized milk were stored at 8°C until end of shelf life and total bacterial counts as well as the dominant species in the microbiota were determined. Containing at least 10^6 cfu/mL 14% of all packages were spoilt. Apart from gram-negative contaminants and high G+C gram-positive bacteria, psychrotolerant spores were the most important group in those end products. Mainly represented by *Paenibacillus* sp. and *Bacillus cereus sensu lato* they were isolated from 52% of all spoilt packages.
In order to determine whether spore forming bacteria in ESL milk originate from raw milk or are contaminants of the manufacturing process six large-scale process analyses were performed. Combined separation, microfiltration and pasteurisation reduced the spore load by 4 $\log_{10}$ units. A comparison of species found in raw milk and end product reveals diverging findings. The most dominant species in raw milk, *Paenibacillus xylanexedens* that represented about 49% of all isolates in biodiversity analyses was not detected in ESL milk at all. The spores were obviously effectively removed by combined microfiltration and pasteurization. In contrast, *Bacillus cereus* accounted for only 1% of psychrotolerant spores in raw milk, but for 40% of isolates in end products. This high percentage is most likely caused by contamination during manufacturing.
Regarding the risk of premature spoilage these data emphasise the role of plant hygiene in ensuring an impeccable microbial quality of ESL milk during the entire shelf life.

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219/LMP

Integrated analysis pipelines for whole genome MLST and whole genome SNP in BioNumerics® 7.6, applied to publicly available *Escherichia coli* isolates

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Question: Enterohemorrhagic *Escherichia coli*O157:H7 occur in the intestinal tracts of warm-blooded mammals, and is a major foodborne pathogen causing severe disease in humans worldwide. Outbreaks have been traced to both food and environment e.g. undercooked meats, apple juice, salad, dairy products and contaminated water.
Molecular subtyping methods for detecting outbreaks of *E.Coli* have mainly been Pulsed-field electrophoresis (PFGE) and Multi Locus Variant Analysis (MLVA). Nowadays, whole genome sequencing (WGS) methods offer an important advantage as the ability to rapidly extract relevant information from large sequence data files can considerably speed up the outbreak detection.
We present two pipelines for high resolution WGS-based molecular typing: whole genome multilocus sequence typing (wgMLST) and whole genome single-nucleotide polymorphism analysis (wgSNP). Both strategies are applied onto public datasets from *E. Coli* O157:H7 outbreaks.
Method: A typing scheme has been created by extracting all coding regions from a set of reference organisms, resulting in a set of discernible wgMLST loci. Two independent allele calling approaches are applied, an assembly-free and a BLAST-based allele calling algorithm, to determine locus presence and detect allelic variants in a quality-controlled manner. A wgSNP pipeline, tuned to reduce false positives while maximizing resolution, detects SNP variants by mapping the WGS reads to a reference sequence. For both methods, calculation-intensive data processing steps are performed on the BioNumerics® Calculation Engine, deployed locally or in the cloud.
Result: We demonstrate that wgMLST is suitable for the rapid analysis of large datasets, making it a useful technique for outbreak surveillance. The added resolution of wgSNP against an internal reference sequence increases the confidence in the detected clusters. BioNumerics® 7.6 offers a powerful platform where both wgMLST and wgSNP analysis can be performed at high-throughput rates. Using this platform, WGS analysis results can be validated against traditional data, rapidly providing a robust, portable and high resolution picture of molecular typing data.
Conclusion: The combination of two complementary approaches, on a virtually unlimited number of samples, managed by a single software platform that also stores metadata, opens many perspectives for food safety and public health monitoring programs. The BioNumerics® solution combines the power of a cluster or cloud implementation with the ease of use of a local database and management software. The possibility to extract 'historical' typing data, resistance and virulence data from wgMLST schemes, moreover reduces the total analysis time and cost, and may lead to more efficient outbreak detection.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

220/LMP

Molecular Interaction of Subtilase Cytotoxin subunits of pathogenic *E. coli*

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Subtilase Cytotoxin (SubAB) is an AB_5 bacterial toxin expressed by several Shiga toxin-producing *E. coli* (STEC) strains. These toxins are composed of an enzymatically active A subunit and a pentamer of B subunits responsible for the interaction of the toxin with cell surface structures of specific target cells. Until today, four different variants of SubAB were described. $SubAB_1$ was the first discovered variant and the only one located on a virulence plasmid (Paton et al. 2004).
Whereas the function of SubAB has been investigated on a cellular level in detail, basic biochemical properties are still open to be addressed. Two questions are of main interest for a better understanding of the biochemical properties of $SubAB_1$ association. First, the mode of association has to be clarified: does the $SubB_1$ subunit bind as a pentamer in one step of association or does the assembly of the holoenzyme occur in a stepwise manner? Second, the binding affinity needs to be examined.
The oligomerization of both subunits was characterized with size exclusion chromatography. $SubA_1$-His and $SubB_1$-His were separately recombinantly expressed and purified via a His-Tag approach followed by a gel filtration step. For $SubA_1$-His, a single monomeric species was found after purification. $SubB_1$-His elutes as a single peak with a molecular size corresponding to a pentamer.
The K_d of the association of $SubA_1$-His und $SubB_1$-His were determined by microscale thermophoresis. This method is based on the detection of changes in the movement of a fluorescently labelled protein in a microscopic temperature gradient when titrated with a non-fluorescent binding partner. For our preliminary analysis presented here we labeled $SubA_1$-His randomly on Lysine

residues with the NT-495 dye and conducted the titration with unlabeled $SubB_1$-His. A K_d in the low micro molar range could be determined with this setup.
These results indicate a binding of a stable $SubB_1$ pentamer in a single step reaction to the $SubA_1$ subunit. The presence of the His-tag can deteriorate the binding affinity between the subunits, however this has to be analyzed in further studies. In addition, we want to investigate if the K_ds of the different variants are related to their cytotoxicity and if the formation of SubAB chimeras displays any peculiarities in respect to their biochemical properties.

Reference
Paton, AW. et al., J Exp Med. 2004 Jul 5;200(1):35-46.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

221/LMP
Usage of cold hydrogen peroxide vapour for inactivation of murine norovirus, a surrogate for human norovirus
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Introduction: Human norovirus (family *Caliciviridae*) is responsible for gastroenteritis outbreaks worldwide. For research studies, surrogates as murine norovirus (MNV) are utilized for studying norovirus infection due to the lack of a cell culture for human norovirus. Hydrogen peroxide vapour (cold fogging decontamination) has been used as effective application for decontamination, e.g. hospital rooms or cleanrooms. Additionally, own investigations indicate a reduction of human norovirus titer on plastic carriers using hydrogen peroxide vapour. As norovirus contamination of food products is a persistent problem, the current study examines, if aerosolized hydrogen peroxide is an effective agent for decontamination of norovirus as contaminant of fruits and vegetables.
Method: For detection of an effect of liquid hydrogen peroxide to MNV particles, MNV aliquots were incubated with liquid hydrogen peroxide (Diosol Pure 19%, DIOP GmbH & Co. KG) for 60 min. Cold hydrogen peroxide vapour procedure was performed in a defined treatment chamber (30 min, 60 min). For treatment of artificial inoculated apple peal, different volumes of Diosol Pure were vapourized while temperature, relative humidity and hydrogen peroxide content of air were recorded. Recovery of MNV was achieved with swabs based on DIN EN ISO 15216-2. After RNase treatment for elimination of free RNA, real-time RT-PCR was used for relative quantification of intact capsids. In parallel, plaque assay was used to determine infectivity of virus particles.
Result: The first approaches were supposed to demonstrate the effect of liquid hydrogen peroxide solution used for vapourization on MNV in general. Incubation of MNV with ≤ 2% hydrogen peroxide hydrogen peroxide for 60 min showed a reduction of MNV titer in relation to increasing hydrogen peroxide concentration detectable by real-time RT-PCR. Using hydrogen peroxide vapour for decontamination of artificially contaminated apple peal, no reduction of MNV was determined by real-time RT-PCR. However, plaque assays led to first assumptions about a slight reduction of MNV infectivity due to cold hydrogen peroxide mist procedure.
Conclusion: The reduction of intact MNV particles with liquid hydrogen peroxide was shown. The first examinations regarding the impact of cold hydrogen peroxide vapour on artificially with MNV inoculated apple peal lead to the assumption that norovirus infectivity is reduced after treatment, although capsid structures seem to remain intact. Further approaches will aim at the confirmation of this hypothesis and the inactivation of MNV on surfaces of fruits and vegetables using aerosolized hydrogen peroxide.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

222/LMP
Occurrence and characterization of *Yersinia enterocolitica* Isolates from wild boars hunted in Lower Saxony, Germany
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Introduction: In Europe, Yersiniosis is the 4th most frequently reported bacterial foodborne disease. Yersiniosis is often associated with the consumption of undercooked pork contaminated with enteropathogenic *Yersinia* (*Y.*) *enterocolitica* of bioserotypes 4/O:3 and 2/O:9. The most important reservoir of the bacteria are domestic pigs which are predominantly asymptomatic carriers of *Y. enterocolitica*. In contrast to the occurrence of *Y. enterocolitica* in domestic pigs, data about the occurrence in wild boars in Germany are rare. In recent years, wild boar populations in Europe considerably increased, leading to increasing hunting activities and consumption of wild boar meat.
Objective: Aim of the study was to get knowledge about the occurrence of (pathogenic) *Y. enterocolitica* isolates in wild boars hunted in Lower Saxony, Germany, and to characterize these isolates.
Materials and Methods: Samples of tonsils from 111 wild boars were microbiologically (according to ISO 10273:2003) investigated for the presence of *Y. enterocolitica* and isolates were confirmed by MALDI-TOF analysis. Subsequently, isolates were bio- and serotyped and the presence of selected virulence factors was determined by PCR and real-time PCR. In addition, macrorestriction analysis and multilocus sequence typing (MLST) were conducted. Antimicrobial susceptibility testing of isolates was performed by using the broth microdilution method.
Result: Tonsils from 17.1% of 111 investigated wild boars were positive for *Y. enterocolitica* by culture methods. The majority of isolates belonged to biotype (BT) 1A and internal nucleotide sequence segments of *ystB* enterotoxin genes were detected by PCR in all but one BT 1A isolates. In addition, the *ail* gene was detected in two of the BT 1A isolates. Only two isolates belonged to BT 1B but did not carry the virulence plasmid. A high degree of genetic diversity of the isolates was determined by macrorestriction analysis and MLST, although the isolates originated from only one geographical region. All isolates were resistant to ampicillin with MIC values of ≥32 µg/ml, but exhibited low MIC values for all other antimicrobial agents tested.
Conclusion: In conclusion, all but two isolates belonged to BT 1A, with the majority bearing a *ystB* enterotoxin gene. Two isolates carried the *ail* gene. The remaining isolates were identified as BT 1B, without carrying the virulence plasmid. The enteropathogenic bioserotypes 4/O:3 and 2/0:9, which are usually found in domestic pigs in Europe, could not be detected.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

319/LMP
Surveillance of extended-spectrum beta-lactamase producing Escherichia coli in dairy cattle farms in the Nile delta, Egypt
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Industrial livestock farming is a possible source of multidrug-resistant Gram-negative bacteria, including producers of extended

spectrum beta-lactamases (ESBLs) conferring resistance to 3rd and 4th generation cephalosporins. Limited information is currently available on the situation of ESBL producers in livestock farming outside of Western Europe. Therefore, a surveillance study was conducted from January to May in 2014 in four dairy cattle farms in different areas of the Nile delta, Egypt.
In total, 266 samples were collected including rectal swabs from healthy cattle (n=210), and environmental samples from the stables (n=56). All samples were screened for ESBL producing Escherichia coli using MacConkey agar supplemented with 1 mg/L cefotaxime. Suspected colonies of putatively ESBL producing E. coli were sub-cultured and subsequently characterized genotypically (DNA-microarray) and phenotypically (VITEK-2). The DNA-microarray based tests CarbDetect AS-1 and E. coli PanType AS-2 allowed the detection of a multitude of genes and their alleles associated with resistance towards carbapenems, cephalosporins and other frequently used antibiotics. In total, 114 (42.8%) ESBL producing E. coli were identified. The ESBL genes blaCTX-M15 (n=105), blaCTX-M9 (n=1), blaTEM (n=90) and blaSHV (n=1) were detected. Alarmingly, the carbapenemase genes blaOXA-48 (n=5) and blaOXA-181 (n=1) were found in isolates that also were phenotypically resistant to imipenem and meropenem.
This study is to the best of our knowledge the first report of a high prevalence of ESBL-/carbapenemase producing E. coli in dairy farms in Egypt. ESBL-/carbapenemase producing E. coli isolates with different underlying resistance mechanisms were common in investigated dairy cattle farms in Egypt. The global rise of ESBL and carbapenemase-producing Gram-negative bacteria is a big concern, and demands intensified surveillance, also in developing/emerging economies such as Egypt.

Presentation: Monday, 12 September 2016 from 17:00 – 18:00 in room Donauhalle.

POSTERSESSION 03
13 Sept. 2016 • 10.30-11.30

Microbial Pathogenicity (FG MP)

223/MPP

Candidalysin - mediated translocation of *Candida albicans* through the intestinal epithelial barrier

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Introduction: The opportunistic fungal pathogen *Candida albicans* is a common and mostly harmless inhabitant of the human gastrointestinal tract. However, this fungus can cause life-threatening blood stream infections in immunocompromised patients by translocating from the gut lumen into the blood stream. To date, the molecular mechanisms of this translocation are mainly unknown. Recently, a lesion-forming peptide toxin (Candidalysin) which derives from the gene *ECE1*, has been described to be critical for mucosal infection.
Aim: We want to analyze which routes of translocation (e.g. paracellular, transcellular) are preferred by *C. albicans*, with a focus on the role of Candidalysin during this process.
Method: For all our *in vitro* experiments differentiated Caco-2 C2BBe1 intestinal cell monolayers were confronted with different *C. albicans* mutants (including an *ece1*Δ/Δ deletion mutant, a *ece1*Δ/Δ+*ECE1* revertant and a mutant lacking only the Candidalysin-encoding sequence) to assess their virulence potential in this model. We quantified barrier function integrity (electrical resistance, TEER), damage (LDH assay) and translocation. We also used different microscopic techniques to get deeper insights into the cellular processes of translocation.
Result: Candidalysin is an important factor involved in the translocation process. Our data suggest that Candidalysin induces necrosis of intestinal cells leading to a barrier breakdown of the monolayer and translocation through these gaps. However, hyphae of *C. albicans* may also penetrate the monolayer via a transcellular mechanism without causing severe damage. The possibility of a paracellular translocation by degrading tight junctions is still under investigation. Apoptosis and induced endocytosis as translocation mechanisms can be excluded based on our results.
Conclusion: We conclude that the *in vitro* translocation of *C. albicans* through intestinal cells is mainly mediated by invasive hyphae penetrating the monolayer and inducing damage by means of the Candidalysin toxin.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

224/MPP

***Yersinia enterocolitica* effectors YopM and YopP have opposite and concordant effects on cytokine gene transcription**

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The capability to inject effector proteins (Yops) into innate immune cells through its type-III-secretion system is essential for the virulence of pathogenic *Yersinia*. YopP/YopJ is well known to block the nuclear factor-kB dependent expression of proinflammatory cytokines in infected macrophages. The effect of YopM on expression of cytokines is less well understood, although its eminent role for virulence of *Yersinia* in infected animals has also been related to the subversion of cytokine gene expression and -production.
To understand the interplay of YopM and YopP on cytokine expression we analyzed the global transcriptional response of

primary human macrophages to infection (6 h) with i) wild type *Yersinia enterocolitica* WA314, ii) a YopM deletion mutant (WA314ΔYopM), iii) a YopP deletion mutant (WA314ΔYopP) and iv) a YopM/YopP double deletion mutant (WA314ΔYopMP). Analysis of RNA-Seq data revealed a great number (from 158 to 480) differentially expressed genes (DEGs) in the four groups of infected macrophages (foldchange 2; adjusted p value<0.05). A Kyoto Encyclopedia of Genes and Genomes (KEGG)-pathway analysis of these DEGs showed that in the presence of YopP (WA314ΔYopM group) YopM upregulated many of the immune response pathways (i.e. Jak-Stat-, Toll-like receptor signaling and Cytokine-cytokine receptor interaction) that YopP downregulated. Thus, YopM can act as an antagonist of YopP under this condition. Surprisingly, however, in the absence of YopP (WA314ΔYopMP group) YopM downregulated many of the immune response pathways that were normally downregulated by YopP and notable also those that it upregulated in the presence of YopP. This YopP-dependent variable gene expression pattern stimulated by YopM in macrophages was confirmed by qPCR for IL-1beta, IL-10, IL-6 and IL-12beta.
We conclude that the effect of YopM on cytokine expression in macrophages depends on the context of infection (i.e. whether YopP is active or not) and includes inhibitory as well stimulatory activities on cytokine expression.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

225/MPP

Identification and characterization of in vivo repressed genes detrimental for colonization fitness of *Vibrio cholerae*

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Vibrio cholerae is a Gram-negative bacterium, which is responsible for the water-borne, epidemic secretory diarrheal disease cholera. The bacterium is able to live in aquatic environment and human host by the oral route of infection. The way of infection, orally ingested *V. cholerae* pass through stomach before they reach the primary site of infection, i.e. the small intestine, for colonization. Thus, they traffic from acidic to alkaline environments, respectively.
In the past, research has primarily focused to identify genes induced during *in vivo* conditions in order to identify important virulence factors, which are required for proper colonization. Here we introduce a novel technique, which provides of identification *in vivo* repressed (*ivr*) genes. We hypothesized that such *in vivo* repressed genes might be detrimental for the colonization fitness and therefore need to be turned off during the in vivo passage. Thus, they might deliver important insights in the *in vivo* physiology of *V. cholerae* and may offer new therapeutic interventions. By screening of *V. cholerae* pools composed of approx. 20.000 random, independent reporter-fusion strains in murine model of cholera, 101 *ivr* genes were identified. Our data confirmed that constitutive *in vivo* expression of some of these *ivr* genes results in severe defects during colonization of the gastrointestinal tract.
Interestingly, one of these *ivr* genes, is annotated as a CIC family member of chloride channel proteins. As characterized in this study by competition and survival assays as well as gene activity assay the encoded gene product is part of an acid detoxification system, which facilitates survival under low pH (stomach), but needs to be turned off under alkaline conditions (small intestine). Besides, it is also shown that the expression of this CIC chloride channel is activated highly under acidic condition, but not under alkaline, by AphB, which is a LysR-type activator that plays a key role to initiate the expression of the virulence cascade by cooperating with the quorum-sensing regulated activator AphA in *V. cholerae*.
The herein presented results highlight that the established method is a powerful tool to identify repressed genes in a given condition *(e.g. in vivo)*. We propose that downregulation of a subset of genes is essential for the colonization fitness of *V. cholerae* and almost as important as the induction of virulence factors. Moreover, chloride channel proteins play an important role for acid tolerance response during lifecycle of *V. cholerae* in the host.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

226/MPP

Key players of a type IV secretion system mediating antibiotic resistance spread among enterococci and staphylococci

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Discovery void of novel types of antibiotics and increasing antibiotic resistances in pathogenic bacteria present a serious health issue worldwide. Conjugative DNA transfer is the most important transmission path for antibiotic resistance and virulence gene dissemination among bacteria and is mediated by a protein complex, generally known as type IV secretion system (T4SS). Enterococci are the third most common cause of healthcare-associated infections, which include urinary tract infections, bacteremia and endocarditis. A conjugative model system in Gram-positive bacteria is the T4SS from broad-host-range plasmid pIP501, frequently encountered in nosocomial pathogens, such as *Enterococcus faecalis* and *Enterococcus faecium*. Postulated key factors of the conjugative transfer complex are the relaxase TraA, two ATPases, TraE and TraI/TraJ, the first putative two-protein T4S coupling protein, the muramidase TraG, the putative channel factors, TraF, TraK, TraL and TraM and the surface factor TraO[1]. To elucidate the role of the pIP501 Tra proteins we generated a number of single marker-less *tra* knock-out mutants in *E. faecalis* harbouring pIP501 by using a method consisting of two homologous recombination steps. Till now we have generated the deletion mutants, *E. faecalis* pIP501Δ*traE*, Δ*traF*, Δ*traG*, Δ*traH*, Δ*traK*, Δ*traL*, Δ*traM* and Δ*traN*. Biparental matings showed that TraE, TraF, TraG, TraH, TraL and TraM are essential for pIP501 conjugative transfer. *In trans* complementation of the respective deleted gene excluded possible polar effects on downstream genes in the *tra* operon. TraN has been shown to be a DNA binding protein, binding sequence-specifically upstream of the pIP501 *oriT nic* site[2]. It acts as a negative regulator of pIP501 transfer, presumably by either interfering with TraA relaxase activity or by negatively regulating *tra* operon expression. Generation of the knock-outs *traB, traC, traD, traI, traJ* and *traO* is in progress and will help decipher the pIP501 conjugative transfer machinery. Here we will present the current state of knowledge of the pIP501 T4S model system.

References

[1] Fercher C, Probst I, Kohler V, Goessweiner-Mohr N, Arends K, Grohmann E, Zangger K, Meyer NH and Keller W (2016). VirB8-

like protein TraH is crucial for DNA transfer in *Enterococcus faecalis*. Scientific Reports 6:24643, doi:10.1038/srep24643.
[2] Goessweiner-Mohr N, Eder M, Hofer G, Fercher C, Arends K, Birner-Gruenberger R, Grohmann E and Keller W (2014) Structure of the double stranded DNA binding type IV secretion protein TraN from Enterococcus. Acta Cryst Section D, Biological Crystallography, 70, 2376-2389.

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227/MPP

Binding partners of the SINC homologue CAB063 in *Chlamydia abortus* infection

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Introduction: *Chlamydia* (*C.*) *abortus* is an obligate intracellular zoonotic pathogen. It is the world's leading cause of ovine enzootic abortion and may lead to serious infections in pregnant women. Gene sequencing projects revealed that chlamydiae use a type III secretion system (T3SS) to deliver effector proteins to the host cell. Among them CAB063 was shown to be localised in the host cell nucleus.
Aim: Our aim is to elucidate the function of CAB063 by identification of putative binding partners.
Materials and Methods: HeLa cells were infected with *C. abortus* and harvested 48 h post infection. Whole cell lysate and nuclear fractions of infected and transfected cells were subjected to immunoprecipitation experiments using a purified anti-CAB063 rabbit serum. Immune complexes were separated by 1- and 2D-SDS-PAGE and protein bands or spots were identified by mass spectrometry based methods. Apoptosis and cell death were investigated in measuring Annexin V, FLICA and PI positive cells via flow cytometry. Morphological analysis of host cell nuclei was performed by indirect immuno fluorescence microscopy (IIF).
Result: We identified a panel of pro- and eukaryotic binding partners of CAB063 including the Heat Shock Protein 70, which is a chaperone and contributes to efficient secretion of T3SS effector proteins. Further prokaryotic binding partners included Polymorphic Membrane Protein D and elongation factor Tu. Possible eukaryotic binding partners include Pre-Lamin A/C that plays an important role in mitosis, the heterogeneous nuclear ribonucleoprotein K, which interacts with RNA p21 and the ATP-dependent RNA helicase DDX5 that is involved in p53/TP53-dependent apoptosis. In experimentally infected HeLa cells a significant increase of apoptotic cells could be detected at the end of the chlamydial developmental cycle. In CAB063-transfected HeLa cells we also identified an increased rate of apoptotic cells compared to untreated or GFP-transfected HeLa cells. In addition characteristic lobular morphological changes of the host cell nucleus were observed in CAB063 transfected cells.
Conclusion: We could confirm that CAB063 is localized in the host cell nucleus. Also we identified HSP70, as a putative prokaryotic and Lamin A and DDX5 as putative eukaryotic binding partners of CAB063. Additionally, our results suggest that the type III secreted CAB063 may induce both host cell apoptosis at the end of the developmental cycle and typical lobular nucleic changes.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

228/MPP

Assembly and protein export mechanisms of the bacterial flagellum of *Salmonella* Typhimurium

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Background: Gastrointestinal infections by *Enterobacteriaceae* pose a serious health risk in developing and developed countries. One causative agent of gastrointestinal diseases is the gram-negative, food-borne pathogen *Salmonella enterica* serovar Typhimurium. *Salmonella* uses rotation of a helical organelle, the flagellum, for directed movement in various environments. The bacterial flagellum is closely related to virulence-associated injectisome systems of many pathogenic bacteria. Both nanomachines utilize a type-III secretion system (T3SS) to export proteins across the inner membrane in a proton motive force (pmf)-dependent manner. Type-III protein secretion is essential for the assembly of the flagellum, the injectisome, as well as for secretion of effector proteins.
Question: The filament of the flagellum is made of several thousand subunits of a single protein, flagellin, and extends several micrometer beyond the cell surface. The T3SS-dependent export of flagellar building blocks is a remarkable fast process and more than 1500 amino acids per second are transported during filament growth. A fundamental problem concerns the molecular mechanism of how the long, external filament grows at a rapid rate in the absence of any cellular energy sources.
Method: We determined for the first time the growth rate of single flagella using *in situ* labeling and real-time immunostaining of growing flagellar filaments. The growth rate data was combined with mathematical modeling to generate a biophysical model of flagellum growth.
Result: Here, we present a molecular mechanism to explain the growth of flagellar filaments based on simple biophysical parameters. We provide experimental evidence to demonstrate that growth of flagella follows a saturated diffusion mechanism and decreases with length. We determined the growth rate of single flagella using *in situ* labelling and real-time immunostaining of growing flagellar filaments. The growth rate data revealed a negative correlation between the rate of filament polymerization and the length of the flagellum. Addition of uncoupling agent that disrupted the pmf prevented filament elongation. Growth was resumed after removal of uncoupler, indicating a major contribution of the pmf in driving flagellin export. Competitive export of flagellin mutant proteins deficient in head-to-tail chain linkage did not impair the flagellum growth rate. While inter-subunit interactions between flagellin monomers might be important during substrate docking, these results suggest that the pulling force of chain of flagellin molecules does not contribute substantially to the filament elongation dynamics.
Conclusion: In summary, we propose a flagellum growth model based on simple biophysical parameters where the filament growth rate is driven by both hindered diffusion and proton motive force-dependent secretion of subunits.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

229/MPP

The impact of the rhomboid protease on virulence of *Listeria monocytogenes.*

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Introduction: Rhomboids belong to a conservative family of serine proteases that target domains reside immersed inside the

membrane (1). In the last years, the rapid progress in the study of biochemical mechanisms allowed an establishment of structure-function relationships of rhomboids and their targets (2; 3). The putative rhomboid protease encoded by the *rpl* gene (for **r**homboid **p**rotease of **L**isteria) was identified in the Gram-positive pathogenic bacterium *Listeria monocytogenes.* rpl is the first gene of the operon, which includes a putative single-stranded nucleic acid-binding protein encoding gene and a putative glucokinase-encoding gene. The mutant strain with an insertion into the rpl gene was created. The mutation caused a noticeable drop in abundance of certain membrane-bound proteins such as elongation factors EF-Tu and EF-G, glyceraldehyd-3-phosphate-dehydrogenase (GAP) and dihydrolipoamide-acetyltransferase E2 subunit (E2 PDH) (4). The revertant that lost an insertion restored the parental phenotype.

Objective: To establish the impact of rhomboid on L. monocytogenes virulence

Materials and Methods: The *L.monocytogenes* EGDe strain and it derivative lacking Rpl (4) were used. *L. monocytogenes* was grown in BHI medium. The lecithinase reaction was carried out as described (Ermolaeva et al., 1997). The haemolytic activity was evaluated as the ability to cause a lysis of red cells according to the dilution of bacterial supernatant in a 96 well plate. Invasion rates in HEK293 cells were determined in "gentamycin assay". To investigate the ability of mutant strain to form biofilms, bacteria were grown in polystyrene microtiter plates for 24h and 48h, then they were stained using 0,01% crystal violent solution. The intensity of formed biofilms was measured with the photometer.

Result: The mutant strain without Rpl showed a ten-fold drop of a haemolytic activity compared with the parental strain. The mutant strain had lower lecithinase activity than parent one.

Invasion rates into eukaryotic cells were 0.10% and 0.05% for the parental and mutant strains, respectively (Fig.1). The intracellular growth was evaluated in 2, 4, 6 hours after infection and was also reduced for the mutant strain.

The mutant strain with an insertion into the *rpl* gene formed the biofilms faster, but on the second day an exfoliation of bacteria occurred, while the parental strain continued to form biofilms (Fig. 2).

Conclusion: The rhomboid protease Rpl is important for L. monocytogenes virulence and biofilm formation**.**

References

[1] Urban, S. (2013). *Biochim. Biophys. Acta* 1828, 2797-2800.

[2] Vinothkumar, K.R. (2011) *J. Mol. Biol.* 407, 232-247.

[3] Ha, Y., Akiyama, Y., Xue, Y. (2013) *J. Biol. Chem.* 288, 15430-15436.

[4] Sysolyatina, E., Chalenko Y. Ermolaeva, S., Surin, A. (2014) *Biospektrum*, 85.

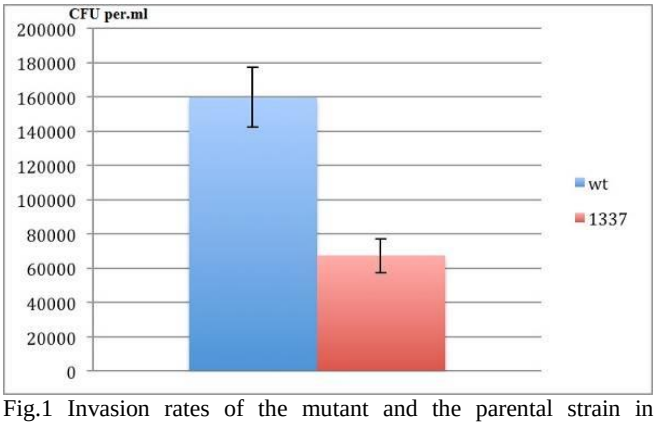

Fig.1 Invasion rates of the mutant and the parental strain in HEK293 cells.

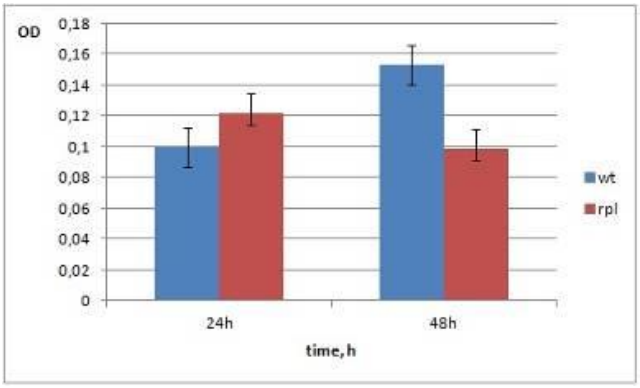

Fig.2 The thickness of the biofilm formed by the mutant and the parent strain

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

230/MPP

Naturally occurring variants of the invasion factor InlB differentially support Listeria monocytogenes infection

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Introduction: The Gram-positive bacterium *Listeria monocytogenes* causes severe infection in humans and animals. *L. monocytogenes* enters mammalian cells, disrupts the phagosome and multiplies within the cytoplasm. The virulence factor InlB induces bacterial uptake. InlB targets the eukaryotic tyrosine kinase receptor Met. InlB has dual functions and behaves as an invasin and a growth factor [1]. Purified InlB was shown to activate Met-regulated signaling pathways, which include but are not restricted to those that induce actin rearrangements and bacterial uptake [2].

We identified 16 InlB variants when we analyzed InlB diversity in *L. monocytogenes* strains isolated from lethal cases of prenatal listeriosis and wildlife [3]. The maximal likelihood analysis demonstrated that InlB variants belonged to 4 distinct clusters.

Objective: To reveal a functional difference between naturally occurring InlB variants.

Materials and Methods: The internal parts of 4 distinct inlB variants were cloned in pTRKH2 [4] that carried the inlAB promoter and the inlB fragment encoding GW-domain. GW-domain is responsible for InlB.

Presentation: bacterial surface and is not important for InlB behavior as a growth factor. Obtained plasmids were introduced into the L. monocytogenes strain EGDeΔinlB. The recombinant and parental strains were tested in gentamycin assay. BALB/c mouse infection was performed by intragastric, intravenous and intraperitoneal routes. To get purified InlB, the same inlB variants were cloned in pET28b vector and His-tagged proteins were purified.

Results: All InlB variants restored invasion of the parental strain in epithelial human HEK293 and murine C26 cells. The invasion rates were similar for recombinant strains and exceeded invasion rates of the parental strain by a factor of 2log10.

Meanwhile, recombinant strains demonstrated different virulence in mice in dependence on infection route. Two InlB variants found in wild rodents were maximally effective when bacteria were applied intragastrically. In contrast, the "non-murine" variants provided highest rates of intravenous and intraperitoneal infection. Then, we purified 4 InlB variants and studied Met activation in Hep-2 cells.

Conclusion: Naturally occurring InlB variants differed in their ability to support infection in mice. This difference weakly correlated with differences in cell invasion rates. InlB behavior as

an invasion factor activating Met-dependent cell response seems to be responsible for observed differences in infection rates.
The work was supported by RSF (grant 16-15-00091).

References
[1] Bierne H, Cossart P. (2002) J Cell Sci 115:3357-3367.
[2] Jiwani S, Wang Y, et al. (2012) Infect Immun. 80:1252-1266
[3] Adgamov R, Zaytseva E, et al.(2012). In: Genetic Diversity in Microorganisms, M. Caliskan (Ed.), InTech, DOI: 10.5772/32913.
[4] O'Sullivan DJ, Klaenhammer TR. (1993). Gene.137:227-231.

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231/MPP

S. aureus α-toxin-dependent xenophagy occurs in the absence of ADAM10

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Introduction: *Staphylococcus aureus* is able to invade various cell types (1). A fraction of intracellular *S. aureus* is consistently found in LC3(microtubule associated protein light chain 3)-decorated compartments, presumably autophagosomes (2, 3). The bacteria seem to subvert autophagy, as they evade killing and divide. Generally, damage of the phagosomal membrane is thought to trigger xenophagy. Notably, efficient recruitment of LC3 depends on α-toxin (3, 4). Together, this implies that α-toxin can cause damage to the phagosomal membrane, suggesting that it binds to a cellular α-toxin receptor on the luminal side of the endosomal membrane. Because ADAM10 is the putative high affinity receptor for α-toxin (5), we investigated its role in α-toxin-dependent xenophagy.
Aim: Aim of this study was to clarify, if binding and action of *S. aureus* strictly depends on its putative high affinity receptor ADAM10.
Materials and Methods: Cells lacking ADAM10 were transfected with EGFP-LC3 and infected with *S. aureus* strains expressing wild type α-toxin or a non-lytic mutant to study requirements for LC3-recruitment to *S. aureus* containing compartments.
Result: Although we confirm that ADAM10 mediates binding of *S. aureus* α-toxin to the cell surface, efficient recruitment of LC3II to *S. aureus* containing vacuoles in cells did not depend on ADAM10 (6), but lytic activity of α-toxin was required. Therefore, ADAM10 appears to be dispensable for α-toxin-mediated damage of internal membranes, suggesting a role of alternative receptors in this process (7).
Summary: ADAM10 is not required for efficient α-toxin-dependent recruitment of LC3 to *S. aureus*-enclosing internal membranes, although lytic activity of α-toxin is required.

References
[1] Fraunholz M, Sinha B. Intracellular Staphylococcus aureus: live-in and let die. Frontiers in cellular and infection microbiology. 2012;2:43.
[2] Schnaith A, Kashkar H, Leggio SA, Addicks K, Krönke M, Krut O. Staphylococcus aureus subverts autophagy for induction of caspase-independent host cell death. J Biol Chem. 2007;282(4):2695-706.
[3] Mestre MB, Fader CM, Sola C, Colombo MI. Alpha-hemolysin is required fot the activation of the autophagic pathway in Staphylococcus aureus-infected cells. Autophagy. 2010;6(1):110-25.
[4] Kloft N, Neukirch C, Bobkiewicz W, Veerachato G, Busch T, von Hoven G, Boller K, Husmann M. Proautophagic signal induction by bacterial pore-forming toxins. Med Microbiol Immunol. 2010;199(4):299-309.
[5] Wilke GA, Bubeck Wardenburg J. Role of a disintegrin and metalloprotease 10 in Staphylococcus aureus alpha-hemolysin-mediated cellular injury. Proc Natl Acad U S A. 2010;107(30):13473-8.
[6] von Hoven G, Rivas AJ, Neukirch C, Klein S, Hamm C, Qin Q, Meyenburg M, Füser S, Saftig P, Hellmann N, Postina R, Husmann M. Dissecting the role of ADAM10 as a mediator of S. aureus alpha-toxin action. Biochem J. 2016
[7] Valeva A, Hellmann N, Walev I, Strand D, Plate M, Boukhallouk F, et al. Evidence that clustered phosphocholine head groups serve as sites for binding and assembly of oligomeric protein pore. J Biol Chem. 2006;281(36):26014-21.

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232/MPP

The pro-domain of ADAM10 is required for efficient binding of _S. aureus_ α-toxin to the plasma membrane of target cells

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Introduction: *Staphylococcus aureus* α-toxin is considered to be an important virulence factor of *S. aureus*. The metalloprotease ADAM10 is thought to serve as the high affinity receptor for α-toxin (1). Moreover, ADAM10-dependent cleavage of E-cadherin and consequential destruction of cell-cell contacts appears to enhance the severity of infections. However, it is unclear how binding, cytotoxicity and activation of catalytic activity are linked.
Aim: Aim of the present study was to delineate domains and sites of ADAM10 required for binding and cytotoxic action of α-toxin.
Materials and Methods: We exploited a panel of ADAM10 expression constructs in complementation experiments with human cells engineered by CRISPR/CAS9 to lack expression of ADAM10. Flow cytometry, surface labeling and pull-down experiments were used to study binding; cytotoxicity was measured using ATP-assays.
Results: This approach revealed that a functional catalytic site is not required for α-toxin binding, although a small molecule inhibitor of ADAM10 thought to block the catalytic site inhibited binding of α-toxin to cells. More importantly, deletion of the ADAM10 pro-domain impaired binding of α-toxin, and prevented toxin-dependent loss of cellular ATP. Although surface expression of the separate pro-domain did not rescue binding and action of α-toxin, co-expression of the pro-domain together with pro-domain deficient ADAM10 sensitized cells. Our data reveal that the chaperone activity of the ADAM10 pro-domain or co-binding of the cleaved pro-domain and additional parts of the metallo-protease is essential for α-toxin binding via ADAM10 (2).
Summary: The presence of ADAM10s pro-domain is indispensable for efficient binding of *S.aureus* α-toxin at the plasma membrane of target cells.

References
[1] Wilke GA, Bubeck Wardenburg J. Role of a disintegrin and metalloprotease 10 in Staphylococcus aureus alpha-hemolysin-mediated cellular injury. Proc Natl Acad Sci U S A. 2010;107(30):13473-8.
[2] von Hoven G, Rivas AJ, Neukirch C, Klein S, Hamm C, Qin Q, Meyenburg M, Füser S, Saftig P, Hellmann N, Postina R, Husmann M. Dissecting the role of ADAM10 as a mediator of S. aureus alpha-toxin action. Biochem J. 2016.

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233/MPP

Identification of a novel regulator controlling *Staphylococcus epidermidis* biofilm formation in the presence of serum

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Background: *Staphylococcus epidermidis* is a leading cause of implant associated-infections, and biofilm formation is thought to significantly contribute to the species' success in this clinical setting. Previous work has shown that the presence of serum induces biofilm formation in some *S. epidermidis* strains.
Hypothesis: The aim of this study was to characterize serum-induced *S. epidermidis* biofilm formation in more detail, and to identify genes contributing to biofilm assembly under this growth condition.
Result: PIA-producing *S. epidermidis* reference strain 1457 formed strong biofilms in TSB as well as TSB containing 50 % serum. In contrast, *S. epidermidis* 1585 was biofilm-negative in TSB, but became biofilm-positive in the presence of serum. Screening of a Tn*917* transposon mutant bank derived from *S. epidermidis* 1585 identified four mutants exhibiting a biofilm-negative phenotype after growth in serum. Sequencing of Tn-flanking chromosomal regions showed that all mutants carried insertions within an open reading frame encoding for a so far uncharacterized transcriptional regulator, preliminaryly referred to as serum response regulator (Srr). Growth in serum induced *srr* expression in *S. epidermidis* 1585, indicating that Srr is relevant for adaptation to this condition. *In trans* complementation of *srr* fully rescued the biofilm positive phenotype, unambiguously linking the biofilm-negative phenotype with inactivation of *srr*. Further analysis of 1585*srr*::Tn917 identified changes in AtlE- and Embp-production. Consistent with phenotypic assays, these changes indicate that *srr* is crucial for expression of mechanisms involved in adherence as well as intercellular adhesion and biofilm accumulation.
Conclusion: This is the first systematic approach to identify genes relevant to serum-induced biofilm formation. *srr* is a novel regulator, influencing biofilm accumulation through pleiotropic effects on adherence properties and intercellular adhesion. In depth analysis of regulatory circuits and functional molecules depending on *srr* expression will provide important insights into mechanisms allowing *S. epidermidis* to adopt to hostile environments to cause device-related infections.

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234/MPP

***Candida albicans* and trace metal**

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Zinc serves as both structural and catalytic cofactor in many enzymes and is required for a multitude of biological processes. The availability of zinc is recognized as a central factor in infections.
In the vast majority of humans, *Candida albicans* co-exists as a harmless commensal. However, this fungus may cause severe infections when the host's immune system is compromised.
We are investigating the transcriptional response of *C. albicans* to zinc limitation (ZL). To this end, we deprived *C. albicans* of zinc *in vitro* and performed transcriptome analyses. As expected, the 'zincophore' zinc acquisition system, consisting of secreted Pra1 and the zinc transporter Zrt1, was up-regulated during our *in vitro* zinc starvation.
Another mutant, lacking the central zinc importer Zrt2, showed a severe growth defect under ZL. Additionally, it demonstrated an up-regulation of the zinc uptake machinery even in the presence of zinc, indicating intracellular zinc starvation. *Zrt2*Δ is hence largely unable to obtain free zinc, but we found that it is still able to utilize zinc citrate. Therefore, we hypothesize that there is an additional zinc citrate uptake machinery in *C. albicans*.
To analyse in details the network of zinc homeostasis, the growth of 165 transcription factor (TF) mutants (Homann *et al.*, PLoS Genet., 2009) was checked under ZL. A severe growth defect was observed in *ssn6*Δ. Moreover, a transcriptional profiling displayed reduced transcript levels of *ZRT2* and *ZRT3*, a vacuole zinc exporter, and increased *CSR1*, a TF essential for zinc up-take genes expression, in *ssn6*Δ in comparison to WT under ZL.The obtained results revealed Ssn6 as a new component of the transcriptional basis for zinc homeostasis. The potential interaction of Ssn6-Csr1 will be checked via Bimolecular fluorescence complementation (BiFC).

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235/MPP

Survival of *S. pneumoniae* in sputum of ICU patients.

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Introduction: *Streptococcus pneumoniae* and *Staphylococcus aureus* are known causative agents of ventilator-associated pneumonia (VAP), which leads to increased morbidity and mortality of intensive care unit (ICU) patients. To date, it is not known to which stresses these bacteria are exposed in airways of ventilated patients.
Objective: The present studies were therefore aimed at establishing an *ex vivo* assay that mimics the *in vivo* situation through incubation of bacteria in sputum samples collected from different ICU patients.
Materials and Methods: Wild-type *S. pneumoniae* TIGR4 and different virulence-attenuated mutants were incubated in sputum samples from mechanically ventilated ICU patients from the University Medical Center Groningen. Next, bacterial survival in the sputum samples was tested by plating. *S. pneumoniae* was also used in a sputum spotting assay, where sputum was spotted on a

blood agar plate and the inhibition zones were compared to those observed for *Streptococcus anginosus* and *S. aureus*.
Results and Conclusions: The tested *S. pneumoniae* strains showed different rates of survival in sputa from different patients. Intriguingly, the spotting assay revealed major differences in the bactericidal activity of sputa from different patients. Our ongoing research is aimed at assessing both patient parameters that affect bacterial survival in sputum as well as the bacterial gene repertoire needed for survival.

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236/MPP
Regulation of phospholipases D virulence factors in *Acinetobacter baumannii*
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Introduction: Nosocomial infections with multiresistant bacteria are a rapidly emerging threat nowadays. Especially infections with multiresistant strains of the opportunistic pathogen *Acinetobacter baumannii*, which is subject of the studies within the DFG research unit 2251, have substantially increased over the last decade. Adaptation of *A. baumannii* to clinical habitats is fostered by its persistence on dry surfaces. Moreover, *A. baumannii* is well adapted to host cells, thriving under iron- and phosphate-limitation at low pH-value and under oxidative stress. However, information with respect to molecular mechanisms of adaptation to clinical environments and the human host is scarce.
Objective: Detection of three phospholipase D (PLD) genes in the genome of *A. baumannii* ATCC 19606 raised the question whether the PLD's play a role in infection and whether transcription of the *pld* genes is regulated by different physiological conditions found in host cells.
Materials and Methods: To analyse the role of phospholipases D in pathogenicity of *A. baumannii* a markerless mutagenesis system was established and used to generate *pld* mutants. These mutants were analysed in infection studies. Transcriptional regulation of the *pld* genes was analysed by promotor activity studies.
Result: All three phospholipases D of *A. baumannii* were found to play a role as virulence factors. Different stress conditions including growth phase and temperature were identified to affect *pld* gene expression.
Conclusion: The three PLD's act in a concerted manner as virulence factors and play a role in host cell invasion. In addition, the three *pld* genes undergo transcriptional regulation.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

237/MPP
Characterization of the *Acinetobacter baumannii* DNA-(adenine N6)-methyltransferase AamA and its regulon
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Questions: DNA methylation is a post-replicative epigenetic mechanism that affects gene expression without changing the DNA sequence. Base modifications regulate various physiological processes such as transcription and mismatch repair with an impact on virulence as well as motility. In γ-proteobacteria the DNA adenine methyltransferase (Dam) transfers a methyl group from S-adenosyl-L-methionine (SAM) to the N-6 position (m6A) of the adenine in GATC sequence. Also the *Acinetobacter baumannii* genome encodes such a methyltransferase, designated A1S_0222 in strain ATCC 17978. We hypothesized the adenine DNA methyltransferase A1S_0222 to impose epigenetic control in *A. baumannii* and approached its characterization.
Method: Making use of the naturally competent *A. baumannii* isolate 29D2 we were able to inactivate the gene A1S_0222. 29D2 wildtype and mutant 29D2 *aamA::Km* were phenotypically characterized and the A1S_0222 protein was recombinantly produced. Moreover, single-molecule real-time (SMRT) sequencing was performed on the 29D2 wildtype and its *aamA::Km* mutant for a comparative methylation pattern analysis.
Result: The phenotypical characterization illustrates a deficiency in surface-associated motility of 29D2 *aamA::Km* and increased susceptibility to antibiotics compared to the wildtype. Further, the mutant is attenuated in the *Galleria mellonella* infection model. A1S_0222 was recombinantly produced as a GST fusion protein and purified to near homogeneity after cleaving off the GST tag. Subsequently, the recognition site of DNA adenine-methyltransferase A1S_0222 could be identified as GAATTC. SMRT sequencing was performed and data were screened for m6A motifs. It could be observed that mutant 29D2 *aamA::Km* lost the A1S_0222-specific methylation pattern.
Conclusion: Taken together, the results demonstrate that A1S_0222 encodes a DNA adenine-methyltransferase which methylates the GAATTC sequence. SMRT sequencing revealed differences in the methylation pattern between 29D2 wildtype and its A1S_0222 mutant which may reflect a regulon that accounts for the phenotypic appearance. We propose the name AamA (*Acinetobacter* DNA adenine-methyltransferase A) for A1S_0222 and *aamA* for the gene.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

238/MPP
The biochemical characterization of two distinct phospholipases C of *Acinetobacter baumannii*
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Introduction: In the past decades A. baumannii became an emerging threat in hospital environments which is subject of the studies within the DFG research unit 2251. Multidrug resistance paired with an array of different virulence factors have led to the development of epidemic lineages with growing mortality rates. Among the virulence factors of A. baumannii are phospholipases C (PLC) and phospholipases D (PLD). Recently, we have identified three PLDs of A. baumannii mediating virulence and being important for cell adhesion and invasion (1). In addition, one of the two PLCs encoded in the genome of A. baumannii has already been shown to mediate cytotoxic effects on epithelial cells (2). Objective: To get insights into the physiological role of both PLCs we performed biochemical analyses.
Method: The A. baumannii plc genes were heterologously expressed as his-tag fusion proteins in E. coli and purified by Ni-NTA affinity chromatography. Biochemical analyses were performed with the purified PLCs.
Result: Both PLCs cleaved the cromogenic substrate p-nitrophenol phosphorylcholine giving rise to the products phosphorylcholine and p nitrophenol. Maximum specific activity of both PLCs was detected between 37 °C and 40 °C. However, the maximum specific activity of PLC2 was 10-fold higher than higher than the one of PLC1. Furthermore, the activity of both PLCs differed significantly in dependence of the pH. The PLC1 exhibited maximum specific activity at pH 7 whereas the optimum for PLC2 activity was pH 9.
Conclusion: The differences of the PLCs in pH optimum and maximum specific activity suggest that the PLCs of A. baumannii play distinct roles under different physiological conditions.

References
[1] Stahl J., Bergmann H., Göttig S., Ebersberger I., Averhoff B., (2015) PloS One 10:e0138360.
[2] Camarena L., Bruno V., Euskirchen G., Poggio S., Snyder M.. (2010) PLoS Pathog 6:e1000834

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239/MPP

Immune evasion of *Borrelia miyamotoi*: CbiA, a novel outer surface protein exhibiting complement binding and inactivating properties

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Question: Relapsing fever is caused by various *Borrelia* species including *B. miyamotoi*. Relapsing fever spirochetes are able to bind distinct complement regulators of the alternative (AP), classical (CP) and lectin pathway via certain outer surface molecules, all of which facilitate serum resistance. More recently, we showed that *B. miyamotoi* resists complement-mediated killing and survived in the presence of human serum. To gain deeper insight into the molecular principles of immune evasion of *B. miyamotoi*, we sought to identify potential determinant(s) contributing to serum resistance of this new emerging human pathogen.
Method: Using comparative sequence analysis, we identified a gene that showed 64% identity to the gene encoding the factor H-binding protein of *B. hermsii*. Due to the complement binding properties and regulatory activity on the CP, AP, as well as the terminal pathway (TP), this protein was termed CbiA (complementbinding andinhibitory protein). To analyze the functional activity of CbiA on complement, ELISA was performed and growth inhibition assays were conducted to investigate CbiA-mediated resistance on serum-sensitive spirochetes.
Result: Our analysis revealed that CbiA is an outer surface molecule displaying multifunctional properties, as this protein interacted with C3, C3b, C4b, and C5 as well as with complement regulator Factor H. Upon binding to CbiA, FH retained its cofactor activity for Factor I-mediated inactivation of C3b. The predominant Factor H-binding site within CbiA was mapped to the short consensus repeat 20 whereby the C-terminus of CbiA was involved in the binding of FH. Of importance, CbiA is able to directly counteract complement activation by inhibiting both the CP and TP. To examine the mode of action under more physiological settings, a serum-sensitive *B. garinii* strain was transformed with a shuttle vector harboring the CbiA encoding gene. Spirochetes producing CbiA on their surface were able to survive in the presence of human serum, indicating that this particular protein facilitates serum resistance.
Conclusion: In conclusion, our data revealed a strategy of *B. miyamotoi* to efficiently combat complement attack by producing an outer surface protein, CbiA that possesses both complement binding as well as complement inhibitory activities. Our data strongly suggest that this protein represents an important serum resistance factor which efficiently protects spirochetes from the destructive attack of complement.

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240/MPP

Export of the Phospholipase PlaB of *Legionella pneumophila*

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Introduction: The gram-negative pathogen *Legionella pneumophila*, transmitted by airborne water droplets, causes Pontiac fever and Legionnaires' disease, a severe and potentially fatal pneumonia. A new family of lipases, first described in *L. pneumophila*, is important for pathogenicity. The discovered phospholipase A, called PlaB, is cell-associated and highly active. In previous studies, PlaB has shown phospholipase A, lysophospholipase A and hemolytic activity. Furthermore, PlaB was found to localize to the bacterial outer membrane where it seems to become active.
Aim: The transport mechanism is still unclear since so far described protein export systems such as type IVB secretion system were not essential for PlaB export. Therefore, the transport mechanism should be analyzed in this work.
Method: To identify the responsible exporter of PlaB, the system "BioID" will be used. Here, the protein of interest (PlaB) is terminally tagged with the *E. coli* biotin protein ligase $BirA_{R118G}$ BirA*. BirA* promiscuously biotinylates proteins in a proximity-dependent manner. The biotinylated proteins will be purified by neutravidin affinity matrix and identified by mass spectrometry. To prevent a high number of biotinylated proteins, the outer membrane will be fractionated by ultracentrifugation. This method enables us to identify interaction partners of PlaB especially in the outer membrane.
Result: PlaB-BirA*-2HA was successfully inserted in *E. coli* and *L. pneumophila*. Thereby, PlaB in *E. coli* is used as a model to analyze the export of *L. pneumophila* PlaB, which can also point to a general transport way in gram-negative bacteria. The expression levels, activity and localization of PlaB-BirA*-2HA were verified and compared with untagged PlaB. Due to a similar behavior of PlaB and PlaB-BirA*-2HA, the establishment and quality control of the BioID assay was done by analyzing the biotinylated proteins in Western Blot after BioID. PlaB-BirA*-2HA seems to biotinylate specific proteins compared to the control BirA*-2HA. Therefore, the samples will be analyzed by means of mass spectrometry.
Conclusion: PlaB-BirA*-2HA seems to biotinylate specific proteins, which may be part of the transport mechanism of PlaB. Knowledge of the transporter, which targets PlaB to the outer membrane, is important for understanding the pathogenicity of *L. pneumophila* and may lead to the identification of a novel protein transport mechanism, which may serve as a novel drug target.

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241/MPP

Impact of *Legionella pneumophila* phospholipases A on modification of phagosomal membranes

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Introduction: Legionellae are intracellular bacteria which can cause a possibly fatal pneumonia called Legionnaires disease. After being inhaled, the bacteria are phagocytosed by alveolar macrophages but evade degradation and trigger a modification of the phagosomal membrane. The resulting Legionella-containing vacuole (LCV) allows replication of Legionellae within a unique membrane-bound compartment. Lipid composition of the LCV and whether phospholipases contribute to the lipid rearrangements is currently not known.

Aim: Legionellae express several phospholipolytic enzymes including three GDSL phospholipases A (PlaA, PlaC and PlaD). We are focused on the modification of phagosomal membranes in response to infection with *Legionella pneumophila*, primarily on the role of Legionella phospholipases in LCV formation.
Method: In a first step we investigate the phospholipolytic and glycerophospholipid:cholesterol acyltransferase (GCAT) activities of the GDSL-enzymes via lipid hydrolysis assay and thin layer chromatography.
Result: The GDSL hydrolases PlaA, PlaC and PlaD were recombinantly expressed and purified, antibodies were generated and phospholipase single-, double- and triple-mutants in *Lpn* were created. It was shown that PlaA and PlaC are secreted from *Lpn* via a type II (Lsp) secretion system and display phospholipase A (PLA) and lysophospholipase A (LPLA) activity. PlaA and PlaC are processed by the zinc metalloproteinase rProA. This leads to an enhanced LPLA activity of PlaA and induces GCAT activity of PlaC. PlaA and PlaC are important for *Lpn* replication in competitive infection assays in *A. castellanii*. rPlaD was shown to exhibit weak LPLA and GCAT activity and possibly needs to be activated by an eukaryotic factor. This and the mode of PlaD translocation is still unknown and is under investigation.
Conclusion: The GDSL hydrolases rPlaA and rPlaC have been shown to process lipids that are commonly present in cellular membranes. As they are secreted from *Lpn* via a type II secretion system they get in contact with the phagosomal membrane and might thus be involved in LCV modification. Therefore we next aim to determine the lipid composition, ultrastructure and biophysical properties of LCV membranes in response to infection with Lpn wildtype versus phospholipase mutants.

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242/MPP
Interactions of *Streptococcus gallolyticus* subsp. *gallolyticus* with human macrophages
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Introduction: *Streptococcus gallolyticus* subsp. *gallolyticus* (SGG) is an inhabitant of the human gastrointestinal tract and a facultative pathogen. It is assumed that SGG gets through colonic malignancies in the bloodstream and is then able to induce an infective endocarditis. It has been observed as pathogen of infective endocarditis in about 20 % of streptococcal-induced cases. The pathogen interaction with the innate immunity is widely unknown. Therefore, this study focuses on the phagocytosis by and interaction of different SGG strains with human macrophages.
Method: For the phagocytosis assay, the monocyte cell line THP-1 was differentiated into macrophages. Bacteria (five SGG isolates and one *Staphylococcus aureus* isolate for comparisons) were added (MOI=5) and phagocytic uptake was enabled for 30 min. Cells were washed and incubated in medium supplemented with antibiotics. After several time points, cells were lysed and vital bacteria were quantified by plating assay. For relative gene expression analysis, RNA from THP-1 cells was isolated, followed by cDNA-synthesis and real-time PCR. The cytotoxicity of the bacteria was determined with a LDH Assay in supernatant after 5 h. The lysozyme resistance was determined in BHI medium with different lysozyme concentrations.
Result: The SGG isolates LMG 17956 and BAA-2069 and *S. aureus* were phagocytized very strongly (50-70 % of the initial added amount) by macrophages. In comparison, only 10 % of the strain UCN 34 was phagocytized. The percentage uptake of the isolates ATCC 43143 and DSM 16831 are between these values. BAA-2069 and UCN 34 were remarkable because they had a constant titer in an observed time period (5-16 h). With regard to these features, the aspects of the phagocytosis like cytotoxicity and lysozyme-resistance of SGG were analyzed. It was shown that SGG isolates led to 20 % lysis of THP-1 macrophages after 5 h of inoculation whereas *S. aureus* led to 30 % lysis. Thereby, *S. aureus* induced 30-fold higher gene expression of interleukin 6 than the SGG isolates. Lysozyme is an important bactericide in humans including in degrading bacteria in macrophages. The SGG strains DSM 16831 and LMG 17956, isolated from animals, show a significant growth inhibition by 10 mg/ml lysozyme in BHI. In comparison, the human isolates UCN 34 and BAA-2069, which were conspicuous in their survival in macrophages, showed no decrease in growth up to 20 mg/ml lysozyme.
Conclusion: The SGG isolates show strain-dependent characteristics in phagocytic uptake by and survival in macrophages. These seem to correlate with their growth ability in the presence of lysozyme. Furthermore, SGG isolates do not stimulate the macrophages as strong as *S. aureus* and they cause lower lysis of macrophages. The inflammatory response in SGG infection could be suppressed which could lead to a better survival of SGG in human tissue.

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243/MPP
Draft genome sequence of the vaccination strain *Mycobacterium bovis* BCG S4-Jena
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Questions: From its initial release at the Pasteur Institute in Paris in 1921, *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) was distributed worldwide and cultivated by continuous serial passage. As a result, cultivated BCG strains accumulated mutations as compared with the original 1921 strain (substrain Pasteur; not available anymore), for example, single nucleotide polymorphisms (SNPs) and genetic loss of regions of difference (RDs). The tuberculosis vaccine strain S4-Jena (strain accession DSM 45071) was brought to Jena, Germany from Gothenburg, Sweden in 1950 by the microbiologist Hans Knöll. Allegedly, it is a derivative of a Swedish BCG substrain that was imported directly from the Paris Pasteur Institute in the 1920ies. From 1950 onward, it was used to prepare tuberculosis vaccine batches to be used in the former German Democratic Republic (GDR) and in Poland. We aimed to genetically characterize this formerly broadly used, phylogenetically old BCG strain.
Result: To determine the draft genome sequence of *M. bovis* BCG S4 Jena, sequencing of a paired-end library on the Illumina MiSeq system was performed. The sequencing run (2 x 250 bp) resulted in 2,570,425 reads yielding approx. 587 megabases of sequence information. A *de novo* assembly of the processed reads by means of the gsAssembler software (version 2.8.) generated 382 contigs accounting for a total length of 4.3 Mb featuring a GC-content of 65.55%. The assembly was validated by applying contig-length vs. read-count analysis (7, 8). Remaining assembled contigs could be arranged in 48 scaffolds with 132 scaffolded contigs by exploiting paired-end sequence information. For gene prediction and functional annotation, the genome annotation platform GenDB (9) was applied. This approach resulted in 4,020 predicted genes. In total, 67 single nucleotide polymorphisms (SNPs) were detected in comparison with the *M. bovis* BCG Pasteur 1173 strain
Conclusion: Phylogenetically different BCG substrains have different clinical outcomes if used to treat bladder cancer. In

comparison to the commercially available BCG substrains Tice (OncoTICE®) and Connaught (immuCyst®) that are licensed to treat non-muscle invasive bladder cancer, this phylogenetically old BCG strain has fewer genome alterations in comparison to wild-type *M. bovis* with a known safety record.

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244/MPP

Toll-like receptor 2 activation depends on lipopeptide shedding by bacterial surfactants

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Sepsis caused by Gram-positive bacterial pathogens is a major fatal disease but its molecular basis remains elusive. Toll-like receptor 2 (TLR2) has been implicated in the orchestration of inflammation and sepsis but its role appears to vary for different pathogen species and clones. Accordingly, *Staphylococcus aureus* clinical isolates are found to differ substantially in their capacity to activate TLR2. Strong TLR2 stimulation depends on high-level production of phenol-soluble modulin (PSM) peptides in response to the global virulence activator Agr. PSMs are required for mobilizing lipoproteins, the TLR2 agonists, from the staphylococcal cytoplasmic membrane. Notably, the course of sepsis caused by PSM-deficient *S. aureus* is similar in wild-type and TLR2-deficient mice, but TLR2 is required for protection of mice against PSM-producing *S. aureus*. Thus, a crucial role of TLR2 depends on agonist release by bacterial surfactants. Modulation of this process may lead to new therapeutic strategies against Gram-positive infections.

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245/MPP

Veterinary and environmental *Vibrio navarrensis* isolates from Germany: A comparison to human pathogenic strains

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Introduction: Isolated during a survey on the distribution of pathogenic *Vibrio cholerae* bacteria in aquatic environments of the Spanish province Navarra, *V. navarrensis* was first described as a new species in 1991 (Urdaci et al., 1991). Some years later, further strains classified as *V. navarrensis* biotype *pommerensis* were reported from the German Baltic Sea (Jores et al., 2007). In 2014, the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia published results on the characterization of *V. navarrensis* isolates associated with human illness (Gladney and Tarr, 2014). Our study aimed to investigate genotypic and phenotypic traits of veterinary and environmental strains from Germany to assess their human pathogenic potential.
Materials and Methods: The study comprised a total of nineteen German isolates and one reference strain. All strains were confirmed as *V. navarrensis* by partial *rpoB* sequencing and characterized in standard biochemical assays. The presence of genes encoding virulence-associated factors like capsule or pilus biosynthesis proteins, hemolysins and the type VI secretion system (T6SS) was examined by polymerase chain reaction (PCR). Furthermore, the hemolytic activity of the isolates was investigated in phenotypic assays. To determine the phylogenetic relationship to clinical *V. navarrensis* strains, MLST analyses were performed.
Result: Nearly all strains possessed genes for capsule and pilus biosynthesis. In addition, all isolates were positive for the majority of hemolysin genes studied and showed hemolytic activity against human erythrocytes. Clear genetic differences were especially observed for the T6SS which was detected in all veterinary isolates while it seemed to be absent in more than half of the environmental isolates. The MLST analyses revealed a close relationship between veterinary and human pathogenic strains.
Discussion: Our study indicates a human pathogenic potential of German *V. navarrensis* isolates, especially veterinary strains and re-emphasizes the need for further investigation of this *Vibrio* species.

References

[1] Gladney, L.M., Tarr, C.L., 2014. Molecular and phenotypic characterization of *Vibrio navarrensis* isolates associated with human illness. J. Clin. Microbiol. 52, 4070-4074.
[2] Jores, J., Appel, B., Lewin, A., 2007. *Vibrio navarrensis* biotype *pommerensis*: a new biotype of *V. navarrensis* isolated in the German Baltic Sea. Syst. Appl. Microbiol. 30, 27-30.
[3] Urdaci, M.C., Marchand, M., Ageron, E., Arcos, J.M., Sesma, B., Grimont, P.A., 1991. *Vibrio navarrensis* sp. nov., a species from sewage. Int. J. Syst. Bacteriol. 41, 290-294.

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246/MPP

Induction of host cell signaling during infection of macrophages with different *S. aureus* strains

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Introduction: Community-associated methicillin-resistant *S. aureus* strains (caMRSA) are quite unique in several aspects of their virulence potential. It has been described in different studies that the caMRSA strain USA 300 avoids killing through professional and non-professional phagocytes by escaping from the phagosomal compartment to the cytoplasm (Fraunholz et al., 2012). In this scenario, staphylococci show a strong intracellular replication ending up in the destruction of the host cells. Several bacterial factors essential for phagosomal escape have been identified, whereas factors on the host site are largely unknown.
Aim: In this study, PMA-differentiated human monocytes (THP-1) and primary blood monocytes were used to study the induction of central host kinases during infection with different staphylococcal strains.
Method: PMA-differentiated professional phagocytes (THP-1 and primary monocytes) were infected with the laboratory *S. aureus* strain SA113 or the caMRSA strain USA300. The rate of internalized bacteria and intracellular survival was monitored using antibiotic protection assays and double immunofluorescence microscopy. The activation of central protein kinases (phosphoinositide 3-kinase, protein kinase AKT and the MAP-kinases ERK1/2 and JNK/SAPK) was analyzed and quantified post infection at different time points by western blotting of cell lysates. Furthermore, the influence of selected kinases on phagocytosis and the intracellular fate of the staphylococci was analyzed in the presence of specific pharmacological inhibitors.
Results and Conclusions: By using the THP-1 phagocytosis model system as well as primary monocytes we were able to provide first insights into the complex signal transduction events

during phagocytosis and the intracellular fate of *S. aureus* SA113 and USA300.

References

[1] Fraunholz, M., and Sinha, B. (2012) Intracellular *Staphylococcus aureus*: live-in and let die. Front Cell Infect Microbiol 2, 43

Figure 1

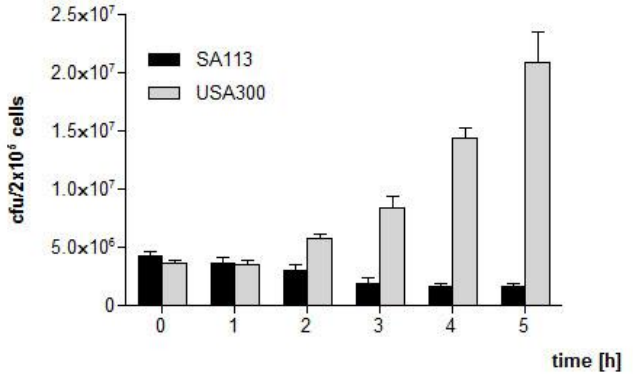

Figure 1: Intracellular fate of S. aureus *SA113 and USA300 in human THP-1 cells. 2x105 THP-1 cells were infected with a MOI of 50. After 60 minutes extracellular bacteria were killed by the addition of Gentamycin (100µg/ml) and Penicillin G (300 U/ml). The intracellular amount of living bacteria was determined at different timepoints after infection by lysing of the cells (using saponin) and plating of the lysates on blood agar plates*

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247/MPP

Role of Sortase A in the pathogenicity of *Staphylococcus lugdunensis*

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Introduction: Bacterial cell surface proteins are required to initiate colonization, enable invasiveness and generate biofilm formation. In *Staphylococcus aureus*, sortase A (Sa-SrtA) has been described as cysteine transpeptidase specific for LPXTG motif-containing proteins. *S. lugdunensis* also harbours a putative sortase A (Sl-SrtA) enzyme that anchors the LPXTG motif-containing proteins to cell surface. Hitherto, 11 assumed Sl-SrtA substrates have been described for *S. lugdunensis* that carry the LPXTG motif.
Intention. Here, we report on the functionality of Sl-SrtA to anchor LPXTG substrates to the cell wall. In particular, the role of Sl-SrtA-dependent surface proteins in biofilm formation and invasion of eukaryotic cells is determined.
Materials and Methods: Sl-SrtA mutants (Mut47 and Mut80) were generated by allelic replacement and were compared with their parent strains Sl44 and Sl253, respectively, for their capabilities to bind to plasma and extracellular matrix proteins and to generate biofilms. Mutants were further investigated for adherence and invasion to human epithelial (lung adenocarcinoma cells A549 and bladder carcinoma cells 5637) and endothelial (umbilical vein cells Ea.hy926) cell lines.
Results: While assimilation tests revealed no differences between the parent strains and the mutants, both mutants did not show agglutination in the Pastorex Staph Plus kit (BioRad) in contrast to the parental strains.Compared to the wild type strains, adherence of both mutants was significantly less to immobilized fibrinogen. Binding to fibronectin-coated surface was also less for both mutants, but not reaching statistical significance. Both mutants showed reduced adherence to the epithelial celllines A549 and 5637 as well as to human fetal lung fibroblast cells. Mut47 appeared higher invasive for human embryonic kidney (HEK) 293 cells and Ea.hy926. Mut80 was more invasive for the fetal lung fibroblast cells, but invasiveness for 5637, HEK and Ea.hy 926 cells was not significantly different compared to the parent strain. Mut80 showed less biofilm generation in TSB. Adhesin genes such as *fbl* and *vWbF* were overexpressed in both mutants when compared to their respective wild types using RT-PCR analysis. However, since the adhesins are not anchored to the cell surface, they are unusable in the case of the *S. lugdunensis* mutants. Thus, in addition to anchoring of surface proteins to the cell wall, Sl-SrtA also regulates the adhesin gene expression on a transcription level.
Conclusion. Sl-SrtA was found as the pathogen´s housekeeping sortase. Disrupting the presentation of surface proteins by blocking the activity of Sl-SrtA could therefore effectively reduce bacterial virulence and, thus, promote bacterial clearance by the host. Although some strain-specific differences may exist, Sl-SrtA could be an attractive target to attenuate *S. lugdunensis* virulence.

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248/MPP

Dendritic cells are target cells for *Clostridium botulinum* C3 toxin

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The C3 exotoxins produced from *Clostridium (C.) botulinum* and *Clostridium limosum* are small protein enzymes that act as mono-ADP-ribosyltransferases in the cytosol of their target cells. Previously, it was shown that the C3 toxins are specifically taken up into the cytosol of macrophages and monocytes via an endocytic pathway where they mono-ADP-ribosylate the GTPases Rho A and B [1]. The C3-catalyzed ADP-ribosylation blocks Rho signaling, which leads to a reorganization of the actin-cytoskeleton and entails impaired macrophage functions, such as phagocytosis and migration. Furthermore, we demonstrated that also specialized macrophages such as osteoclasts are target cells for C3 toxins [2] whereas C3 toxins are not efficiently taken up into other cell types like epithelial cells.
Prompted by these results, we investigated dendritic cells (DC), a further member of the monocyte/macrophage family, as target cells for clostridial C3 toxins. To address this question, we explored the uptake of C3 toxins into a human DC line and primary DC derived from monocytes of healthy human blood donors. DC are antigen-presenting cells and play a central role in the innate immune system of mammals and are essential for the development of immunologic memory and tolerance. We were able to show the specific uptake of C3 toxins into the cytosol of DC by performing different approaches such as Western blot analyses or fluorescence microscopy. Immature as well as mature DC efficiently internalized C3 toxin, which leads to ADP-ribosylation of Rho and the characteristic “C3-morphology” with long protrusions of the cell body. In contrast, the C2I enzyme from *C. botulinum* is not taken up into DC, indicating the specificity of the C3 uptake into these cells. Currently, the molecular mechanisms underlying the uptake of C3 toxin into the cytosol of DC are examined in more detail. Moreover, an enzymatically inactive C3 mutant [2] will be used for the targeted delivery of pharmacologically active compounds including enzymes and peptides into the cytosol of DC

to specifically modulate cell functions, such as antigen presentation and T-cell activation in these immune cells.
In conclusion, we identified human DC as novel target cells for clostridial C3 toxins and demonstrated the efficient uptake of these proteins into the cytosol of DC. These findings might serve as a starting point for targeted drug delivery into DC to modulate their functions or to establish novel vaccination strategies.

References
[1] Fahrer, J., Kuban, J., Heine, K., Rupps, G., Kaiser, E., Felder, E., Benz, R., Barth, H. (2010). Selective and specific internalization of clostridial C3 ADP-ribosyltransferases into the cytosol of macrophages. Cell. Microbiol. 12, 233-247.
[2] Barth, H., Fischer, S., Möglich, A. and Förtsch, C. (2015). Clostridial C3 toxins target monocytes/macrophages and modulate their functions. Front. Immunol. 6:339. doi: 10.3389/ fimmu.2015.00339.

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249/MPP

Auranofin, a thioredoxin reductase inhibitor, prevents intoxication of cells with disulfide bond dependent bacterial toxins

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Bacterial AB-type toxins act as enzymes in the cytosol of mammalian cells and modify cellular target molecules, which leads to severe human and animal diseases. These toxins show defined enzymatic activity and sophisticated modes of cellular transportation, that often abuse intracellular mechanisms of the target cells for intoxication. One of these host cell dependencies is the reducing environment in the cytosol, which is necessary for the reduction of disulfide bonds in certain toxins, which connect structurally relevant parts within the protein, like the enzymatically active (A-)subunit and the binding/transport (B-) subunit in diphtheria toxin (DT), or within the A-subunit in *Pasteurella multocida* toxin (PMT). Such disulfide bonds must be reduced during cellular uptake of the toxin for either release of the A-subunit from the B-subunit at the cytosolic side of endosomal vesicles to reach its target molecules in the host cell cytosol or for activation of the A-subunit. Auranofin, an organic gold compound used for the treatment of rheumatoid arthritis, influences the redox state of treated cells by specifically inhibiting thioredoxin reductase [1]. Therefore, this compound is an attractive tool to investigate the mechanisms underlying cleavage of the disulfide bond of bacterial toxins and a potential lead compound for novel therapeutics to prevent the uptake of the A-subunit into the host cell cytosol and to protect human and animal cells from intoxication.
We demonstrated that human epithelial cells pretreated with Auranofin are significantly less susceptible to DT and PMT. The toxicity of DT was significantly reduced in a cell-based assay. This inhibitory effect could be specified for the translocation step via mimicking acidic conditions of early endosomes, indicating relevance for the endosome to cytosol translocation [2]. For PMT we found that the enzymatic activity in the host cell cytosol is reduced, as detected by specific Western blot analysis of the modified cellular substrate of PMT. This indicates that a reduction of the disulfide bond is necessary for PMT to be fully functional.
In conclusion, these results suggest that intoxication of cells by DT and PMT depends on the redox conditions of targeted cells and that the targeted pharmacological modulation of this potential to a less reducing state prevents toxin uptake into the cytosol or reduces enzymatic activity, thereby protecting mammalian cells from intoxication with these toxins.

References
[1] S. Gromer, S. Urig, and K. Becker, “The thioredoxin system - From science to clinic.,” *Med. Res. Rev.*, vol. 24, no. 1, pp. 40-89, Jan. 2004.
[2] L. Schnell, L. Dmochewitz-Kück, P. Feigl, C. Montecucco, and H. Barth, “Thioredoxin reductase inhibitor auranofin prevents membrane transport of diphtheria toxin into the cytosol and protects human cells from intoxication.,” *Toxicon*, Apr. 2015.

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250/MPP

Biochemical characterization of the D-serine deaminase from *Staphylococcus saprophyticus*

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Introduction: *Staphylococcus saprophyticus* is the only species of the staphylococci that is typically uropathogenic and the genome is the only of all sequenced staphylococci that possesses a D-serine-deaminase. The amino acid D-serine is present in relatively high concentrations in human urine and is toxic or bacteriostatic to several non-uropathogenic bacteria. The D-serine deaminase converts D-serine to pyruvate and ammonia. Therefore its function is twofold - detoxification and provision of a carbon and energy source. Here we describe the biochemical characterictics of the D-serine deaminase of *Stapyhlococcus saprophyticus*.
Materials and Methods: To study the properties of the D-serine-deaminase from *Staphylococcus saprophyticus*, we cloned the *dsd*A gene into the vector pQE70 which allowed expression of the DsdA as fusion protein with a His-tag at the C-terminus in *E. coli* M15 (pREP4) cells. DsdA was purified by Ni^{2+}-chelating column chromatography and anion exchange chromatography. The activity of the D-serine-deaminase was estimated by measuring the formation of a colored dinitrophenylhydrazone of the pyruvic acid which is liberated from D-serine by the D-serine-deaminase.
Result: The purified enzyme showed a single protein band upon the SDS-PAGE with a molecular mass of about 50 kDa, which is compatible with the calculated mass from its amino acid sequence (47.89 kDa). This band was also detectable in Western Blot analysis with an antibody against the His-tag. Besides D-serine as a preferred substrate, DsdA could also convert D-threonine. The pH-optimum of the enzyme was at pH 7.8 and the temperature optimum was at 37 °C.
Discuccion: Compared to other D-serine deaminases, the D-serine deaminase of *Staphylococcus saprophyticus* was not able to convert L-serine and its affinity for D-serine as substrate is lower but the rate of turnover is higher.

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251/MPP

Intoxication of monocytes/macrophages with clostridial C3 toxins results in disturbed chemotaxis and migration.

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C3 toxins (approx. 25 kDa) are produced and secreted by various Gram-positive bacteria and selectively mono-ADP-ribosylate the small GTPases Rho-A, -B and -C by catalysing the covalent transfer of ADP-ribose from NAD^+ onto Asn-41 of Rho (1). This inhibits the exchange of GDP to GTP and the ADP-ribosylated Rho-GDP binds more efficiently to GDI, which traps Rho in the Rho-GDI complex. As a result the activation of Rho by extracellular signals is prevented and the Rho-mediated signal transduction in cells is blocked (1;2). Rho GTPases control a wide variety of signal transduction pathways in all eukaryotic cells, e.g. regulating the actin cytoskeleton (1).

The clostridial C3 ADP-ribosyltransferases C3bot and C3lim, produced by *Clostridium* (*C.*) *botulinum* and *Clostridium limosum* respectively, are selectively internalised into the cytosol of monocytes and macrophages most likely by an endocytotic mechanism via acidified endosomes. C3bot and C3lim are not efficiently taken up into the cytosol of other cell types, including epithelial cells and fibroblasts (1).

We and others demonstrated that the C3-catalysed Rho-inhibition inhibited migration, adhesion and phagocytosis in macrophages. The C3-treatment of macrophages resulted in characteristic morphological changes due to a reorganization of the actin cytoskeleton following Rho-modification. The C3-mediated impairment of Rho-signalling also inhibited essential macrophage functions, such as chemotaxis and migration. The specific inhibition of characteristic functions of monocytes and macrophages suggest that clostridial C3 enzymes have an immunosuppressive effect on such immune cells and thus could play a role in pathophysiology (1). The inhibition of chemotaxis and migration of cultured macrophages as well as primary human monocytes by C3 toxins were demonstrated by performing *in vitro* and *ex vivo* scratch and trans-well-chemotaxis assays.

Remarkably, a recombinant C3lim fusion protein (C2IN-C3lim), which contains C2IN, an enzymatically inactive portion of C2I, the enzyme component of the binary actin ADP-ribosylating *C. botulinum* C2 toxin, is more efficiently internalised into the cytosol of macrophages than wild-type C3 (1).

We showed that C2IN-C3lim, in contrast to the clostridial wildtype C3 toxins, acts reversible and macrophages recover from the intoxication with this recombinant toxin.

C2IN-C3lim shows high specificity to monocytes and macrophages and shows a reversible mode of action. Hence, this fusion toxin should represent an attractive tool for the selective and targeted inhibition of pathologically enhanced monocyte and macrophage migration and chemotaxis, as observed in the context of inflammatory and trauma-associated diseases.

References

[1] Barth, H., et al., Frontiers in Immunology, 2015. 6.
[2] Vogelsgesang, M., et al., Naunyn Schmiedebergs Arch Pharmacol, 2007. 374(5-6): p. 347-60.

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252/MPP

***N. meningitidis* uptake of functionalized ceramide analogs and incorporation into its outer membrane**

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Introduction: *Neisseria meningitidis*, an obligate human pathogen, is a causative agent of septicemia and meningitis worldwide. Recent studies demonstrated that distinct membrane microdomains, named lipid rafts, and ceramide play an important role in infectious biology. Ceramide can be generated via the salvage pathway through the action of sphingomyelinases (neutral or acid sphingomyelinase), or the *de novo* synthetic pathway through the action of ceramide synthases.

Background: To better understand the dynamics of actin and association of cortical plaque structure with ceramides, we applied live cell imaging and made use of the functionalized ceramide-analogs. Unexpectedly, we observed that ceramide-analogs are efficiently incorporated into both host cell and *N. meningitidis*

Materials and Methods: We employed four different functionalized ceramide analogs, including: ω-azido-C6-ceramide and ω-azido-C16-ceramide, α-azido-C6-ceramide and α-azido-C16-ceramide. Acquisition of the fluorescent click-labeled compounds was estimated by flow cytometry and visualized using direct stochastic optical reconstruction microscopy (*d*STORM). Moreover, growth kinetics were applied to determine toxicity of the functionalized ceramide-analogs.

Result: *N. meningitidis* incorporated all four tested functionalized ceramide-analogs in a dose dependent manner. Interestingly, toxicity analyses revealed that ω-azido ceramides (ω-azido-C6-cer and ω-azido-C16-cer) are more toxic than α-azido ceramides (α-azido-C6-cer and α -azido-C16-cer Cer6 or Cer16). Moreover, incorporation of these molecules by *N. meningitidis* could be visualized by *d*STORM analysing bacteria feed with ω-azido-C6-ceramide and clicked with Dibenzylcyclooctyne (DBCO)-sulfo-Cy5 and suggested an incorporation in the outer membrane.

Conclusion: Our results indicate incorporation of functionalized ceramide-analogs into *N. meningitidis.* Here we demonstrate that biorthogonal functionalized ceramide probes and super-resolution imaging can be used as a strategy to analyse lipid uptake by bacteria.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

253/MPP

Construction of a phage integration vector for *Francisella tularensis*

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Background: *Francisella tularensis* (*Ft*), the causative agent of tularemia, is found in a wide range of wild animals and is able to infect humans, causing a variety of clinical expressions. We recently identified and described the genomic island (GI) FhaGI-1 and showed that its att sites in combination with the site-specific integrase are sufficient to generate the episomale form of the GI in *E. coli* and *Ft* subsp. *holarctica* after transformation (Rydzewski, Tlapák et al., 2015; IJMM 305: 874-880).

Result: Here we report the utilization of this information for the development of a phage integration vector for use in *Francisella*

research. To allow for propagation of the vector in *E. coli* we used vector pUC57-Kan as a basis and inserted the essential parts of FhaGI1, a second antibiotic marker as well as a multiple cloning site, leading to the Francisella Integration Vector-tRNA^{Val}-specific (pFIV1-Val). We could show that a sequence of positive and negative selection steps of transformants of *Ft. holarctica* LVS leads to 10-20% of positive clones. With the help of a gfp^{+}-construct of pFIV1-Val we could demonstrate that the integrated gene was active and stable even after 10 passages in mediumT without antibiotic treatment. We also used the vector to successfully integrate *gfp* into other *Francisella* species (*Ft. novicida* Fx1, *F. philomiragia*).

Conclusion: The results show that pFIV1-Val can be used as a genetic tool in *Francisella* research, e.g. for stable complementation of specific gene- deletion mutants or to stably label bacteria.

Method: *Francisella* strains were cultivated in medium T or on enriched cystine-heart agar. Construction of the vector was done by genetic cloning. Transformation of *E.coli* and *Francsiella* strains was performed by electroporation.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

254/MPP

Influence of D-serine on regulation systems of *Staphylococcus saprophyticus*

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Introduction: *Staphylococcus saprophyticus* is an opportunistic pathogen which is a common cause of urinary tract infections. Several virulence factors are known and described e.g. a urease, the surface proteins Aas, SdrI and UafA, a surface-associated lipase Ssp and a D-serine-deaminase DsdA. Since D-serine is prevalent in urine and has a toxic or bacteriostatic effect on many bacteria, possession of the D-serine-deaminase seems to be an important adaption for colonization of the urinary tract. It was shown that the lipase Ssp is upregulated in presence of D-serine. However we do not know anything about the mechanisms of regulation of the D-serine-deaminase or any other virulence factor. Bacteria often use regulation systems to modulate expression of virulence factors as a reaction to certain environmental influences. It is supposed that D-serine has influence on the expression of a regulation system, thus leading to different expression of virulence factors. For *Staphylococcus aureus* it is known that three regulation systems, which often interact with each other, play a major role in regulation of virulence factors: the agr (accessory gene regulator)-system, the sar (staphylococcal accessory regulator)-system and the alternative sigma-factor sigB. Existence of the agr-system is also described for *S. saprophyticus*. Genome analysis show that the sar-system and sigB also exist in *S. saprophyticus*. However the functionality, the actual role in regulation and a possible influence of D-serine on these systems remains still unclear. The physiological significance of the agr-system, the sar-system and sigB and the influence of D-serine on these systems should be investigated by construction of agr-, sar-, and sigB knock-out mutants, followed by comparative physiological tests and real time qRT-PCR between wild type strain and knock-out mutant, with and without influence of D-serine.

Method: Cloning of agr, sar and sigB knock-out constructs was accomplished by use of a yeast recombination system. Therefore the erythromycin cassette and a 500 bp fragment upstream and downstream of the corresponding gene were amplified and transformed with the linearised shuttle vector pRS426 into yeast strain PJ69-4a. The assembled mutagenesis construct was cloned into the temperature sensitive replacement shuttle vector pBT2. Plasmids were purified from E. coli DH5α and transformed into *S. saprophyticus* strain 7108 by protoplast transformation, followed by a plasmid curing step.

Results and Conclusions: Knock-out mutants were successfully constructed. In a next step the influence of the regulation systems on the expression of virulence factors will be investigated by real-time qRT-PCR and different physiological assays such as urease activity testing, d-serine-deaminase activity testing, lipase activity testing or binding and biofilm assays.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

255/MPP

Host cell surface proteins and cholesterol are involved in the formation of multinucleated giant cells mediated by the *Burkholderia* type VI secretion system 5

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The pathogen *Burkholderia pseudomallei* and the related species *Burkholderia thailandensis* are facultative intracellular bacteria able to escape from the phagosome into the cytosol of host cells and induce the formation of multinucleated giant cells (MNGCs). MNGCs are mediated by the bacterial type VI secretion system 5 (T6SS-5), which is required in both *B. pseudomallei* and *B. thailandensis* for virulence in mice. The physiological role of multiple host cells fusing into an MNGC remains unknown; however, it may facilitate localized spread of the bacteria and access to nutrients, while at the same time protect against phagocytic cells and several antibiotics. Similarly, the mechanisms of MNGC formation and the target(s) of the T6SS-5 are unknown. In the present study we sought to gain insight into the nature of host cellular factors involved in MNGC formation that potentially function as targets of the T6SS-5. To this end, we focused on cholesterol and cell surface proteins. First, we analyzed if the removal of cholesterol affects MNGC production using methyl-b-cyclodextrin (MbCD), an oligosaccharide that extracts cholesterol from cellular membranes. For this, RAW 264.7 macrophages infected with *B. thailandensis* wild type were treated with 5 mM MbCD and the formation of cell-cell fusions was monitored and quantified. Treatment with 5 mM MbCD reduced the total cholesterol content of macrophages by 39%. The extraction of cholesterol reduced MNGC formation by 58% as compared with infected, untreated cells. Notably, MbCD treatment did not have a significant impact on the viability of host cells and intracellular bacteria. Next, we determined if an increase in cholesterol levels influences the fusion of host cells by treating infected macrophages with a water-soluble cholesterol substitute at various concentrations. Compared with cells that were infected but untreated, a relationship between increasing amount of cholesterol and a decreasing amount of MNGC formation was observed. Whereas a 4 mg/L dose decreased MNGC formation by 57%, a 0.04 mg/L dose only caused a 34% decrease. The addition of cholesterol had no significant effects on cell viability. Finally, we examined whether proteins located on the host cell surface contribute to cell-cell fusions. For this, we treated *B. thailandensis* infected RAW 264.7 macrophages with 0.05% trypsin followed by an agar overlay assay to bring the non-adherent cells into contact. Trypsinized, infected macrophages displayed a 56% decrease in MNGC formation compared with infected and EDTA treated cells at 5 h post treatment, indicating that the temporary depletion of surface proteins influences MNGC formation. In conclusion, our data show that cholesterol and host cell surface proteins contribute to MNGC formation and we speculate that a surface protein(s) that binds to cholesterol or is located in lipid rafts is targeted by the T6SS-5 to mediate host cell fusions.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

256/MPP

The role of Zn for streptococcal Lmb proteins

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Streptococcus agalactiae or group B streptococcus (GBS) is a primary cause for neonatal sepsis and meningitis. Efficient colonization and invasion of GBS requires expression of cell surface adhesins mediating microbial attachment to host extracellular matrix proteins (ECM) such as laminin. Lmb and its close homologues have been detected in pyogenic streptococci as well as in viridans streptococci from the *S. anginosus* group. The structure of the laminin binding protein (Lmb) of GBS has been resolved to contain a central Zn-binding site. Since Lmb displays similarities to the solute binding proteins of bacterial transporters, our aim was to gain further insights into the role of Lmb in laminin-binding and Zn-hemostasis in different streptococcal species. Investigations were carried out in GBS as well as in *Streptococcus anginosus* (SAG), an emerging pathogen in cystic fibrosis and a frequent isolate from blood and abscesses. Mutational analysis of Zn-binding histidine residues in Lmb of GBS and SAG resulted in impaired binding abilities and abrogation of adhesion functions to laminin. To investigate if Lmb is also involved in Zn transport, growth experiments of Lmb mutants of SAG and GBS were performed in Zn-depleted growth medium in comparison to the wild-type strains. No growth differences could be observed in growth medium with different Zn concentrations, indicating that Lmb is not involved in Zn-homeostasis. In addition expression analysis documented an induction of Lmb with increasing Zn concentrations, while bacterial transporters are often inhibited in the presence of excess substrate concentrations. Our data support the conclusion that Lmb, a widely distributed streptococcal surface protein, is primarily involved in the adhesion to laminin, which harbours a high concentration of Zn binding residues, and that the presence of Zn^{2+} is only structurally needed in order to maintain correct folding and adequate protein conformation of Lmb.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

257/MPP

Regulation and high resolution imaging of Yersinia translocator proteins YopB and YopD during cell infection

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Pathogenic species of the genus *Yersinia* inject effector proteins into host cells via a type 3 secretion system. YopB and YopD are intermediate substrates of the T3SS. These hydrophobic proteins are believed to integrate into the host cell membrane and form an entry pore for the late T3SS substrates, i.e. the effector Yops. We aimed at visualizing and quantifying YopB and YopD in *Y. enterocolitica* infected cells, employing immunofluorescence- and detergent extraction techniques. Upon cell contact, YopB and YopD could be visualized in close proximity to the bacteria forming irregular distributed structures. By confocal immunofluorescence microscopy around 10 YopB/YopD clusters were detected per bacterial cell, which could be further resolved by super resolution microscopy (STED) revealing around 50 spots per bacterial cell. Co-staining of YopB and YopD revealed almost perfect co-localization within the spots. The YopB- and YopD clusters were sensitive to protease digestion and could be extracted by detergents unable to permeabilize bacteria, suggesting that they are located at bacteria cell contact sites. The proportions of cell associated bacteria displaying YopB clusters were 4 % in wild type *Yersinia*, 22 % in a *Yersinia* strain deficient for YopD, 27 % in a *Yersinia* strain expressing the T3SS but no effector Yops and 41 % in a strain missing the effector YopE. This suggests that both, pore components (YopD) and effector Yops such as those downregulating the Rho GTP binding protein Rac1 control release of translocon components during cell infection. Furthermore, the percentage of YopB/YopD positive wild type bacteria was increased approximately 5-fold when interacting with cells expressing a permanently active form of Rac1 compared to control cells. In summary, the *Yersinia* translocation pore could be visualized at the bacteria cell contact site by high resolution fluorescence microscopy. Release of its components is controlled both by bacterial and host cell factors whereby the cellular signals leading to enhanced translocation pore release involve the Rho GTP-binding protein Rac1.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

258/MPP

Inhibitors of macrophage infectivity potentiator-like PPIases affect neisserial and chlamydial pathogenicity

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The pathogenic bacteria *Chlamydia trachomatis* and *Neisseria gonorrhoeae* express the surface-exposed macrophage infectivity potentiator-like protein (MIP), which has been shown to play a role in their pathogenicity. MIP belongs to the family of FK506-binding proteins (FKBP), exhibiting a peptidylprolyl isomerase (PPIase) activity that can be inhibited by rapamycin and FK506. Using high throughput microscopy and a chymotrypsin-coupled PPiase assay we tested pipecolic acid derivatives, which are known to inhibit *Legionella pneumophila* and *Burkholderia pseudomallei* MIP, for their activity against the chlamydial (Ctr-) and neisserial (Ng-) MIP. We were able to identify two MIP inhibitors termed PipN3 and PipN4 that inhibited PPIase activity of Ctr-MIP and Ng-MIP.

These inhibitors decreased chlamydial inclusion size in a dose-dependent manner and affected the pathogen's developmental cycle in HeLa cells. Furthermore, we could show the importance of MIP for survival of gonococci in the presence of neutrophils, which was reduced after the deletion of Ng-MIP, or after the addition of the two MIP-inhibitors. Our results confirm the importance of MIP-like proteins in infection and indicate the relevance of pipecolic acid derivatives as antimicrobials against *C. trachomatis* and *N. gonorrhoeae*.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

259/MPP

Multidrug-resistant organisms detected in refugee patients admitted to a University Hospital, Germany June-December 2015

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Introduction: Current data provided by Federal Agency of Migration and Refugees, Germany, indicate a dramatic increase in migration, with most people arriving from Syria, Albania, Afghanistan and Iraq. These countries are known as countries with high prevalence for multidrug-resistant Gram-negative bacteria (MDR-GN) (*Enterobacteriaceae*, *Acinetobacter baumannii*) and for meticillin-resistant *Staphylococcus aureus* (MRSA). Systematic studies regarding prevalence of multidrug-resistant organisms (MDRO) in refugees are not yet available in the scientific literature. In order to fill this gap, we investigated the prevalence of MDR-GN and MRSA in patients admitted from refugee accommodations to the University Hospital Frankfurt am Main (UHF), Germany between June and December 2015 and compared it with prevalence in resident patients.
Method: The prevalence of MDR-GN and MRSA in the first n=143 patients admitted from refugee accommodations (REF) to University Hospital Frankfurt, Germany (UHF) in the observational period. REF were identified on admission and screened for MDR-GN by rectal swabs and for MRSA by nasal swabs. MDR-GN screening was undertaken for extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, and *Enterobacteriaceae and Acinetobacter baumannii* resistant to piperacillin, any third generation cephalosporin, and fluoroquinolones +/- carbapenems. These data were compared to 1,489 resident patients without refugee history admitted to UHF within the same period.
Result: Multidrug-resistant gram negative bacteria (MDR-GN) were found to colonize 60.8% (95% confidence interval: 52.3-68.9) of n=143 refugee patients (REF) admitted to University Hospital Frankfurt, Germany, between June and December 2015. This percentage exceeds the prevalence of MDR-GN in resident patients by four-fold.
Discussion: The current high refugee movement is posing a serious risk for local health care systems and infection control measures have to be adapted to this situation.

- This is a contribution of the DFG research group 2251 "Acinetobacter -

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

260/MPP

AciAssembler - a comprehensive workflow for assembling, scaffolding and annotating bacterial genomes

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Introduction: Genome sequences form the data basis for virtually all *in silico*, *in vitro*, and *in vivo* studies on living organisms. They provide direct access to the species specific composition of genes, their arrangement along the genome sequence as well as their organization in regulatory and functional units. The comparison of genomes and of the genes therein between different strains within a species and between different species further facilitate an integration of analyses on the contemporary sequences and species with the evolutionary process that molded them from their common ancestors. It is therefore, that the generation and annotation of genome sequences is a crucial first step in analysis and has to be carefully monitored, tested and documented. Only then can downstream analyses rest on a solid data fundament.
Objective: Individual methods abound for accomplishing the individual steps in assembling and annotating genome sequences from whole genome shotgun data. Few solutions however exist that integrate these tools into a controlled and automated workflow. This is due to the plethora of different demands on such a workflow that vary among others with the species, the method of data generation, and the precise scientific question of a particular project.
Materials and Methods: We have generated a comprehensive workflow that designed to assemble, annotate and classify Acinetobacter genome sequences. The modular structure comprises standardized routines for (i) whole genome shotgun sequence data pre- and post-processing, (ii) genome assembly into contigs and a subsequent scaffolding integrating de-novo scaffolding with reference sequence based approaches, (iii) the automated annotation of genes, (iv) the screen for assembly completeness, and (v) strain typing.
Result: AciAssembler is a fully automated pipeline facilitating a standardized assembly and annotation of bacterial genomes with particular focus on the genus Acinetobacter. Intermediate results of the individual analysis modules together with the relevant metrics are carefully documented allowing for a seamless quality control from the raw sequence read to the final annotated genome sequence. Due to its modularity, AciAssembler can be easily extended on demand to include additional functionalities. The resulting genome sequences form a homogenous and stable data basis for downstream functional and evolutionary analyses.
Conclusion: AciAssembler is a novel genome assembly and annotation tool facilitating a standardized and comprehensive workflow from the raw sequence reads to the final genome sequence.

- This is a contribution of the DFG research group 2251 "Acinetobacter –

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

261/MPP

Acinetobacter baumannii of international clone II and *Acinetobacter indicus* carrying OXA-23 and OXA-58 carbapenemases in cattle

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Acinetobacter (*A.*) *baumannii* is a frequent cause of nosocomial infections and strains with resistance to carbapenems have been particularly accounted for therapeutic failures and increased mortality rates. This study aimed to determine the occurrence, molecular characteristics and antimicrobial susceptibility of *A. baumannii* and other *Acinetobacter* spp. in cattle in order to assess their zoonotic relevance.

From 01/2015 to 02/2016 randomized nasal and rectal swabs (NS, RS) from cattle (n=410; 353 farms) and composite fecal samples (FS) were screened for *Acinetobacter* spp. using blood, Gassner and ESBL agar. Species were identified by MALDI-TOF MS, *gyrB* PCR and 16S rRNA sequencing. MIC data (VITEK2) were interpreted according to breakpoints provided for human *Acinetobacter* spp. by the CLSI. Beta-lactamase and carbapenemase genes ($bla_{\text{OXA-23/24/58;}}$ bla_{VIM}, $bla_{\text{NDM-1;}}$ bla_{KPC}) were detected by PCR and sequence analysis. Whole genome sequences were used to identify resistance genes and their genetic environment which was further demonstrated by I-CeuI digestion of whole-cell DNAs. *A. baumannii* isolates were assigned to international clones (IC) 1-3 by a PCR approach.

A. baumannii was isolated from 79 cattle and 73 farms, respectively. The isolates were obtained from NS (n=71), FS (n=43) and RS (n=6). Out of 71 possible copy strains tested so far (i.e. isolated from same sample locations, animals, or farms), 60 isolates showed unique PFGE patterns while 11 isolates were excluded from the study due to their clonal nature. IC2, which globally emerged in humans, was determined in 18 non-duplicate cattle isolates. None of the *A. baumannii* isolates carried a carbapenemase gene or revealed acquired phenotypic resistances. Eight *A. indicus*-like strains carrying $bla_{\text{OXA-23-like}}$ (n=6) and $bla_{\text{OXA-58-like}}$ (n=2) genes were identified, thereof one showing high MICs to imipenem (8 mg/L) and to other antimicrobial substances, including piperacillin, tetracycline, and trimethoprim-sulfamethoxazole. Whole genome sequencing of *A. indicus*-like isolate IHIT31215 revealed high similarity to human clinical *A. indicus* isolates previously described in The Netherlands and France. In this isolate $bla_{\text{OXA-23}}$ was localized on the chromosome and surrounded by truncated and incomplete Tn*2008* transposon structures.

Our data suggest cattle to be a common source of *A. baumannii*. The presence of IC2, a dominant clonal lineage in humans, points towards a shared genetic background of bovine and human strains, which needs to be clarified. The frequent finding of OXA-23 and OXA-58-positive *A. indicus*-like isolates - a novel species published in 2012 and demonstrated to be implicated in human infection in 2014 - requires further investigations on genomic characteristics, clinical relevance antimicrobial resistance and zoonotic relevance of this new *Acinetobacter* species.

Presentation: Tuesday, 13 September 2016 from 10:30 – 11:30 in room Donauhalle.

262/MPP

Small RNA-mediated phase-variable gene expression control in the gastric pathogen Helicobacter pylori

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Based on a global RNA-sequencing approach we have identified more than 60 sRNAs in Helicobacter pylori. We are now functionally characterizing these sRNAs and are especially interested in their mechanisms and roles in stress response and virulence control. For example, we could show that the highly abundant and conserved sRNA, RepG, directly base-pairs with a homopolymeric G-repeat in the mRNA leader of the chemotaxis receptor TlpB and that length variation of this G-repeat determines the outcome (repression or activation) of RepG-mediated post-transcriptional tlpB regulation [1]. Phase variation at such hypermutable simple sequence repeats increases phenotypic variation in bacterial populations and thereby can contribute to host adaptation. More recently, we have identified additional RepG targets. One of these RepG targets is co-regulated with tlpB and turned out to be a glycosyltransferase that is required for H. pylori LPS O-chain and Lewis x antigen production. Deletion of this glycosyltransferase completely abolished mice stomach colonization and increased the sensitivity of H. pylori to salt and antibiotics stress. We demonstrate that RepG co-regulates expression of tlpB and the glycosyltransferase and that the G-repeat length in the tlpB mRNA leader determines the outcome of RepG-mediated regulation of both TlpB and the glycosyltransferase, and in turn O-antigen expression. This phase-variable sRNA-mediated regulation allows for a gradual, rather than ON/OFF, control of glycosyltransferase expression and LPS biosynthesis. The fine-tuning of O-antigen expression might be advantageous for H. pylori colonization and persistence within the host. The RepG example shows that identifying and studying sRNAs in bacterial pathogens can provide novel insights into pathogenicity and host colonization control.

Reference

[1] Pernitzsch SR, Tirier SM, Beier D, Sharma CM (2014). A variable homopolymeric G-repeat defines sRNA-mediated posttranscriptional regulation of a chemotaxis receptor in Helicobacter pylori. PNAS, 111(4):E501-10

POSTERSESSION 04
13 Sept. 2016 • 17.00-18.00

Infection Epidemiology and Population Genetics (FG MS)

263/MSP

Staphylococcus aureus nasal carriage among children in the Ashanti region of Ghana

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Question: Nasal carriage with *Staphylococcus aureus* is a common precondition for invasive infections, indicating the necessity to monitor prevalent strains, particularly in the vulnerable paediatric population. This surveillance study identifies carriage rates, subtypes, antimicrobial susceptibilities and virulence markers of nasal *S. aureus* isolates collected from children living in the Ashanti region of Ghana.
Method: Nasal swabs were obtained from children < 15 years of age on admission to the Agogo Presbyterian Hospital between November 2013 and January 2015. *S. aureus* isolates were characterized by their antimicrobial susceptibility, the presence of genes encoding for Panton-Valentine leukocidin (PVL) and toxic shock syndrome toxin-1 (TSST-1) and further differentiated by *spa*-typing and multi-locus-sequence-typing.
Result: Out of 544 children 120 (22.1%) were colonized with *S. aureus*, with carriage rates ranging between 15% below one year of age and 32% in the 5-9 year old age group. The 123 isolates belonged to 35 different *spa*-types and 19 sequence types (ST) with the three most prevalent *spa*-types being t355 (n=25), t84 (n=18), t939 (n=13), corresponding to ST152, ST15 and ST45. Two (2%) isolates were methicillin-resistant *S. aureus* (MRSA), classified as t1096 and t4454, and 16 (13%) were resistant to three or more different antimicrobial classes. PVL and TSST-1 have been detected in 71 (58%) and 17 (14%) isolates respectively.
Conclusion: Despite different living conditions in West Africa, this study reveals carriage rates and an age-dependent colonization pattern similar to those in industrialized countries. In contrast the prevalence of PVL and TSST-1 is substantially higher and of serious concern as these strains might serve not only as a source for severe invasive infections but as a reservoir for genes, potentially leading to highly virulent MRSA clones.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

264/MSP

The primary transcriptome of the human commensal pathogen _Neisseria meningitidis_ and its interaction with the RNA chaperone Hfq

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Despite experimental evidence that differences in gene regulation among strains as well as the expression of small non-coding RNAs (sRNAs) affect meningococcal virulence [1-3], the organization of its transcriptome including in particular the biogenesis of sRNAs and their mode of action are only poorly understood so far.
Here, we used differential RNA sequencing (dRNA-seq) to uncover a single-nucleotide resolution map of the primary transcriptome of *Neisseria meningitidis* strain 8013. We further combined co-immunoprecipitation of sRNAs bound to the RNA chaperone Hfq with RNA sequencing (RIP-seq) to determine the set of Hfq-bound sRNAs along with their target mRNAs on a transcriptome-wide level.
For the 1918 annotated protein coding sequences in strain 8013 dRNA-seq analysis predicted 1,625 transcriptional start sites (TSSs) with the majority of TSSs utilized in mid as well as late exponential growth in rich medium. The majority of the 706 primary TSSs (pTSSs) were generated for proteins with 382 pTSS obtained for single genes and 240 pTSSs obtained for genes located in operons. Promotor analyses of the upstream regions of our 1,625 TSSs further demonstrated that a classical *Escherichia coli*-like σ70 promoter is absent in most of the protein coding genes in meningococci.
dRNA-seq further revealed 65 sRNAs of which 45 were not previously identified, and the expression of over 20 was also confirmed by northern-blot analysis. By Hfq RIP-seq we could identify a large Hfq-centered post-transcriptional regulatory network comprising 24 validated sRNAs, and rifampicin treatment experiments demonstrated that Hfq binding confers enhanced stability on sRNAs.
Using a green fluorescent protein based plasmid system we further validated the predicted interactions between selected sRNAs and their mRNA targets as indicated by Hfq RIP-seq analysis. Thereby, we could so far identify *prpB* (NMV_0472) as an mRNA target which is post-transcriptionally repressed by two structural highly similar sRNAs (NMnc0017/NMnc0018) thus indicating a role of riboregulation in proprionate metabolism in *N. meningitidis*.
In conclusion, this large expression compendium allows a deeper understanding of meningococcal transcriptome organization and riboregulation thus providing a valuable resource for the scientific community.

References

[1] Del Tordello E, et al. *J Bacteriol* 2012, **194:**6217-6232.
[2] Fagnocchi L, et al. *PLoS One* 2015, **10:**e0126325.
[3] Joseph B, et al. *J Bacteriol* 2010, **192:**5363-5377.

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265/MSP

The Pangenome and Variome of _Coxiella burnetii_

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Introduction: Analysis of genetic differences (gene presence, nucleotide polymorphisms, insertions/deletions and structural variations) among strains of a bacterial species is crucial to understand molecular mechanisms of pathogenesis and to select targets for novel antibacterial therapeutics. Up to date, no such comprehensive comparative genome analysis was done for the Q fever pathogen *Coxiella burnetii*, even though, there are more than twenty whole genome assemblies and ten raw sequence datasets available. To address this issue, we constructed the pangenome and variome of 27 *C. burnetii* strains.
Materials and Methods: We expanded the repertoire of only five complete genomes by one finished reference genome and two newly sequenced genomes. Further, all publicly available genomes and sequence read data were downloaded from the NCBI GenBank database and assembled if necessary.
All assemblies were quality filtered according to sequencing coverage and fragmentation. New assemblies without gene annotation were annotated with Prokka (rapid prokaryotic genome annotation). For six *C. burnetii* reference genomes our experimental evidence-based re-annotation was used. For the other

genomes the annotation was obtained from Genbank. Afterwards, all genome annotation sets were filtered for split genes and insertion sequences were removed to avoid multiple hits.
The pan-, core and accessory genome was constructed with Roary, the Pan Genome Pipeline. Then we performed a whole genome variation analysis based on whole genome and orthologous gene and protein alignments and determined unique genomic regions, strain-specific genes, SNPs and InDels, insertion elements, and conserved structural elements and summarized it as the variome.
Results and Conclusions: After adding three new assemblies (Ohio, S4, C2) now 27 out of 35 strains met the quality criteria for downstream analysis. The new pangenome comprises 4,354 genes, whereof 1,197 genes are present in almost all strains and 148 genes are missing in up to two strains. Nearly half of the genes are showing a unique pattern (only present in up to three strains). Our phylogenetic classification confirms existing SNP-based phylogenetic trees and 627 core genes showed no point mutations (SNPs).
Here, we present the first preliminary pangenome (as a matrix of the core and accessory gene pool) and variome (multiple sequence alignments, SNP and InDel annotation, etc.) of *C. burnetii*. It will serve as the basis for future work like construction of species-specific metabolic networks linking the annotations to observed phenotypes to allow the development of new diagnostic and therapeutic tools in the different Q fever entities seen in Germany and worldwide.

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266/MSP
Sequence-based metagenomics for the determination of antibiotic selection pressure
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The human gut is a dynamic reservoir of antibiotic resistance genes (ARGs). Antimicrobial agents impact significantly the intestinal microbiome and thus also the resistome by the means of enhanced horizontal transfer and selection of resistance. Estimations of this selection pressure can help to design powerful antibiotic stewardship programs, adapted for different settings and their local epidemiological situation. However, studies that investigated the effect of antimicrobial agents on resistance rates have been primarily culture-based, thus only representing a minority of the intestinal microorganisms and mechanisms of resistance.
To provide a more comprehensive picture, we have examined the development of the intestinal ARG composition in two healthy individuals over a six-day course of ciprofloxacin (Cp) treatment by using sequenced-based metagenomics and different ARG quantification methods. Stool samples from both volunteers were collected before treatment (day 0), during treatment (day 1, 3 and 6), and 2 and 28 days after treatment. Metagenomic shotgun sequencing was done on an Illumina HiSeq 2000 platform, using paired-end sequencing with a read length of 100 bp and an insert size of 180 bp. Fixed- and random-effects models were performed to determine the alteration in ARG abundance per defined daily dose of Cp as an expression of the intestinal selection pressure.
Cp treatment led to significant shifts in the composition of the intestinal resistome. In one individual, a strong positive selection for class D beta-lactamases was determined. One member of the class D beta-lactamases, blaOXA-347, was located on a mobile genetic element as shown by a metagenomic assembly approach. Of note, negative selection has also been observed. For instance, the class A beta-lactamases showed a trend in that direction (-2.66 hits per million sample reads / defined daily dose; $p = 0.06$). Four weeks after treatment, a reconstitution towards the initial ARG composition has been observed in both individuals, but to a different degree.
Our work presents a novel analysis algorithm for the determination of antibiotic selection pressure that can be applied to different body sites, not just the human intestine. It can furthermore be used in clinical settings to compare therapeutic regimes regarding their impact on the human resistome. Such information is of major relevance for clinicians in order to choose antimicrobial agents which have a low selective force on the patients' intestinal ARGs. That kind of measures, for instance applied within the frame of antibiotic stewardship programs, are likely to result in a diminished spread of resistance and an attenuated burden of hospital-acquired infections with multidrug resistant pathogens.

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Infection Epidemiology and Population Genetics (FG MS)

267/MSP
Whole genome based typing of *Clostridium difficile (Peptoclostridium difficile)* by a novel cgMLST scheme
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Clostridium difficile was recently renamed to *Peptoclostridium difficile*, however, this name is not widely used. *C. difficile* is a major cause of nosocomial infections; in addition to complications due to an antibiotic therapy, infections are also occurring in the community and in animals. The prevalence and severity of *C. difficile* infections have increased significantly in the last years worldwide. Currently different typing methods for determining strain relatedness in a nosocomial setting are used for *C. difficile*, (e. g. PFGE and MLVA) and the current standard method is PCR ribotyping. However, these methods are difficult to standardize and to compare between different laboratories.
To allow subtyping of *C. difficile* for infection control purposes and to overcome the drawbacks of current methods, we developed a genome-wide typing scheme based on gene-by-gene comparisons (core genome [cg]MLST approach).
We first determined the population diversity based on all available MLST data of 347 *C. difficile* sequence types (ST) (http://pubmlst.org/cdifficile/, March 31, 2016) using the Bayesian analysis of genetic population structure (BAPS). Subsequently, cgMLST target genes were defined using *C. difficile* strain *630* (NC_009089.1) as reference and genome sequences of representative isolates for all BABS groups and the six *C. difficile* clades using the cgMLST target definer of the Ridom SeqSphere$^+$ software (Ridom GmbH, Muenster, Germany). Subsequently, we evaluated the cgMLST scheme with genome sequences originating from a diverse collection of reference strains for toxinotyping and 8 clinical *C. difficile* isolates from two published clusters (Eyre DW et al., BMJ open 2(3):e001124 [2012]).
BAPS analysis generated six *C. difficile* groups. Creation of the cgMLST scheme based on genome sequences of representatives of the six BAPS groups and six *C. difficile* clades resulted in 1528 cgMLST targets using the target definer with relaxed parameters (≥

75 % similarity and 95 % overlap) reflecting the high diversity within the *C. difficile* species. Of the cgMLST targets ≥ 95.5 % were present in all strains investigated (clinical isolates and reference strains). Furthermore, cgMLST based grouping of strains was concordant to both, *C. difficile* clades and BAPS groups. In both *C. difficile* clusters, cgMLST showed an equivalent grouping of strains comparable to single nucleotide variant (SNV)-typing: in cluster 1, cgMLST differentiated 2 ST37 isolates with 3 alleles distance (SNV-typing: no differences), and in cluster 2, cgMLST differentiated 2 ST5 isolates with 52 alleles distance (SNV: 144 sites differences). All other isolates within both clusters were only distantly related with both methods.
This cgMLST scheme covers the diversity within *C. difficile* and enables a high discriminatory typing with the advantages of sequence-based methods, i. e. reproducibility and portability of data.

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268/MSP

Unexpected genomic relationships between *Bacillus anthracis* strains from Bangladesh and central Europe

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Introduction: The zoonosis anthrax caused by the bacterium *Bacillus anthracis* has a broad geographical distribution. Active enzootic areas are typically located away from central and northern Europe where cases of the disease occur only sporadically and in limited numbers. In contrast, regions of Bangladesh are hyper-endemic for anthrax, also known as 'Torka' in the national language, where the disease causes major losses in live-stock, especially in cattle and goats. While human disease occurs upon exposure to infected animals, or by handling anthrax spore-contaminated animal by-products, wild and domestic herbivores are infected upon direct exposure to *B. anthracis* in feed and soil. During 2009 and 2010 several outbreaks in districts with the country's largest cattle population, Pabna, Sirajganj and Tangail, led to 140 reported animal and 273 human cutaneous cases of anthrax.
Materials and Methods: Using the improved GABRI method we isolated eight new *B. anthracis* strains from environmental samples of these districts. We genotyped these strains, sequenced a variety of genomes and conducted a comparative genome analysis.
Result: All isolates from Bangladesh belonged to canSNP group A.Br.001/002 differing only in a few of 31 MLVA-markers. Whole genome sequences were obtained from five of these strains and compared with genomic information of *B. anthracis* strains originating from various geographical locations. Characteristic signatures were detected defining two "Bangladesh" clusters potentially useful for rapid molecular epidemiology. From this data high-resolution PCR assays were developed and subsequently tested on additional isolates from Bangladesh and Central Europe.
Conclusion: Remarkably, this comparative genomic analysis focusing on SNP-discovery revealed a close genetic relationship between these strains from Bangladesh and historic strains collected between 1991 and 2008 in The Netherlands and Germany, respectively. Possible explanations for these phylogenetic relationships are discussed.

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269/MSP

Current status of *C. difficile* infection epidemiology in communities of Africa, Indonesia and Germany

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Clostridium difficile infections (CDI) lead to nosocomial diarrhea with complications like pseudomembranous colitis or toxic megacolon resulting in high mortalities worldwide. Routinely used antibiotics that destroy the indigenous intestinal microbiota are known as a major risk factor for CDI. Towards the prevalence of *C. difficile* infections between geographical regions that differ in regional use of antibiotics less data are available.
In order to assess the epidemiology in communities in Ghana, Tanzania, Indonesia and Germany, a total of 608 patients with diarrhea and 594 asymptomatic control individuals of different ages were screened for the presence of *C. difficile* in stool samples. Cultured *C. difficile* strains were further subtyped using PCR ribotyping, MALDI-TOF MS, genome analysis, detection of toxin genes and production, as well as antibiotic susceptibility testing.
CDI was present in all countries, with prevalence rates that ranged from 5% in Africa to 15% and 27% in Indonesia and Germany in diarrhoeal patients, respectively. Nontoxigenic strains of ribotype 084 were most abundant in Africa in contrast to toxin A+/B+ ribotypes 001/072 and 078 that predominated in Germany. In Indonesia, most strains belonged to toxin A-/B+ ribotype 017. Differences were obvious for the genome size and the abundance of phages and plasmids according to the geographical region. Antibiotic susceptibility patterns revealed major differences between the regions. Especially in Germany a high rate of strains (67%) was resistant to Moxifloxacin, followed by Indonesian strains with 24% of resistance. In comparison, in all African *C. difficile* isolates the Moxifloxacin resistance was completely absent.
The study indicates that CDI is a global health threat with geographically different prevalence rates that might reflect the distinct use of antibiotics. The investigated strains differed significantly for the distribution of mobile genetic elements, ribotypes, toxin production, as well as the antibiotic resistance pattern. If diagnosis relies on detection of toxin A only, several CDI cases especially from Africa or Asia might be left undetected.

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270/MSP

Spontaneous mutation rates in diverse strains of *Staphylococcus aureus*

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Introduction: Recently, population genomic analyses have provided estimates of short-term evolutionary rates for a number of human pathogenic bacteria. For as yet unknown reasons, annual rates of base substitution differ markedly between different species of bacteria. The spontaneous mutation rate is the expected number of mutations that a bacterial cell will sustain during its lifetime. It is a basic parameter for understanding the dynamics of bacterial evolution. We hypothesize that spontaneous mutation rates might partially explain these differences.
Objective: To better understand mechanisms and dynamics of evolution in *S. aureus*, we determined spontaneous mutation rates for methicillin-susceptible and methicillin-resistant *S. aureus* isolates affiliated to a variety of phylogenetic lineages, including ST398, CC8, CC30, CC22, CC1 and CC5.
Materials and Methods: We performed classical fluctuation analyses in trypticase soy yeast extract medium. In brief, thirty parallel cultures per each strain were inoculated with overnight culture and following 6 hour incubation at 37^{0}C in plate reader 100 µl from each culture was plated on selective plates. Mutation rates were calculated employing the MSS Maximum Likelihood Method with the web tool FALCOR (http://www.keshavsingh.org/protocols/FALCOR.html).
Result: Our results showed a largely uniform spontaneous mutation rate among isolates from different clonal complexes, including both MRSA and MSSA.
Conclusion: The uniform mutation rate correlates with a universal short-term evolutionary rate among diverse *S. aureus* strains. It implies similar evolutionary constraints and average growth rates among different clonal complexes.

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271/MSP

The population genetics of structural genomic variation in *Clostridium difficile* ribotype *027*

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Introduction: Population genomic analyses have provided unprecedented insights into the evolution of several bacterial pathogens, including *Clostridium difficile*. The steady accumulation of single-nucleotide polymorphisms (SNPs) has been observed repeatedly and been exploited to estimate the timescales of bacterial spatial spread and population expansion. However, the evolutionary dynamics of structural genomic variation (including insertions, deletions, and the gain and loss of genetic material) are less well understood.
Objective: We intended to comprehensively detect structural genomic variation in a set of *C. difficile* genomes, and to investigate the dynamics of its evolutionary change on the basis of a time-calibrated phylogenetic analysis.
Materials and Methods: By using Illumina technology, we re-sequenced the genomes from 57 clinical *C. difficile* ribotype 027 isolates, which had been collected between 1990 and 2012. In addition, we sequenced to completion the genomes from eight selected isolates by applying a combination of PacBio and Illumina technologies.
Result: SNPs constituted the most abundant sequence variation that had been accumulated over few years, but we also detected considerable numbers of insertions and deletions, including length differences of homopolymeric runs and tandem repeat regions, single-nucleotide deletions, and larger indels, several of which were likely to have functional impact.
Conclusion: Integration of Illumina data with complementary PacBio genome sequence data from selected isolates provided a comprehensive picture of structural genomic variation, including the gain and loss of mobile genetic elements, and revealed the timescale over which this diversity got generated.

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272/MSP

Typing of SCC elements in Staphylococcus aureus and other staphylococci

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Methicillin-resistant Staphylococcus aureus (MRSA) are a global and serious problem to infection control and healthcare for many years. MRSA carry *mecA* and *mecC* genes on SCC*mec* elements. These are potentially mobile genetic elements in Staphylococci that additionally may comprise other genes such as recombinase genes and a variety of accessory genes including other resistance markers. Twelve main types and several variants have yet been described.
In order do subtype MRSA strains based on variations within their SCC*mec* elements, 81 markers were selected from published SCC sequences for an assay based on multiplexed primer extension reactions followed by hybridization to 113 specific probes. This panel included *mecA* and *mecC*, *fusC*, regulatory genes, recombinase genes, genes from ACME (arginine catabolic mobile element) and heavy metal resistance loci as well as several genes of unknown function. For validation and development of a stringent hybridization protocol, real hybridization experiments with fully sequenced reference strains were performed modifying protocols until experimental results were in concordance to theoretical predictions based on the known sequences. After validation, approximately 800 Staphylococcus aureus isolates from different parts of the world including Western Europe, the Middle East, the Caribbean and Australia were characterised. These comprised mostly mecA MRSA, but also mecC MRSA and methicillin susceptible strains with SCC elements that did include other markers than mecA/C. Within the study, nearly 200 subtypes or variants of SCC elements as well as composites thereof were identified. Several common epidemic strains showed a remarkable variability of SCCmec subtypes. These included ST239-MRSA-III, CC5-MRSA-IV, CC22-MRSA-IV or CC398-MRSA-V/VT. These strains might have evolved during their spread to carry divergent

SCC variants, or they might have been polyphyletic, i.e., originating from related clones of parental MSSA that independently acquired different (although similar or related) SCCmec elements.
Using the microarray, variations within SCC elements can be evaluated for typing purposes. Moreover, the array is a high-throughput screening tool that can be used for epidemiological typing and also for selecting strains that warrant further investigation, e.g., by genome sequencing.

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273/MSP

Whole genome core gene typing of *Bacillus anthracis*

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Introduction: In the event of bioterrorism or infectious disease outbreaks, high-resolution genetic characterization is critical for determining the identity of the pathogen and possibly attributing it to a specific source. *Bacillus anthracis* is a gram-positive, obligate pathogenic bacterium and the etiologic agent of anthrax. The diagnosis of anthrax and trace-back analysis is mainly based on PCR targeting virulence-plasmid markers, variable-number tandem repeat (VNTR) regions and single nucleotide polymorphisms (canSNP, tag SNP). With these markers it is only possible to define major phylogenetic branches and particular subclades of limited phylogenetic resolution. Here, we present a comprehensive whole genome core gene SNP typing approach (cgMLST) which is based on all available *B. anthracis* genome assemblies and raw data.
Materials and Methods: We collected all *B. anthracis* genomes available in October 2015 as well as raw sequence datasets of currently unassembled genomes from the NCBI databases. Raw sequence datasets where assembled with SPAdes. The well annotated type strain "Ames Ancestor" was used as reference. To define the core genome, the genes of the reference strain were initially filtered and then mapped against all the other genome assemblies using SeqSphere+. Only those assemblies having a good assembly coverage and additionally comprised more than 85% of reference genes were used in the final dataset to determine gene presence/absence and allelic differences. This data set was used for phylogenetic and gene diversity analyses.
Results and Conclusions: Out of the 114 available *B. anthracis* assemblies and 52 raw sequence data sets, 134 strains were finally used to build the core genome based on 1,286 target genes (4,490 targets built the canSNP reference core genome, out of 5,029 genes after quality filtering). About 290 of the core targets were highly conserved and did not contain any SNPs, but only about one-quarter of them had annotated functions. The backbone phylogenetic tree showed high congruence with established canSNP- and MLVA grouping. Further, possible new sub-branches were identified. Notably, cgMLST showed the same discriminatory power as other SNP-based typing schemes. In contrast or additionally to whole genome SNP calling, this novel approach enables a simple standardization and data exchange between different laboratories. Besides, one further advantage of cgMLST is that a completely closed genome is not required. Even if some target genes were missing, comparison is possible and time- and cost-consuming re-sequencing can be avoided without any loss of resolution.

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274/MSP

***E. coli* functional genotyping: predicting phenotypic traits from whole genome sequences**

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Introduction: Foodborne bacteria like *E. coli* pose a major threat to public health. To prevent widespread infections due to these bacteria, as well as to detect outbreaks, rapid and accurate identification and characterization of these bacteria are of key importance.
Objective: Conventional methods for serotyping, virulence profiling, and antimicrobial susceptibility testing are time-consuming and often require complex workflows. Alternatively, whole genome sequence (WGS) data provides highly detailed genotypic information and could have the potential to replace some of these conventional methods. In this study, we present a genotyping plugin for the BioNumerics® 7.6 software, that predicts O and H serogroups and pathotype, and detects virulence and resistance genes as well as prophages and plasmids, starting from *E. coli* WGS data.
Materials and Methods: The BioNumerics® genotyping plugin contains public databases for e.g. serotype, virulence and resistance prediction downloaded from the Center for Genomic Epidemiology (www.genomicepidemiology.org), combined with private knowledge. The various *E. coli* genotyping tools start from the assembled genomic sequences and use a blast-based approach to detect and identify the genes of interest. Detection parameters were set to 90 % sequence identity and 60 % sequence coverage.
Result: For a variety of clinically relevant *E. coli* phenotypes, publically available sequence reads were assembled and whole genomes were analysed by the BioNumerics® *E. coli* genotyping plugin. The genotyping tools were evaluated versus traditional typing information. Typing results were recorded both as database information fields and/or phenotypic datasets. In addition, exploratory reports on the detailed genotyping results, including locus name, similarity score, and descriptive information on the detected genes, are easily made available from within the software.
Conclusion: The prediction of phenotypic traits from WGS can greatly improve the efficiency and effectiveness of molecular surveillance. The BioNumerics® functional genotyping plugin proofs to be an effective predictive tool for *E. coli* phenotypes, including serotype, antibiotic resistance, and virulence. This user friendly WGS-workflow combined with the integrated read quality assessment, wgMLST and wgSNP tools of BioNumerics®, can lead to more efficient outbreak characterization.
This tool can be adapted to other bacterial pathogens in the future.

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275/MSP

Where are you from? Trace-back analysis of *Brucella melitensis* isolated from humans in Germany

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Introduction: On a global scale human brucellosis is one of the most common bacterial zoonotic diseases. However, Germany is considered a brucellosis-free area with less than 50 cases reported annually, mainly due to travellers to or immigrants from endemic regions like the Mediterranean basin. Increasing numbers of brucellosis cases in 2014 and 2015 raised the question of the geographic origin of strains isolated in Germany and the probable origin of infection.

Material and methods: All patient records of culture-positive cases of the national Brucella consultant laboratory from 2014 henceforward were evaluated with regard to patient history and clinical presentation. Strains already confirmed to be B. melitensis by realtime and conventional PCR were selected for whole genome sequencing (WGS) using Nextera XT Library kit and MiSeq platform. Additional strains from the strain collection of the Bundeswehr Institute of Microbiology were included as reference and outgroup. After de-novo and reference-based assembly, genome comparison with genome-wide single nucleotide polymorphism (SNP) analysis was applied and genetic determinants of antibiotic resistance were investigated. Moreover, in silico multilocus variable number of tandem repeats analysis (MLVA) was performed and compared to a public MLVA database containing entries of 2,215 B. melitensis strains.
Results: All strains were straightforwardly sequenced and phylogenetic analysis based on whole-genome SNPs revealed spatial clustering. MLVA led to the same grouping of isolates and predominantly matched the East Mediterranean genetic clade. Genetically encoded resistance determinants with phenotypic effect on the susceptibility of commonly used antibiotics for brucellosis treatment were not observed.
Conclusions: This study used a comprehensive collection of B. melitensis strains isolated in Germany during the last three years and corroborates WGS to be a suitable tool for trace-back analysis pointing to the geographic origin of a given strain. Compared to MLVA WGS achieves a higher resolution and at the same time genotype-phenotype correlations like resistance mechanisms can be determined. However, the lack of reliable metadata in public databases often hinders a resolution below geographic regions or country level and corresponding precise trace-back analysis. Hence epidemiological findings and patient histories remain essential despite the novel role of WGS-derived bacterial typing in cluster investigation.

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National Reference Centres and Laboratories of Consultancy (StAG RK)

276/RKP
Antibiotic resistance of German invasive *H. influenzae* isolates against Ampicillin and Imipenem
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Introduction: The National Reference Laboratory for Meningococci and *Haemophilus influenzae* (NRZMHi) carries out laboratory surveillance of invasive *H. influenzae* infections in Germany. Monitoring of antibiotic resistance is one of its tasks. Resistance to ampicillin due to beta-lactamases and *fts*I mutations has been described for 2009-2012 (Lam et al., IJMM 2015) and will be reported here for another three years. Resistance to imipenem has been recognized in the past ten years (Cerquetti et al., AAC 2007), but data on its epidemiological relevance are limited. The genetic mechanisms are unresolved, but limited evidence suggests beta-lactamase-independent *fts*I mutations.
Aim: The aim of this study was to assess resistance rates towards ampicillin and imipenem in H. influenzae isolates from blood or CSF in Germany.
Materials and Methods: Strains submitted to the NRZMHi were analysed by Etest using Eucast breakpoints and *fts*I sequencing.
Result: Antibiotic resistance data for ampicillin are available starting from 2009. Ampicillin resistance (MIC > 1µg/ml) was found in 15% of all tested isolates with no clear temporal trend (2009: 11%, 2010: 9%, 2011: 18%, 2012: 14%, 2013: 15%, 2014: 21%, 2015: 17%). Beta-lactamase negative ampicillin resistant (BLNAR) isolates with *fts*I mutations were found at low rates, ranging from 1% (2010) to 6% (2014). Surveillance of imipenem resistance in invasive isolates commenced in 2016. So far 134 isolates were analysed, of which 32 (24%) were imipenem resistant. A major number, but not all of the imipenem resistant isolates were beta-lactamase negative ampicillin resistant (BLNAR or lowBLNAR). Molecular analysis of the *fts*I gene revealed multiple mutations that have been described as characteristic for BLNAR with no clear specificity for the trait imipenem resistance. In imipenem resistant isolates, the susceptibility to third generation cephalosporines was not affected. Most imipenem resistant isolates were sensitive towards meropenem.
Conclusion: Treatment of invasive infections due to *H. influenzae* with aminopenicillins without beta-lactamase inhibitors is no longer acceptable. The observed 24% resistance rate for imipenem for the time being is not affecting treatment decisions, however, unravelling its epidemiological importance and mechanisms could become very important once cephalosporin resistance emerges in this species. This study, therefore, provides the basis for future enhanced analysis of carbapenem resistance.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

277/RKP
Fluorescence in situ hybridization for the identification of Treponema pallidum in tissues
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Fluorescence in situ hybridization (FISH) is a microscopy tool that allows simultaneous identification, visualization and localization of bacteria in clinical samples, in particular for fastidious and yet uncultured spirochetes like Treponema pallidum. In this study we applied FISH for the diagnosis of inconclusive cases of syphilis.
A species-specific fluorescently labelled probe, targeting the 16S rRNA of Treponema pallidum, was designed in silico and evaluated by comparison to all available sequences in EMBL and GenBank databases. The probe was tested and validated in tissue positive controls from experimentally infected rabbit models and negative controls from oral treponemes cultures. Sections of methacrylate embedded clinical biopsy samples from three different Syphilis cases were hybridized using the species-specific probe TPALL and revealed number and distribution of T. pallidum in the following tissue:
1. Preputial tissue from a patient misdiagnosed and treated for phimosis.
2. Lymphatic tissue obtained from an HIV positive patient with inconclusive serology.
3. Gingival tissue from a patient showing oral lesions of unknown origin.
4. Tonsil tissue from a patient with cervical inflammation and tonsillitis with follicular hyperplasia.
FISH contributes to the understanding of the invasiveness of treponemal infections by allowing simultaneous visualization,

identification and localization of individual microbial cells within tissue. FISH may be a valuable tool in research models and for diagnostic procedures, in particular when standard routine diagnostics failed to deliver conclusive results.

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278/RKP

Outcome of salvage Helicobacter pylori eradication therapies based on genotypic susceptibility testing

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Objective: To evaluate treatment outcome of in H. pylori infected individuals who had undergone salvage eradication treatments tailored by genotypic susceptibility testing
Method: From October 2004 to December 2013, 124 H. pylori positive patients with prior unsuccessful eradication treatments were administered eradication treatments based on genotypic susceptibility testing by PCR and/or sequencing. Six months on, treatment outcome was assessed by urea breath test; stool-antigen ELISA; and microbiology/histopathology. Clinical improvement of symptoms was asked and documented by attending physicians.
Result: Salvage eradication treatments based on genotypic testing achieved eradication rates of nearly 70% in these patients. No particular regimen was significantly better than another.
Conclusion: Genotypic antimicrobial susceptibility testing prevents prescription of inefficient antimicrobials and enables individualized and promising salvage treatments in patients with prior unsuccessful eradication treatments.

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279/RKP

EU-wide external quality assessment study to establish performance characteristics of different amplification protocols for detection of *Borrelia burgdorferi* sensu lato

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Background: Laboratory diagnosis of lyme borreliosis (LB) the most important vector borne disease in the northern hemisphere is based mainly on antibody detection. However, nucleic acid amplification techniques (NAT) are an important additional tool for diagnostic and research. A major challenge for NATs is the enormous genetic heterogeneity of the causative agent *B. burgdorferi* s.l.: to date >20 different genospecies have been described of which nine are assured ore probably pathogenic for humans. Broad comparative studies for NAT-based methods currently used in diagnostic settings for lyme borreliosis including all relevant Borrelia burgdorferi sl species have not been conducted.
The major aim of the study is to compare analytical sensitivity and specificity of the various PCR protocols (commercial, in-house) using identical material.
Materials and Methods: A panel of pre-prepared identical 50 µl-aliquots of dilution series of DNA extractions (corresponding to 10^4 to 10^{-1} genome equivalents / 5µl) from 15 *B. burgdorferi* s.l. strains representing nine genospecies as well as relapsing-fever spirochetes, *Leptospira* and *Treponema* were distributed blinded on dry ice to different labs to test amplification protocols and their outcome at a EU-wide scale. Results were collected using a standardised questionnaire.
Result: So far data for 72 protocols from 29 Laboratories were collected. Limit of detection of most protocols for most strains was between 1 and 10 GE per PCR. However, differences in analytical sensitivity of a factor up to >10.000 were found between different protocols regarding a single strain, but also within a single NAT protocol when using different *B. burgdorferi* strains. Cross amplification was frequently observed using relapsing fever borreliae, but was rare with Treponema and Leptospira.
Conclusion: Overall analytical sensitivity and specificity performed well with most protocols.
The data obtained in these experiments will allow to compare the analytical sensitivity and specificity of different loci and primers, their performance in various laboratories, to judge the robustness of different protocols and finally to find out the most suitable detection protocols for the NAT-based diagnosis of Lyme borreliosis.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

280/RKP

A case of pharyngeal diphtheria in Germany, June 2015

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Introduction: Diphtheria is rarely observed in Europe today with 10 to 31 cases per year. It is caused by toxin-producing *Corynebacterium (C.). diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis* that are spread by droplets or direct contact. A 45-year-old man suffering from acute necrotic tonsillitis and throat phlegmon with a greyish thrush covering the tonsils was hospitalized in Nuremberg including emergency coniotomy and intubation. Throat swabs grew a toxigenic *C. diphtheriae* strain. Due to several job-related events all over Germany 10 days before the patient was symptomatic he had diverse close contacts.
Question: Intensive contact tracing was performed by the local health department to rule out secondary cases. Multilocus sequence typing (MLST) and Next Generation Sequencing (NGS) was performed to characterize the toxigenic strain and find the possible source of infection.
Methods: Strain identification was performed by biochemical differentiation (API Coryne; bioMèrieux, Germany) and MALDI-TOF analysis (MALDI Biotyper; Bruker Daltonics, Germany). Susceptibility testing was performed according to the CLSI guidelines. Toxigenicity was verified by real-time PCR and a modified Elek test. MLST based on seven housekeeping loci was performed and analysed using the respective database (http://pubmlst.org/cdiphtheriae). Next generation sequencing analysis was carried out with the isolate using Illumina Nextera XT libraries and an Illumina MiSeq.
Result: A tox+ *C. diphtheriae biovar mitis* strain sensitive against penicillin G and erythromycin was identified. MLST data extracted from the NGS data yielded sequence type (ST) 255. NGS data also revealed insertion of a corynephage similar to the ωtox+ prophage found in e.g. *C. diphtheriae* PW8 (PMID: 22505676), carrying the full diphtheria toxin gene.
The patient recovered quickly under antibiotic treatment and was discharged after 25 days. He had no protective immunity and the

most probable source of infection was a 2-week beach holiday in Thailand 19 days prior to his first symptoms. A number of 80 close contacts (3 family members, 26 health care workers, 32 work colleagues, 19 airplane passengers) were identified and PEP and/or immunization was offered. No secondary cases were found.

Conclusion: An origin of the patient's infection in Thailand with an incubation period of 19 days seems unlikely, but it cannot be ruled out completely. ST 255 was not previously reported from South East Asia.

Diphtheria is rarely observed in Europe but a recent fatal case of a 6 year old boy in Spain commemorates this ancient life threatening disease and highlights the problem of diphtheria antitoxin (DAT) availability.

People travelling outside Europe should always update their diphtheria vaccination and diphtheria must be kept in mind especially after journeys to endemic regions and in current coherency also in asylum seekers with skin or respiratory infections.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

281/RKP

Toxigenic *Corynebacterium ulcerans* causing exudative otitis externa in a man and his pet dog.

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Introduction: *Corynebacterium (C.) ulcerans* is an emerging zoonotic pathogen in several industrial countries. Toxigenic strains may cause both wound infections and classical respiratory diphtheria in humans. Companion animals are recognized as an important reservoir with documented transmission to humans. In contrast, person-to-person transmission seems to be extremely rare, although not completely unlikely. In 2015 all 20 human *C. ulcerans* isolates investigated at the German National Consiliary Laboratory on Diphtheria (CLoD) were toxigenic.

In November 2015, a health 55-year-old care worker presenting with productive bronchitis and exusative otitis externa at his general practitioner. A swab taken from the ear canal grew a toxigenic *C. ulcerans* strain.

Question: Extensive outbreak investigations were performed to find the potentially source of infection and to monitor probable secondary cases. Multilocus sequence typing (MLST) was performed to characterize the toxigenic strain.

Materials and Methods: A number of 66 nasal and pharyngeal swabs of 30 asymptomatic close contacts (2 family members, 28 clients) and 3 asymptomatic pet animals were investigated. Strain identification was performed by biochemical differentiation (API Coryne; bioMèrieux, Germany) and MALDI-TOF analysis (MALDI Biotyper; Bruker Daltonics, Germany). Toxigenicity was verified by real-time PCR and a modified Elek test. Multilocus sequence typing based on seven housekeeping loci was performed and analysed using the respective database (http://pubmlst.org/cdiphtheriae).

Result: The patient was treated with amoxicillin for 7 days and recovered quickly. He had no vaccination card, no history of foreign travel, farm visits or consumption of unpasteurized dairy products, but reported having two dogs and one pet cat. All human throat and nasal swabs taken from close contacts were negative, but a *tox*+, penicillin G and erythromycin sensitive *C. ulcerans* strain was isolated from a throat swab of one dog. MLST showed an identical patterns of the human and the dog isolate. Clonal identity was confirmed as both strains were typed as ST 332, a sequence type that was formerly also found in cats and humans.

Conclusion: We report proven transmission of a toxigenic *C. ulcerans* strain between a dog and his owner in view of the increasing evidence for the emergence of toxigenic *C. ulcerans* as an important zoonotic pathogen for humans in Germany and other industrial countries.

National and international guidance should specifically recommend investigation of animal sources in collaboration with veterinary authorities to reduce the risk of infection in the population.

The proportion of toxigenic vs. non-toxigenic *C. ulcerans* strains from humans analysed at the German CLoD has continuously increased in the last years.

Although in this case systemic toxin-induced symptoms did not occur this case underlines the need for a reliable supply of diphtheria antitoxin in European countries.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

282/RKP

Report of the National Reference Laboratory for Multidrug-Resistant Gram-negative Bacteria on Carbapenemases in Germany in 2015

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Question: Multidrug-resistance in *Enterobacteriacea*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since hardly any innovative antimicrobial drug against gramnegative bacteria will be introduced into clinical practice within the next years. Among all resistance mechanisms the worldwide spread of carbapenemases is the most worrisome development. However, the correct identification of carbapenemases is challenging for the microbiological laboratory.

Material and Methods: The National Reference Laboratory for Multidrug-Resistant Gramnegative Bacteria offers the free service of carbapenemase detection in bacterial isolates with elevated carbapenem MICs. All isolates are tested by a wide array of phenotypic and molecular methods. A bioassay based on cell-free extracts allows the detection of still unknown β-lactamases.

Result: A total of 6522 isolates were investigated for carbapenemases in the National Reference Laboratory in 2015. Specimen sources were mostly rectal swabs (21.9%), urinary (21.2%) and respiratory samples (19.1%). Carbapenemases were found in 1125*Enterobacteriaceae* strains (41.8%), 305 *P. aeruginosa* (22.9%) and 534 *A. baumannii* (95.7%). The most frequent carbapenemases in *Enterobacteriaceae* were OXA-48 (39.2%), VIM-1 (16.8%), NDM-1 (14.1%), KPC-2 (9.3%), KPC-3 (5.2%), OXA-181 (2.9%), OXA-232 (2.4%) and NDM-5 (2,3%). GES-5, IMI-1, IMI-2, IMI-10, NDM-5, NDM-7, NDM-9, GIM-1, VIM-2, VIM-4, VIM-5, VIM-31, OXA-162, OXA-244 and others were found in less than 1% each. In *P. aeruginosa* VIM-2 was the most frequent carbapenemase (81.0%), followed by VIM-1 and GES-5 (2.9%). VIM-4, VIM-11, VIM-28, GIM-1, IMP-1, IMP-2, IMP-7, IMP-10, IMP-13, IMP-15, IMP-22 and NDM-1 were found in less than 2.7% each. OXA-23 was the most frequent carbapenemase in *A. baumannii* (64.5%) followed by OXA-72 (10.4%) and OXA-58 (6.0%). GES-11, NDM-1, NDM-3, VIM-2, GIM-1, OXA-40 were found in less than1.5% each.

Conclusion: A variety of different carbapenemases arrived in Germany. However, the molecular epidemiology in Germany with a predominance of OXA-48 differs significantly from observations made in other countries like Greece, Italy or the USA. Compared to previous years, OXA-181 and OXA-232are on the rise.

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283/RKP

Bartonella spp. - a chance to establish One Health concepts in veterinary AND human medicine

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One Health concepts aim to establish interdisciplinary collaborations between medical, veterinary and environmental researchers as well as public health officials for the early detection of health hazards affecting both humans and animals and to fight them on multiple levels. The genus *Bartonella* represents a highly relevant example of the synergistic benefits that can arise from such combined approaches as they infect a broad variety of animals, are linked with a constantly increasing number of human diseases and are transmitted via arthropod vectors. In this study we employ molecular immunological and next generation sequencing methods, in a One Health approach to examine *Bartonella* prevalence in vectors as well as the infection status of animals and humans. In a preliminary case, a female, adult, half-engorged *Ixodes ricinus* tick (Black Forest area, Germany) was removed from a cat after feeding for ~1-2 days. *Bartonella*-DNA was detected via a specific nested 16S-rDNA -PCR. Amplificate was sequenced and revealed a 99 % species homology with *B. henselae*. Immunofluorescence testing of serum samples from the cat and its owner (taken because of unspecific illness) revealed *B. henselae* antibody titers of 1:640 in the cat but no specific *B. henselae*-antibodies in the pet owner. Following this preliminary case, our experimental algorithm will be applied to the further testing of 200 arthropod vectors, their animal hosts and humans who come in contact with them to provide evidence about the prevalence of *Bartonella* in vectors and the rate of transmission between different hosts.

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Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

284/RKP

The Consultant Laboratory for *Clostridium botulinum* at the Robert Koch-Institute: Tracing the deadliest toxin with modern immunological, functional and spectrometric technologies

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Botulinum neurotoxins (BoNTs) are produced by six morphological and phylogenetical different Clostridia: *Clostridium botulinum* Groups I-IV, *C. baratii* and *C. butyricum* with distinct physiologically and phylogenetical properties. BoNTs cause the rare but life-threatening disease botulism. Three main forms of botulism have been described: Most common in Germany is food botulism which is caused by ingestion of BoNT contaminated food. Wound botulism, which is often associated with injecting drug abuse, is caused by a toxinfection of wounds. Infant botulism, which can occur in infants below one year of age, is caused by colonization of the intestinal tract and toxin production after uptake of spores.

After intoxication BoNTs migrate to neuromuscular nerve terminals and are taken up via recycling endosomes by a double receptor binding mechanism. Upon acidification, the light chain is translocated into the cytosol through a pore formed by the translocation domain. Finally, the light chain, a Zn^{2+}-dependent endopeptidase, cleaves essential proteins involved in endosomal recycling thereby blocking acetylcholine release into the synaptic cleft consequently inducing flaccid paralysis and - eventually - death due to respiratory failure if untreated.

Due to the high potency of BoNT detection is challenging. Assay requirements involve a high sensitivity in the low pg/mL range as well as the ability to detect BoNT in a large variety of complex matrices, such as clinical and food samples. Most importantly, BoNTs occur in seven serotypes including more than 40 different subtypes and mosaic forms complicating BoNT diagnostics. Moreover, BoNTs are not secreted as pure neurotoxins but associated with accessory proteins which shield BoNT from the environment in form of high molecular weight complexes. Thus, different forms of the toxin have to be detected as well.

As new Consultant Laboratory for *C. botulinum* we offer a broad spectrum of modern technologies for highly sensitive detection of BoNTs and *C. botulinum*. For toxin detection, we employ the mouse bioassay according to DIN10102 along with different immunological methods such as multiplex, suspension array-based, and conventional ELISA enabling the direct detection of BoNTs. In addition, we use functional methods for monitoring the catalytic cleavage activity and perform unambiguous identification of BoNTs by mass spectrometry. These protein-based methods are complemented by qPCR for the detecting BoNT and accessory protein genes from a broad range of clinical, veterinary and environmental samples.

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285/RKP

Rising Case Counts of Refugee-Imported Brucellosis Observed by the German Consultant Lab for Brucella

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Introduction: Brucellosis is a zoonosis which is highly endemic in the Mediterranean area and the Arab world. In Germany brucellosis plays a role as an imported infectious disease. Since 2001, it is a notifiable disease with 30 cases reported annually on average. In 2014 and 2015 increasing numbers were reported to the Robert-Koch-Institute. The large influx of refugees from endemic areas of brucellosis to Germany may be a major cause of rising case numbers.

Aim: We report on the characteristics of the brucellosis cases observed by the national consultant laboratory during the last two years. Variance in case numbers, clinical signs, origin of infection and diagnostic results (PCR, serology and antimicrobial susceptibility testing) were analyzed. Characteristics of the refugee-associated cases were compared to those of other imported cases.

Method: State-of-the-art methods were applied for detection and antimicrobial susceptibility testing of *Brucella* spp. and for diagnosis of brucellosis from clinical specimens. These include genus- and species-specific PCR assays, bacteriological culture, MIC determination by microdilution and detection of anti-*Brucella* IgM and IgG antibodies by means of ELISA and Brucellacapt®.

Result: The consultant lab was involved in 22 cases of brucellosis in 2014 and 33 cases in 2015. Confirmation of diagnosis was performed in most of the cases by subcultivation of strains from bacterial cultures sent in by other labs, and species identification was done by means of PCR. Serology was available from 13 and 7 patients for 2014 and 2015, respectively. Species identification of cultivated organisms yielded *B. melitensis* in 51 cases and *B. suis* in one case (2014).

Overall sex distribution of patients was 75% male and 25% female. There were 16 refugees among the 2015 cases, most of them from Syria. There were only one case in 2014 but 8 cases in 2015 in the

age group below 18 years, six of the 2015 cases originating from Syria. In 2014, most of the infections were associated with an origin in Turkey (n=16/22), in 2015 Syria was the country with the highest case numbers (n=12/33). As compared to literature, our cases showed a high rate (45% and 58% in 2014 and 2015, respectively) of chronic infections as characterized by typical patterns of clinical manifestations, serology and cultural detection of the agent. Frequently observed manifestations in these cases were bone or joint infections and epididymoorchitis. The AST of all isolates showed no resistance against antimicrobial substances typically used for therapy.
Conclusion: In the last two years, brucellosis case counts showed an increase. In 2015 there was a formerly unseen group of refugees, most of them young and male individuals originating predominantly from Syria among the brucellosis cases. They presented with a high rate of unusual and also chronic clinical manifestations.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

286/RKP
Effectiveness of pneumococcal conjugate vaccines (PCV7, PCV10 and PCV13) against invasive pneumococcal disease among children under two years of age in Germany
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Backgrounds and Aims: A general recommendation for vaccination with pneumococcal conjugate vaccine (PCV) with a 3+1 schedule was issued for German children ≤2 years in 2006. As of 2009, PCV7 was replaced by higher-valent PCVs, mostly PCV13. We calculated vaccine effectiveness (VE) of PCVs using the indirect cohort method.
Method: Pneumococcal isolates from children with IPD were serotyped at the GNRCS using the Neufeld-Quellung reaction. VE was estimated by comparing the odds of vaccination among IPD cases due to vaccine serotypes with the odds of vaccination among IPD cases cases due to non-vaccine serotypes. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using Firth's bias-reduced logistic regression and adjusted for age and season.
Result: For PCV7, the adjusted VE against all 7 serotypes including 6A was 80% (95% CI: 63-89) for at least one dose, 97% (89-100) after three primary doses and 95% (57-100) after the booster dose. For PCV13, the adjusted VE was 86.0% (74-93) for at least one dose, 85% (62-95) post primary, and 91% (61-99) post booster. For the additional serotypes included in PCV13, VE was 82% (66-91), 80% (46-93) and 90% (54-98) respectively. VE (at least one dose) for serotype 1 was 83% (15-97), serotype 3: 74% (2-93), 6A: 96 (56-100), 7F: 84% (18-98), 19A: 77% (47-90). During the analysis period, no cases with serotype 5 were reported. Vaccination rates increased from 25.6% in 2006-2007 to 83.8% in 2014-2015, but there was considerable delay in administration of doses. Over 90% of vaccine serotype cases were in non- or incompletely vaccinated children.
Conclusion: Our data show high VE for all included serotypes. A disturbing finding is the considerable delay in administration of vaccine doses. About 90% of the remaining vaccine type IPD cases in children <2 years could have been prevented by timely vaccination.

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287/PWP

Abstract withdrawn.

Microbiota, Probiota and Host (FG PW)

288/PWP
RNA-based stable isotope probing of resistant starch degrading bacteria from the digestive tract of mice
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Resistant starch (RS) is an effective nutrient for shifting the structure and activity of the large bowel community towards a more beneficial profile for overall human health and well-being [1, 2]. While the effects of RS on the large bowel microbiota have been inferred using enrichment studies, little is actually known about the bacteria directly involved in the fermentation of RS. This information is needed to fully elucidate the impact of RS on host health and disease [3, 4]. In the present pilot-study, 16S rRNA-based stable isotope probing (RNA-SIP) [5, 6] was applied in a murine model system, to identify bacteria that utilize potato-derived RS within an intestinal community. Murine faecal samples were diluted in minimal media and cultured anaerobically with either 40 mM [U-^{13}C]-RS or native RS (^{12}C-control) as sole carbon source. After 0, 2 and 4 h, total RNA was isolated and separated into labelled and non-labelled fractions by density gradient ultracentrifugation. Labelled and non-labelled fractions were analysed by 16S rRNA gene sequencing (Fig. 1). This revealed a microbial community with *Parabacteroides* and *unclassified Prevotellaceae* as the primary degrader of the ^{13}C-labelled RS as their proportion was significant higher in the 'dense' fractions compared to the 'light' fractions ($p < 0.05$) after 2 h of incubation. After 4 h incubation, the relative abundance ($p < 0.05$) of *unclassified Ruminococcaceae* and *Butyricicoccus* was increased in the 'dense' fractions indicating a secondary role of these bacterial groups in RS degradation. HPLC-IRMS analysis of cultural supernatant samples showed generation of acetate, propionate and butyrate during RS metabolism with metabolic by-products constituting up to 17.3 % of the ^{13}C -pool. This study shows that RNA-SIP can be used to identify microorganisms associated with specific metabolic processes. An *in vivo* mice feeding trial using [U-^{13}C]-potato starch is currently in process.

References
[1] Birt DF et al. (2013) *Adv Nutr* **4**: 587-601.
[2] Higgins JA & Brown IL (2013) *Curr Opin Gastroenterol* **29**: 190-194.
[3] Egert M, et al. (2006) *Trends Microbiol* **14**: 86-91.
[4] Flint HJ et al. (2012) *Gut Microbes* **3**: 289-306.
[5] Manefield M et al. (2002) Rapid Commun Mass Spectrom **16**:2179-2183.
[6] Dumont MG & Murrell JC (2005) *Nat Rev Microbiol* **3**: 499-504.

Figure 1

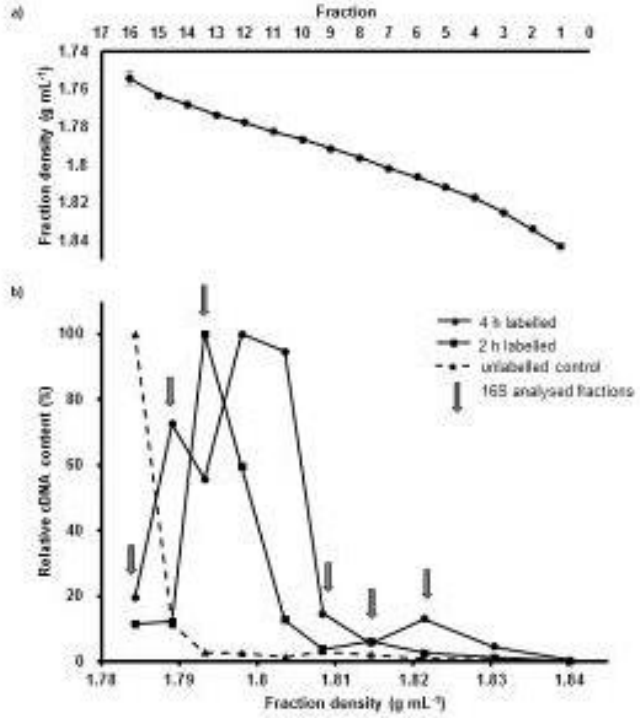

Fig. 1: a) Density curve averaged over three isopycnic buoyant density gradients
b) Relative content of cDNA in fractions collected from density gradients determined by qPCR

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

289/PWP

Genomic and transcriptomic analysis of *Staphylococcus hominis*, a frequent human skin inhabitant

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Introduction: The human associated microbial community has gained tremendous attention due to increasing reports showing the relevance of commensals in triggering and influencing the immune system of the host. The skin, as the physical barrier protecting the human body from its environment, is not only a highly immunocompetent organ but represents an easily accessible target for therapeutic interventions.
Aim: To be able to manipulate the human microbial community for therapeutic measures, the community structure has to be evaluated and the function of particular members needs to be determined.
Materials and Methods: The microbial community structure at different body sites was investigated by 16S rRNA amplicon sequencing. A strain of *Staphylococcus hominis* was isolated from the skin of a healthy volunteer and subjected to genome sequencing on the Illumina MiSeq and PacBio RS II systems. This genome was then used as a template to characterize the bacterial transcriptome under *in vivo* and *in vitro* conditions through RNASeq using the Illumina HiSeq system.
Result: Microbial community structure analysis of different skin habitats revealed *Staphylococcus hominis* as an important community member. The first closed genome of a *S. hominis* isolate inhabinting the human skin was compiled in this work where the 2.16 Mb genome encodes 2126 genes. The transcriptomic profiles show significant difference between *in vivo* and *in vitro* conditions and revealed the adaptation of the strain to the changing environmental conditions on the skin. Although being a commensal, some virulence factors were shown to be transcribed *in vivo*, raising the question of the role those factors.
Conclusion: 16S rDNA amplicon sequencing revealed the importace of staphylococci in colonizing the human skin. A common representative is *S. hominis*, but little was known about its genome and function in vivo. The availability of the complete genome sequence allowed to gain insights into this bacterium's activity on the human skin for the first time.

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290/PWP

The Respiratory Tract Microbiome in Patients Weaning from Ventilation

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Introduction and Aim: Mechanical ventilation is an essential part of modern intensive care medicine, but it may result in a prolonged weaning process, which is then often associated with infectious complications. Whereas several studies analyzed the lung microbiome in chronic lung diseases, the microbiome of weaning patients has not been described. Thus, we intend to elucidate the lung microbiome / virome of weaning patients, for example, whether specific pathogens play a role in and might predict the course and outcome of the weaning process.
Patients and Methods: 29 patients were included, with 3.2 +/-2.4 samples per patient (93 samples overall). Because weaning patients represent a heterogeneous population, patient demographics (age, sex, prior ventilation duration, underlying lung disease, outcome) varied across the study population. Tracheal aspirates were collected on a weekly basis, and analyzed with conventional methods (culture, PCR) and innovative next generation sequencing (NGS) techniques (amplicon-NGS following 16S rDNA amplification, V1-V3 region, and shotgun-NGS following viral enrichment; Illumina MiSeq). For comparison with the microbiology data, clinical metadata such as spontaneous breathing time and clinical and laboratory signs of infection were recorded in parallel.
Result: In conventional microbiology, *P. aeruginosa* was frequently detected (72.4% of patients), often as a continuous colonization. Enterobacteriaceae were also common (55.2%), whereas *S. aureus* and typical respiratory pathogens such as *S. pneumoniae* played a minor role (13.8%/10.3%). In conventional virology, herpes viruses were frequently found (62.1%) with CMV and HHV-6 being most common (each 34.5%), followed by EBV (31.0%) and HSV (6.9%). Adenoviruses were also common (20.6%), but typical respiratory viruses were rather rarely detected. Regarding preliminary comparison of conventional microbiology data and clinical parameters, weaning outcome and clinical signs of infection seem to be unrelated to *P. aeruginosa*, Enterobacteriaceae and virus detection. However, the presence of infiltrates might be correlated with a shorter spontaneous breathing time. Interestingly, the frequency of enterobacteriaceae (but not *P. aeruginosa*) detection was higher in samples collected after a long ventilation duration, and, unexpectedly, the frequency of *P. aeruginosa* detection was higher in samples from patients with a long spontaneous breathing time.
Summary: To our knowledge, this is the first lung microbiome study in patients weaning from ventilation. Our and similar studies might provide interesting insights in the microbiome composition of weaning patients and its potential changes in relation to the course and outcome of the weaning process.

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291/PWP

The probiotic *E. coli* strain Nissle 1917 inhibitis Shiga Toxin production in EHEC and protects *E. coli* K-12 Strains against *stx* phage infection.

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Enterohemorrhagic *E. coli* (EHEC), which are transmitted by contaminated food, have become a significant threat for humans as these pathogens can lead to the development of severe gastrointestinal disease and life threatening complications such as HUS. Since the large outbreak in Germany in 2011 a lot of research addressed the pathogenicity of EHEC and the development of new treatment strategies. The most important EHEC virulence factor is Shiga toxin (Stx), an AB_5 exotoxin. Once secreted this toxin can bind with its B subunits to the globotriaosylceramide receptors (Gb3) of e.g. enterocytes and enter the cells by endocytosis. The A subunit has a specific N-glycosidase activity and cleaves an adenine base from the 28S rRNA of the ribosome by which the protein synthesis is blocked and the cells die due to apoptosis. Treatment of patients with antibiotics is not recommended as this is linked to an increase of released Stx [1]. Previous studies with probiotics showed *E. coli* Nissle 1917 (EcN) to inhibit both growth of EHEC strains and Stx production, which can only be traced back in part to the production of antibacterial operating microcins [2]. In the course of our experiments with a transwell permeable system we could elucidate that no direct cell to cell contact is necessary for EcN to downregulate the expression of Stx by EHEC strains. Furthermore, we could reveal during in vitro studies that EHEC strains can convert *E. coli* K-12 strains to become Stx producers themselves which however, can be blocked by the presence of EcN but not by other commensal *E. coli* strains. This rescuing effect could be explained by a reduction of *stx* phage expression of EHEC provoked by the probiotic EcN. Our in vitro results might reflect the in vivo situation where *stx* phages can infect commensal bacteria in the human gut and turn them into Stx producers themselves. These findings encourage us to elucidate the mechanism of the downregulation of the Stx production in EHEC strains by EcN and support the idea of applying EcN as a medication in the treatment of EHEC infections as supplementary probiotic treatment during a human EHEC infection.

References

[1] DGI *EHEC und Antibiotikabehandlung*. 2011. 3.
[2] Rund, S.A., et al., *Antagonistic effects of probiotic Escherichia coli Nissle 1917 on EHEC strains of serotype O104:H4 and O157:H7.* International Journal of Medical Microbiology, 2013. **303**(1): p. 1-8.

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292/PWP

Influence of an altered microbiome on infections with *Chlamydia muridarum in vivo*

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Infections with the intracellular pathogen *Chlamydia trachomatis* are the most common sexually transmitted bacterial disease worldwide. While doxycycline and azithromycin are the recommended antibiotics in clinical treatment, up to 10% treatment failures occur. Asymptomatic or mistreated infections can lead to ectopic pregnancy, pelvic inflammatory disease and infertility. To study the course of infection *in vivo*, murine models gain in importance. Due to the formation of characteristic pathologies, such as hydrosalpinx, the murine pathogen *Chlamydia muridarum* is frequently used. A potential role of the microbiome on the course of infection is speculated.

The aim of this study was to determine changes of the course of chlamydial infection in mice and its outcome due to unspecific alterations within the commensal microbiome. This gives hints for which bacterial communities might be either beneficial or disadvantageous for the course of infection.

8-week old C57BL/6J mice were divided into different treatment groups. Specific groups were synchronized to the same stage of the estrous cycle with 2.5 mg medroxyprogesterone-acetate (Depo) per mouse. Mice were infected with 10^6 IFUs *Chlamydia muridarum* Nigg II 7 days later. Definite groups were treated with doxycycline (50 mg/kg BW) at three different time points (before, during and after infection) and ampicillin (200 mg/kg BW) before infection. Vaginal swabs and stool samples were collected every 6 days. The bacterial shedding was monitored via recovery of viable *C. muridarum* from vaginal swabs. The microbiome is currently analyzed via DNA isolation from swab and stool samples, amplification of the 16s rRNA (V3-V4 region) gene and subsequent Illumina MiSeq sequencing.

The bacterial shedding showed differences between the treatment groups. Unsynchronized mice had a lower bacterial burden in comparison to the synchronized mice. Within the groups of synchronized mice, the ones treated with doxycycline or ampicillin before infection had a higher shedding than the (untreated) control animals. No more chlamydia were detectable after 24 days in the groups of the unsynchronized mice (doxycycline treated even at day 18). The groups of synchronized mice ended the natural bacterial shedding at day 30 post infection.

To characterize the course of chlamydial infection, we focus on the composition of the microbiome and its influence on the success of chlamydial invasion and survival in the female urogenital tract. The outcome of an altered microbiome will be assessed in more detail. Sequencing based analysis of the microbiome is currently used to correlate specific alterations of the microbiome with the outcome of infection.

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293/PWP

Comparative transcriptome analysis of *E. coli* Nissle 1917 (Mutaflor®) in various conditions

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Introduction: *E. coli* Nissle 1917 (EcN) is one of the best characterized probiotics and it is the active component of the probiotic preparation "Mutaflor®", which is used in the treatment of various gastrointestinal disorders. The non-virulent nature, increased genetic stability, fitness factors and safety aspects make EcN an ideal probiotic. Recently, studies have been reported on antagonistic activity of EcN against various Enterohaemorrhagic *E. coli* strains (EHEC) such as the classical EDL933 and also isolates from the 2011 outbreak (Rund et al, *IJMM*, 2013) which emphasize EcN's anti-pathogenic capability. EcN is currently produced and sold by Ardeypharm GmbH, Germany.

Objective: We believe that the industrial culturing conditions determine the properties of EcN as Mutaflor. These characteristics of the "starter culture" in the gut might be important for EcN's ability to colonize and exert the beneficial effects on the host Therefore, the transcriptome of EcN under production conditions was analyzed.

Materials and Methods: In order to identify genes which are highly expressed in the fermenter culture we compared the transcriptome of LB over-night-cultures in our lab with the transcriptome of fermenter cultures from the Ardeypharm Company. This was achieved by isolating RNA from conventional liquid LB culture and the commercial fermenter culture in their stationary phase by QIAGEN RNAeasy midi kit. The RNA was sequenced by differential RNA sequencing (Sharma CM et al., 2014) and quantified using the DESeq software (Anders et al., 2010). Read numbers were compared between the two different cultivation methods.
Result: Preliminary analysis of changes in gene expression of the fermenter culture indicates that there is a strong iron deprivation which is evident from several fold up regulation of genes that code for different siderophores. In addition there are also changes in gene regulation of important metabolic pathways such as glycolysis, citric acid cycle and urea cycle which might indicate stress due to the varying level of key ingredients like phosphate, sulfur and nitrate in the fermenter. Also there is indication of glucose saturation in the fermenter which is evident from reduced glycolysis activity and increased osmotic stress.
Future perspectives: In addition, owing to the efficient antagonistic activity of EcN against pathogenic strains, we are interested in the trascriptome analysis of EcN after co-incubation with various Enterohaemorrhagic *E. coli* strains (EHEC) and also with human host cells. *E. coli* is very economical and effectors that affect host cells or pathogenic bacteria are believed to be produced only when necessary. These effectors ("tools") of EcN which are up regulated in the presence of host cells or pathogenic bacteria could be essential for its probiotic efficacy. The importance of some of these genes will be evaluated by generating deletion mutants and corresponding complemented strains and testing these constructs in assays for Stx inhibition and host cell protection.

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294/PWP

Purity makes the difference - Strategy of using LPS in *Galleria mellonella*

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The lepidopteran greater wax moth *Galleria mellonella (G.m.)* is a new and promising *in vivo* model organism in the field of host-microbiota interaction. *Galleria mellonella* larvae are suitable for several questions in innate immunity research including local or systemic infection. Low costs, easy handling and no conflicts with animal protection law or ethic guidelines raise the community of scientific users according to increasing numbers of publication. This organism is an auspicious candidate to reduce scientific used amounts of rodents in infection biology and contributes to the 3R strategy (Reduction, Refinement, Replacement) of animal experiments.
Lipopolysaccharides (LPS) of Gram negative bacteria are one of the most potent components for the activation of the host innate immune system and therefore LPS recognition is crucial for the host organism to clear infections of invading bacterial pathogens. LPS is also a common used stimulator of *Galleria mellonella* immune system and often used as prior stimulus in survival studies. In our experiments we demonstrate high differences in using “standard” and “ultrapure” LPS of *Escherichia coli* in *Galleria mellonella*. To investigate whether varied compositions of LPS including ultrapure and standard LPS (contaminated with TLR2 ligands i.e. peptidoglycans according to manufactures description) influence the activation of innate immune system we stimulated *G.m.* larvae via hemolymph injection. The immune status after injection was analysed via several assays. For instance significant differences in mRNA expression levels of six antimicrobial peptides four hours after injection were detectable. Further an injection of high doses of standard LPS for more than 4 days leads to significant reduces survival rates while ultrapure does not. Therefore experiments with ultrapure LPS mixed with TLR2 ligands (synthetic and biological origin) to generate lethality were performed.
The induction of immune processes leading to death are probable not inducible with an ultrapure ligand. Thus these data suggest that induction of lethality is a multicomponent event. Furthermore the application of LPS as prior stimulus in standard quality and the effect on following survival studies should be scrutinized.

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295/PWP

Upscaling of the cell-free *in vitro* expression of microcin S for characterization of its antibacterial spectrum

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Probiotic bacteria utilize - among others - the mechanism of antimicrobial effective peptide production (AMP) to ensure themselves nutritional and locational advantages in an overcrowded biological niche. At the same time the secreted AMPs may have a beneficial side effect to the bacteria’s hosts by defending them against pathogenic microorganisms. Thus, the antibacterial properties of AMPs are under increasing interest of research, since they may serve as supportive therapy as well as new antibiotic substances against today’s resistant bacteria.
Since the probiotic *Escherichia coli* strain G3/10 - a component of the drug Symbioflor2 - is producing such a new AMP, called microcin S (MccS), we have started its characterization in more detail. As recombinant intrabacterial expression of MccS failed - due to its assumed toxicity - a bacteria based but cell-free *in vitro* expression system was developed and optimized, synthesizing MccS quantitatively but in small laboratory scale. Here we present the data generated during upscaling of this cell-free *in vitro* expression system. Biologically active MccS produced in large scale reactions was used to determine the antibacterial spectrum of this microcin against selected indicator strains.
The reducing cell-free *in vitro* expression system consists of a cell extract of *E. coli* BL21 Rosetta 2(DE3), a fructose-1,6-bisphosphate based energy buffer enriched with ATP and NAD, amino acids and a glutathione buffer system. DNA templates contain all up- and downstream elements required for efficient protein expression. Upscaling was conducted by increasing the volume of the reaction mixture step by step, beginning with 10 µl. Simultaneously all reaction conditions were adjusted for best expression rates. Performance of MccS synthesis was measured by dot blot followed by immune detection. Activity of MccS was determined by an agar-diffusion assay against a sensitive indicator strain. A panel of enterobacterial strains, closely related to *Escherichia coli* G3/10, was used for characterization of the antibacterial spectrum of MccS.
Shaking as well as the size of the reaction tubes appeared to be the most determining factors of MccS expression rates. This could be explained by an assumed optimal oxygen entry into reaction mixtures during incubation. It was possible to increase the reaction volume several times with consistent expression rates. Also biological activity of MccS did never drop regardless of the reaction volumes used. In an agar-diffusion assay several strains showed inhibition zones and, thus, they could be identified as being sensitive to MccS.
Our results confirm that we were able to develop an upscaled system to generate high yields of active MccS *in vitro* under

reducing and oxygen optimized conditions. MccS concentrations reached with this system could be used to characterize the antibacterial spectrum of this microcin for the first time.

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296/PWP

Impact of growth conditions on microcin S gene expression in probiotic *Escherichia coli* G3/10

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Introduction: Antibacterial peptides such as bacteriocins and microcins are a promising field of research concerning the growing number of resistances against antibiotics. In 2012, Zschüttig *et al.* described microcin S (MccS) as a new antibacterial peptide produced by probiotic *Escherichia coli* G3/10 [1]. However, regulation of MccS synthesis and expression is not well understood yet. In depth knowledge of this process is necessary, if antibacterial peptides like MccS will be used for instance in therapeutic approaches for treatment of specific bacterial infections.
Aim: Our work focused on the examination of *mcsS* gene expression levels in *E. coli* G3/10 under different *in vitro* growth conditions and bacterial growth phases.
Method: *E. coli* G3/10 was cultivated at 37 °C for 24 hours in either LB-broth nutrient medium or in M9 minimal medium. In every growth phase mRNA was isolated from culture material and transcribed into cDNA followed by real-time PCR (qPCR) targeting the MccS gene. We compared the amount of target cDNA coding for *mcsS* with the housekeeping gene *adk* - encoding adenosine kinase - using TaqMan probes and FAM/TAMRA fluorophores. The gene expression factor R_Q was calculated from the resulting C_T values using the equation depicted in figure 1 [2].
Result: We observed two different patterns of gene expression depending on the used medium. In LBB the R_Q values increased constantly during the exponential growth phase, reaching their maximum at the beginning of the stationary phase. The elevated gene expression persisted during stationary phase. In M9 minimal medium however, the R_Q values showed their maximum values during exponential growth and decreased rapidly in the following, reaching base level again. Direct comparison between the two media showed an almost overall increased level of gene expression in M9 compared to LBB.
Discussion: We were able to show the influence of growth conditions such as bacterial growth phase and availability of nutrients on the gene expression of *mcsS*. A decreased nutrient supply rather stimulates transcription of the microcin S encoding gene, which leads to the assumption that under such conditions *E. coli* G3/10 expresses increased amounts of MccS to prevail against competing bacterial strains. However, the correlation between gene transcripts and actual amount of synthesized MccS protein has yet to be investigated.

References

[1] Zschüttig, A. *et al.* (2012), PLoS One 7(3): e33351.
[2] Pfaffl, MW. (2001), Nucleic Acids Res 29(9):e45.

Figure 1

$$RQ = \frac{E_{gene\ of\ interest}^{\Delta CT_{GOI}(control\ -\ sample)}}{E_{housekeeping\ gene}^{\Delta CT_{HKG}(control-sample)}}$$

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

297/PWP

Survival of probiotic *E. coli* and *Ent. faecalis* in the human host after oral uptake: results from *in vitro* and *in vivo* studies

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The aim of this study was to determine if probiotic strains belonging to the species *Ent. faecalis* and *E. coli* bacteria were able to survive passage through the human stomach and colonise the gastrointestinal tract (GIT). Survival of bacteria following exposure to gastric pH levels was assessed using a dynamic *in vitro* model resembling conditions in a fasting stomach, as well as the SHIME® model more closely mimicking the upper GIT environment (stomach and small intestine) during fed conditions. Viability of both *Ent. faecalis* DSM 16431 and *E. coli* DSM 17257 decreased during acid exposure. However, subsequent exposure to simulated small intestine conditions indicated no further decrease.
Auman volunteer study was performed to assess the colonisation properties of *E. coli*, which resulted in long-term colonisation following a single dose. Viable bacteria were detected in stool samples by means of strain-specific probes. For *Ent. faecalis*, a single dose was tested on one human volunteer, which resulted in transient colonisation only, again determined by means of targeted cultivation and identification of colonies using species- and strain-specific PCR primers.
In conclusion, probiotic *E. coli* and *Ent. faecalis* are susceptible to gastric pH, which reduces their viability with several logs. However, sufficient numbers survive to colonise the gut, so that the bacteria are detected in the stool for several days (*Ent. faecalis*) and multiple weeks (*E. coli*) following a single dose.

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298/PWP

Characterization of three different Serine Protease Autotransporters of Enterobacteriaceae (SPATEs) secreted by the probiotic Escherichia coli strain Nissle1917 (EcN)

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The SPATE (Serine Protease Autotransporters of Enterobactericeae) family was originally described in the context of pathogenicity and seems to have an important role as virulence factors in pathogenic E. coli strains like uropathogenic E. coli (UPEC) [1,2], avian pathogenic E. coli (APEC) [3], enteroaggregative E. coli (EAEC) [4] and others. SPATEs show a broad range of functions (adhesion, protease, lipase, etc.) [5] in the extracellular space. Beside the different functions outside the cell, all of them comprise the same domains (N-terminal signal sequence, passenger domain including the active motive GDSGS, linker domain and the C-terminal translocator domain) and share the same export mechanism via the Type 5a secretion system [6,7]. We could show that the probiotic E. coli strain Nissle1917 harbors at least the genes for three different secreted serine autotransporter proteases which belong to the SPATE family. By genomic comparisons between EcN and the close related pathogenic UPEC strain CFT073 we were able to identify the proteases as protease involved in intestinal colonization (Pic), secreted autotransporter toxin (Sat) and vacuolating autotransporter toxin (Vat).
Because those proteases are typically described in the context of pathogenicity, the question arises why the probiotic strain EcN harbors these genes, if they are expressed and if so, whether these

proteases show the expected activity and whether they are responsible for certain properties of EcN related to its probiotic potential e.g. inhibiton of Shiga toxin expression in EHEC strains [8] or the reduction of the invasion rate of Salmonella typhimurium into human epithelial cells [9].
To address these questions we test EcN's cell-free supernatant as well as supernatant of E. coli KRX overexpressing (T7 expression system) each of the cloned SPATE genes of EcN for protease activity by the Azocasein-assay. After successful construction of single, double and the tribble SPATE-gene mutants of EcN, these mutants will also be tested for protease activity and for inhibition of Stx expression and S. typhimurium invasion. The results of our investigation will help in understanding the role of SPATEs for EcN.

References

[1]: Parham N.J., Srinivasan U., Desvaux M., Foxman B., Marrs C.F., Henderson I.R. PicU, a second serine protease autotransporter of uropathogenic Escherichia coli. FEMS Microbiol. Lett. 2004;230:73–83.
[2]: Guyer D.M., Henderson I.R., Nataro J.P., Mobley H.L. Identification of Sat, an autotransporter toxin produced by uropathogenic Escherichia coli. Mol. Microbiol. 2000;38:53–66.
[3]: Salvadori M.R., Yano T., Carvalho H.E., Parreira V.R., Gyles C.L. Vacuolating cytotoxin produced by avian pathogenic Escherichia coli. Avian Dis. 2001;45:43–51.
[4]: Henderson I.R., Czeczulin J., Eslava C., Noriega F., Nataro J.P. Characterization of Pic, a secreted protease of Shigella flexneri and enteroaggregative Escherichia coli. Infect. Immun. 1999;67:5587–5596.
[5] Henderson I.R., Nataro J.P. Virulence functions of autotransporter proteins. Infect. Immun. 2001;69:1231–1243.
[6]: Henderson I.R., Navarro-Garcia F., Nataro J.P. The great escape: structure and function of the autotransporter proteins. Trends Microbiol. 1998;6:370–378.
[7]: Dautin N., Bernstein H.D. Protein secretion in gram-negative bacteria via the autotransporter pathway. Annu. Rev. Microbiol. 2007;61:89–11.
[8]: Rund, S.A., et al., Antagonistic effects of probiotic Escherichia coli Nissle 1917 on EHEC strains of serotype O104:H4 and O157:H7. International Journal of Medical Microbiology, 2013. 303(1): p. 1-8.
[9]: Altenhoefer, A., S. Oswald, U. Sonnenborn, C. Enders, J. Schulze, J. Hacker, and Oelschlaeger, T.A. (2004) The probiotic Escherichia coli strain Nissle 1917 interferes with invasion of human intestinal epithelial cells by different enteroinvasive bacterial pathogens. FEMS Immunol. Med. Microbiol. 40: 223-229.

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Zoonoses (FG ZO)

299/ZOP

Fluorescence *in situ* hybridization of *Campylobacter fetus*

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Introduction: The microbial species *Campylobacter fetus* divides up into three different subspecies: *C. fetus ssp.* f*etus (Cff), C. fetus* ssp. v*eneralis (Cfv)* and *C. fetus* ssp. *testudinum (Cft)*. While it is well known that enzootic abortion in sheep (*Cff*) and cattle (*Cfv*) is caused by *C. fetus* less is known about *Cft*, which was recently discovered in reptilians. Infrequently, all three subspecies are isolated from human blood of patients suffering from *C. fetus*-caused bacteremia/sepsis.
Objective: The aim of this study was to develop a fluorescence *in situ* hybridization (FISH) assay that can be used to detect specifically all three known *C. fetus* subspecies and that is able to distinguish *C. fetus* from related *Campyobacter* and *Arcobacter* species in order to perform rapid diagnosis, e.g., from bovine or ovine abortion samples as well as blood samples.
Method: For FISH-analysis, two different *C. fetus*-specific probes (a 23S-rRNA-derived and a 16S-rRNA-derived), one *Campylobacter*-genus-specific, one *Campylobacter*/*Arcobacter*-genus-specific, and an Eubacteria-specific probe were used and a strain collection of 33 *C. fetus* isolates of different origin were tested. Twenty isolates originated from colonized cattle, five from aborted calf fetuses, two from aborted sheep fetuses, one from a colonized pig and five were isolated from human blood. At all 21 *Cff*, 11 *Cfv* and 1 *Cft* have been analyzed. Additionally we used fourty isolates of non-*C. fetus* species including *C. jejuni* ssp. *jeuni*, *C. jejuni* ssp. *doylei*, *C. coli*, *Arcobacter butzleri* to test the species specificity of the FISH probes.
Result: After optimization of hybridization conditions: formamide concentration, and hybridization temperature, optimal fluorescence intensity was obtained for both *C. fetus*-specific probes. The *C. fetus*-probe designed to bind in the 23s rRNA-encoding region was highly specific for *C. fetus*, showed no cross-detection of related *Campylobacter*-/*Arcobacter* species and was able to recognize all three *C. fetus* subspecies. In contrast, the FISH-probe designed to bind the 16s rRNA region recognized besides all three *C. fetus* subspecies also related Campylobacter species, especially *C. jejuni* ssp. *doylei*.
Conclusion: We developed a highly *C. fetus*-specfic FISH-assay based on a 23S rRNA-binding probe that can be used to detect all three known *C. fetus* subspecies in abortion materials and blood samples.

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300/ZOP

Glycosphingolipid receptors of Shiga toxin expressed by primary human brain microvascular endothelial cells and their association with *lipid rafts*

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Introduction: Injury of microvascular endothelial cells of the brain frequently accompanies the onset of hemolytic-uremic syndrome (HUS) caused by Shiga toxin (Stx)-producing enterohemorrhagic *Escherichia coli* (EHEC). Stx1a, Stx2a or both Stx-subtypes elicit endothelium damage after uptake through receptor-mediated endocytosis, abusing the glycosphingolipids (GSLs) globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer) as preferred and less effective receptors, respectively.
Objective: In this project we analysed the expression profiles of Gb3Cer and Gb4Cer of primary human brain microvascular endothelial cells (pHBMECs), determined the various receptor lipoforms and investigated their association with plasma membrane microdomains known as *lipid rafts.*
Materials and Methods: Upon propagation of pHBMECs in endothelial cell medium, lipids were extracted from confluent grown cells and the neutral glycosphingolipids (GSLs) were

isolated by anion-exchange chromatography. Stx-receptors Gb3Cer and Gb4Cer were identified by thin-layer chromatography (TLC) immunostaining with specific antibodies as well as with Stx1a and Stx2a in combination with mass spectrometry. Major lipids of detergent-resistant membranse (DRMs), which were prepared by ultracentrifugation of sucrose density gradients and used as *lipid raft*-analogous microdomains, and the association of Stx-receptors Gb3Cer and Gb4Cer with DRMs were determined in detail according to a previous protocol.

Result: Expression profiles of Stx-receptors Gb3Cer and Gb4Cer of pHBMECs, obtained by TLC immunostaining, revealed classical doublets of each GSL, indicating substantial structural heterogeneity due to variable fatty acid substitution in the ceramide moieties. The upper band of each GSL harboured the respective GSL species with long-chain (C22 and C24) and the lower band that one with short-chain fatty acid (C16). The analysis of DRMs, resembling the liquid-ordered phase of the plasma membrane, showed preferential distribution of GSLs, sphingomyelin and the *lipid raft* marker protein flotillin to DRM fractions of sucrose gradient, giving evidence for prevalent localization Stx GSL-receptors in *lipid rafts*. On the other hand, phosphatidylcholine (PC) distributed equally to DRM and nonDRM fractions, while lyso-PC was exclusively present in the nonDRM fractions suggesting this truncated phospholipid as a marker of the liquid-disordered membrane phase.

Conclusion: Increasing knowledge on the molecular mechanisms of Stx-mediated cellular damage and the assembly of plasma membrane microdomains of Stx target cells may help to develop novel therapeutics to treat EHEC-infections.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

301/ZOP

Extended-spectrum beta-lactamase and carbapenemase encoding Enterobacteriaceae isolates harbouring the novel *mcr-1* gene

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Background: Multidrug resistant Enterobacteriaceae, especially those harbouring extended-spectrum beta-lactamases (ESBL) or carbapenemases, are high risk pathogens, in particular for patients with an impaired immune system. A relevant treatment option, especially for infections involving carbapenemase-encoding Enterobacteriaceae (CRE), is the polypeptide antibiotic colistin. However, colistin resistance in bacteria can occur, but was mediated by chromosomal mutations so far. Recently, a plasmid encoded, transferable resistance gene called *mcr-1* was identified in China.

This study was performed to investigate the presence of *mcr-1* in German ESBL-producing Enterobacteriaceae and CRE isolated from 2010 to 2014.

Materials and Methods: Whole genome sequencing of 600 Enterobacteriaceae isolates from different sources (human, livestock, companion animal, environment, food), was performed using Illumina MiSeq. The sequencing data was deposited in a local database, which was searched for the presence of the *mcr-1* gene using blastn. The genetic environment of the *mcr-1* gene was investigated using ISFinder and blastn. Conjugation experiments were performed to identify the transfer rates of the *mcr-1*-encoding plasmids.

Result: Nine *Escherichia coli* isolates harboured the *mcr-1* gene. Four isolates, originating from swine samples, were *bla*$_{\text{CTX-M-1}}$ positive. Four other isolates had been isolated from food and harboured either a *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-15}}$ or a *bla*$_{\text{TEM-52}}$ ESBL gene. The ninth isolate originated from a human patient sample, harbouring a *bla*$_{\text{KPC-2}}$ carbapenemase gene. Colistin resistance was transferable to a susceptible *E. coli* recipient strain by conjugation, confirming plasmidic location. Conjugation rates ranged from 10^{-1} to 10^{-7} cells per recipient. The genetic environment of *mcr-1* was diverse, including variants with an upstream copy of the insertion sequence IS*Apl1*, as well as variants which did not harbour any IS*Apl1*. All isolates harboured *mcr-1* on plasmids of other incompatibility groups (IncX4 or IncHI2 instead of IncI2) as described in the original publication from China (Liu et al., 2015).

Conclusion: The colistin resistance gene *mcr-1* is present in Germany at least since the year 2010, which was the earliest isolation time point of *mcr-1* positive isolates. The presence of a human CRE isolate with the *mcr-1* gene is clearly worrisome, as this represents a further reduction of treatment options on the way to a postantibiotic era. The presence of *mcr-1* in a larger set of isolates (from other locations, e.g. environment) has to be tested to investigate the epidemiology of the *mcr-1* gene in Germany in greater detail.

References

[1] Liu et al. 2015. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect. Dis. doi:10.1016/S1473-3099(15)00424-7.

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302/ZOP

Comparison of Extended-spectrum betalactamase producing *Enterobacteriaceae* from pig farmers and isolates from the farm environment

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Backgrounds and Aims: Extended-spectrum betalactamase (ESBL) producing *Enterobacteriaceae* (ESBL-E) affect >50% of all German pig holdings. In this study, we assessed colonization of pig farmers with ESBL-E and compared isolates from farmers with those from the farm environment.

Method: At 51 pig holdings in North Rhine-Westphalia five faecal samples were collected per holding. From the farmers working on

these farms a stool sample was obtained. After non-selective broth enrichment, all samples were streaked on ESBL chromogenic agar. Species confirmation was done by MALDI-ToF MS, susceptibility testing and ESBL confirmation by VITEK2 automated systems using EUCAST clinical breakpoints. Whole genome sequencing data were used for characterization of ESBL-E by classical multilocus sequence typing (MLST), identification of ESBL-encoding genes (BLAST searches against ESBL determinants from Lahey.org database) and an *ad hoc* core genome MLST (cgMLST). cgMLST analysis was performed using Ridom SeqSphere$^+$ software (version 2.3 beta) and minimum-spanning tree was constructed using the parameter "pairwise ignore missing values" for distance calculations.
Result: ESBL-E were detected on 61% of the farms; 5/84 farmers (6%) from 4/51 farms were colonized. All ESBL-E isolates were *Escherichia coli*. ESBL-*E. coli* isolates from farmers were associated with MLST STs/ESBL-genes ST10/CTX-M-1, ST196/TEM-52, ST278/TEM-52, ST410/CTX-M-15 and ST453/CTX-M-1, respectively. For one farmer, the ESBL-E isolate was clonally identical to isolates from pig faecal sampling of his farm (ST10/CTX-M-1); in the other four cases, isolates from humans were clonally diverse compared with faecal samples, but typing results indicated potential exchange of resistance determinants.
Conclusion: Molecular typing indicated that exchange of ESBL producing strains or resistance determinants/genes in pig holdings occurs.

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303/ZOP
Relapsing Fever in Refugee
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In summer 2015 two young male refugees were admitted shortly after each other to the region-hospital near the primary asylum seekers location in the north-east of The Netherlands. The symptoms were episodes of high fever, headache, stomachache and myalgia. Coming from Eritrea and travelling for weeks through different African countries the first suspection was malaria. Bloodsmears and antigen tests were negative. The laboratory analist however noticed spiralshaped micro-organisms in between the cells of the bloodspecimen of the first patient. The doctor on duty, it was a sunday, started ceftriaxone intravenously. The patient's condition deteriorated, he became unstable and was transferred to the university hospital in Groningen, in the Northern provincie of The Netherands, 50 km from the region hospital. On the ICU patient stabalised, antibiotics were switched to doxyxycline and Jarisch-Herxheimer reaction was diagnosed, a typical reaction in relapsing fever after administration of antibiotics. The second patient was send directly to the university hospital a few days later with similair symptoms by the general practitionar of the same refugee camp. Diognosis Borrelia recurrentis causing relapsing fever was confirmed by sequencing.
Since WWII relapsing fever, only transmitted by louse or tick, didn't occure in the Netherlands. Meanwhile refugees with relapsing fever were reported from Germany and Sweden. Only under very poor circumstances and crowding louse can thrive and spread Borrelia recurrentis from person to person. Scratchmarks facilitate the louseborn bacteria entering the bloodstream of humans.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

304/ZOP
Toll-like-Receptor-4 is essential for *Arcobacter butzleri* induced intestinal and systemic immune responses in gnotobiotic IL-10$^{-/-}$ mice
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Background: The gram-negative bacterium *Arcobacter butzleri* has been shown to be responsible for sporadic cases of human gastroenteritis with abdominal pain and acute or prolonged watery diarrhea. Information about the underlying immunopathological mechanisms of infection *in vivo*, however, is limited. We have recently shown that following *A. butzleri* infection, gnotobiotic IL-10$^{-/-}$ mice exhibited intestinal and systemic pro-inflammatory immune responses. For the first time we investigated the role of Toll-like-Receptor (TLR) -4, the main innate receptor for lipopolysaccharide and lipooligosaccharide of gram-negative bacteria, in murine *Arcobacter* infection in the present study.
Materials and Methods: The intestinal microbiota of TLR-4 IL-10 double deficient (TLR-4$^{-/-}$ IL-10$^{-/-}$) and IL-10$^{-/-}$ control mice was depleted by broad-spectrum antibiotic treatment. The resulting gnotobiotic (i.e. secondary abiotic) mice were then perorally infected with two different *A. butzleri* strains isolated from a diseased patient (CCUG 30485) or fresh chicken meat (C1), respectively.
Result: Until day 16 after infection gnotobiotic TLR-4$^{-/-}$ IL-10$^{-/-}$ and IL-10$^{-/-}$ control mice were stably colonized with either *A. butzleri* strain at high concentrations. During the course of infection, bacterial fecal loads, however, were slightly lower in the TLR-4$^{-/-}$ IL-10$^{-/-}$ as compared to IL-10$^{-/-}$ control mice. *A. butzleri* infected IL-10$^{-/-}$ mice lacking TLR-4 displayed less pronounced colonic apoptosis that was accompanied by lower numbers of innate and adaptive immune cells including macrophages and monocytes, T lymphocytes, regulatory T cells and B lymphocytes within the colonic mucosa and lamina propria as compared to IL-10$^{-/-}$ control mice. Furthermore, large intestinal pro-inflammatory mediators including nitric oxide, TNF, IL-6 and MCP-1 and, remarkably, of systemic pro-inflammatory cytokines such as IFN-γ and IL-12p70 were lower in *A. butzleri* infected TLR-4$^{-/-}$ IL-10$^{-/-}$ versus IL-10$^{-/-}$ mice.
Conclusion: TLR-4 is involved in mediating *Arcobacter* infection *in vivo*. Further studies are needed to investigate the molecular mechanisms underlying arcobacteriosis in more detail.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

305/ZOP
Analysis of the Cultivable Part of the Nasal Microbiota of Livestock-Associated MRSA CC398-Colonized Pigs
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Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) have emerged in Europe, especially in areas with high densities of pig farms. These bacteria are able to colonize pigs and humans alike and have demonstrated their capability to cause infections attributed to *S. aureus* in general, including fatal courses. Investigations of the composition of the porcine bacterial communities - the so-called microbiota - could lead to additional insights into the factors responsible for the successful spread of the clonal lineage CC398 in European livestock husbandry.

Here, the composition of the cultivable part of the porcine nasal microbiota has been studied.
Samples were acquired from nine pigs by intranasal and superficial snout swabbing (each, n = 9) of pigs, transported in Amies medium, and processed within 24 hours. Swabs were directly streaked as well as in 1:10, 1:100, and 1:1000 dilutions onto blood, chocolate, CAP, and MacConkey agar plates for aerobic incubation whereas chocolate agar plates were incubated with 5 % CO_2. Anaerobically incubated samples were diluted and streaked onto Schaedler, Schaedler + K/V, chocolate and CAP agar plates. All plates were incubated at 35 °C for 48 hours. Identification was performed by MALDI-TOF mass spectrometry (MS) analysis. After successful identification, one isolate per habitat and all isolates that could not be identified using MALDI-TOF MS were stored at -80 °C; the latter will be subjected to 16S rRNA gene sequence analysis.
Overall, 18 microbiotas were subjected to analysis. In total, 104 different bacterial species have been detected comprising 638 isolates. Thirty-six percent could not be identified by the MALDI-TOF MS approach. The ten most frequent species in each habitat and pig were *Rothia nasimurium* (18/18), *Corynebacterium xerosis* (14/18), *Aerococcus viridans* (12/18), *Staphylococcus epidermidis* (10/18), *Lactococcus lactis* and *Streptococcus hyovaginalis* (each 9/18), followed by *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus equorum*, and *Staphylococcus hyicus* (each 8/18).
The culturomic approach applied here allows an in-depth-inventory of the cultivable part of the porcine nasal microbiota. It will be the basis for subsequent inter-species interaction experiments. Additionally, the generated data will be compared to the results of molecular approaches such as metagenomic or next-generation sequencing approaches in order to shed further light on the differences, advantages, and disadvantages of each technique.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

306/ZOP
Species Composition and Seasonal Activities of Malaria Vectors in an Area at Reintroduction Prevention Stage, Khuzestan, South-Western Iran
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Background: The most part of Iran become malaria-free region and fall in prevention of re-introduction stage. These regions however are struggling with imported of malaria cases where malaria vectors exist. Therefore, understanding the situation of mosquito vectors is crucial. This study was carried out to find out the present situation of malaria vectors and malaria transmission potential in a malaria-free area.
Method: The study was conducted in a malaria free area, Izeh County, Khuzestan Province during 12 months in 2011-2012. Five villages, including 2 in highlands and 3 in plain area, were selected randomly. The mosquito sam- pling methods were conducted using spray sheet and hand catch collection methods from indoor/outdoors, window trap and larvae collections.
Result: In total, 3352 female Anopheles were captured, 1826 mosquito from highland and 1526 from plain areas. Five species, An. stephensi, An. fluviatilis s.l., An. dthali, An. superpictus and An. pulcherrimus were identified. The seasonal activities were started from April to March. The abdominal conditions of collected mosquitoes from in- door/outdoor places pointed to exophilic propensity of An. fluviatilis.l. s.l. and endophilic behaviour for rest of the vectors. The results of window trap also confirmed these behaviors. The larval habitats of four species were widely dispersed and included spring, margin of rivers, irrigation channels, stagnant water and rice filed.
Conclusion: Understanding the present situation of malaria vectors in free-malaria area is crucial particularly where is struggling with imported cases. The results of present study can be expanded to other area of northern Khuzestan for malaria vector control planning in reintroduction prevention stage.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

307/ZOP
Tick infestation rate of sheep, goat and distribution of ticks in Bashagard District, Hormozgan Province, Iran during year 2012
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Background: Ticks are the main vectors for transmission of different pathogens to human and animals such as relapsing fever, babesiosis, thaileriosis and the fatal disease Crimean-Congo Hemorrhagic Fever. Hormozgan province including Bashagard city is one of the most important husbandry center in southeast region of Iran. This study was carried out to find the fauna of different species of ticks belonging to the family Ixodidae in Bashagard city in the Hormozgan province, Iran during year 2012.
Method: A total number of 8 regions from both mountainous and plateaus zone of the center were selected randomly. 94 sheep's and 355 goat's were tested for tick infestation. Ticks were collected from the body of infested animals and transported to the laboratory of Medical Entomology in School of Public Health of Tehran University of Medical Sciences and then were identified to species level using valid identification key.
Result: A total of 90 hard ticks were c
ollected from the hosts including sheep and goat. The frequency of ticks on domestic ruminants was 71%. The ticks were classified into three genera and 8 species, including: *Rhipicephalus sanguineus* (58.9%), *H. anatolicum* (13.34 %), *H. nym* (10%), *R. bursa* (7.77%), *H. asiaticum* (3.33 %), *R. sp* (2.22%), *H. inermis* (1.11%), *H. punctata (*1.11%), *H. sp* (1.11%) *and R. nym* (1.11%). *R. sanguineus* with 58.9% was the most abundant species.
Conclusion: Since the collected ticks might have veterinary and public health importance and play an important role for transmission of vector borne diseases to human, the results of this study will provide a clue for implementation of disease control on tick-borne diseases in the region for local authorities.

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308/ZOP
Biosensor-based real-time interaction analysis of Shiga toxins with glycosphingolipid receptors in model membranes
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Introduction: Shiga toxins (Stxs) of enterohemorrhagic *Escherichia coli* (EHEC) are responsible for damage of the microvascular endothelium of human kidneys and the brain. The infection starts with diarrhea and can evolve to hemorrhagic colitis and, in some cases, to a hemolytic-uremic syndrome with fatal outcome. The clinical symptoms originate from the capability of the B-subunits of Stx (AB_5-toxin) to bind to its preferential glycosphingolipid (GSL) receptor globotriaosylceramide (Gb3Cer) and to a lesser extent to globotetraosylceramide (Gb4Cer) exposed

on endothelial cells. The GSL-receptors of Stx preferably localize in membrane microdomains known as *lipid rafts*. Such clustered GSLs promote initial binding to the plasma membrane and subsequent internalization of the toxin, whereby the exact interaction mechanism is poorly understood.

Objective: In this study we developed a lipid bilayer model membrane system, which allows for Stx-GSL interaction analysis and calculation of binding kinetics.

Method: Small unilamellar vesicles (SUVs) containing negatively charged 1,2-diacyl-sn-glycero-3-phospho-L-serine (diacylphosphatidylserine, DAPS), cholesterol, sphingomyelin, phosphatidylcholine and the Stx GSL-receptors Gb3Cer or Gb4Cer were produced by extrusion. The gold layer of the biosensor was coated with 11-mercaptoundecanoic acid, whereby the sulfur of the mercapto group sticks to the layer and the carboxyl group is exposed on the surface. SUVs were adsorbed onto the biosensor surface *via* divalent bridging Mg^{2+} cations to form stable lipid bilayers. Insertion of GSL-receptors into the lipid bilayer was confirmed using anti-Gb3Cer and anti-Gb4Cer antibodies. Label-free real-time interaction analyses were done with Stx1a and Stx2a from EHEC wild-type strains to determine the dissociation constant K_D.

Result: The formation of stable lipid bilayer model membranes on the biosensor surface with the fixed carboxyl groups of undecanoic acid residues was optimized by varying the relative content of DAPS in SUVs and the amount of "connecting" bivalent Mg^{2+} cations. Real-time interaction analysis with GSL- specific antibodies revealed successful integration of GSL-receptors into the lipid bilayer. Affinity-purified Stx1a and Stx2a were employed for kinetic analyses. The dissociation constant K_D, which describes the strength of binding between Gb3Cer and Stx, was calculated for Stx1a and Stx2a. K_D-values of Stx1a and Stx2a were in nanomolar range and indicated minor but clear differences in receptor binding of the two toxins.

Conclusion: Our approach is suited for a systematic investigation of the numerous variants of the various Stx-subtypes being helpful for the development of novel Stx-blockers.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

309/ZOP

Neoglycolipids of vegetable origin are able to attenuate the cytotoxic effect of EHEC-based Shiga toxins on Vero cells

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) represent the clinically most important subspecies of Shiga toxin (Stx)-producing *E. coli* (STEC). EHEC are able to elicit life-threatening illnesses like fulminant hemorrhagic colitis or the hemolytic-uremic syndrome (HUS). When being transferred into the circulation, Stx binds to the glycosphingolipid (GSL) globotriaosylceramide (Gb3Cer), which represents the preferential receptor of Stx1a- and Stx2a-subtypes on the surface of microvascular endothelial cells. The ensuing internalization of the toxin initiates a cascade of biochemical reactions, at the end of which the target cells are destroyed.

Objective: The main idea of this project was to produce neoglycolipids (neoGLs) having the potential to inhibit Stx and to prevent Stx from interacting with microvascular endothelial cells. This could be an efficient means to attenuate the cytotoxicity of Stx, which could ideally be neutralized completely.

Materials and Methods: To create neoGLs, pectins (polygalacturonic acids) of vegetable origin were used as starting material. After chemical reduction of the carboxyl groups of the polymer, the resulting galactopolysaccharides were split into oligosaccharides by acidic hydrolysis. Chemical coupling of these oligosaccharides with the amino group of phosphatidylethanolamine (PE) was the final step of the production of neoGLs, followed by electrospray ionization mass spectrometry to analyze in detail the structures of the newly produced glycoconjugates. Cell culture supernatants of Stx1a-, Stx2a- and Stx2e-producing STEC were employed for thin-layer chromatography (TLC) overlay binding assays of separated neoGLs and Vero-B4 cells served as target cells in cytotoxicity assays.

Result: The backbones of the neoGLs were α1-4-linked galacto-oligosaccharides, each unit comprising 2 to 7 galactose molecules. TLC overlay analysis of neoGLs revealed distinguishable binding patterns for each of the analysed Stx1a-, Stx2a- and Stx2e subtypes. These patterns can be taken into account to classify Stxs simply with the supernatant of an EHEC culture. In addition to that, the Stx-binding neoGLs were able to significantly reduce the cytotoxic effects of Stxs on Vero-B4-cells. Precise testing of cytotoxicity was performed with neoGLs inserted into liposomes representing one of several options to apply galacto-oligosaccharides based on pectins.

Conclusion: Here we show that neoGLs have protective effects on Vero-B4 kidney epithelial cells when exposed to Stxs. This aspect certainly makes them a promising tool in the development of potentially efficient glyco-pharmaceuticals which could help to attenuate illnesses caused by EHEC.

Presentation: Tuesday, 13 September 2016 from 17:00 – 18:00 in room Donauhalle.

310/ZOP

Prevalence of antibiotic-resistant bacteria among healthy horses in North Rhine-Westphalia (Germany)

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Introduction: Infections caused by antibiotic-resistant bacteria have become a major problem in human and veterinary medicine. Specifically, beta-lactam resistance acquired by *Staphylococcus aureus*, coagulase negative staphylococci (CoNS) and *Enterobacteriaceae* are of particular importance for human healthcare. The importance of livestock for transmission of antibiotic-resistant bacteria to humans was extensively studied, but data for companion animals are rare.

Intention: We assessed carriage of methicillin-resistant *S. aureus* (MRSA) and extended spectrum beta-lactamase producing (ESBL) producing *Enterobacteriaceae* (ESBL-E) among horses to understand whether they are sources for zoonotic transmission.

Method: Deep intranasal and perianal swabs were taken from healthy horses in the Muensterland region (North Rhine-Westphalia, Germany). Nasal swabs were streaked onto chromogenic agar for *S. aureus* identification (SAID) as well as suspended in tryptic soy enrichment broth (TSB) supplemented with 6% NaCl and cultured for 24 h at 37°C. TSB cultures were used for inoculation of SAID and MRSA chromogenic agars and incubated for another 48 h. In a subset of samples (n = 20), CoNS were additionally isolated from SAID medium and identified. Perianal swabs were enriched in TSB for 24 h (37°C). To detect ESBL producers, 0.01 ml of the culture were streaked onto a selective ESBL chromogenic medium and incubated for 24 h (37°C). Bacterial isolates were identified by MALDI-TOF mass spectrometry or 16S rRNA gene sequencing and genotyped.

Result: Overall, 222 horses from 21 different horse barns were included in the study. A total of 15 (6.8%) horses carried *S. aureus* and nine (4.1%) horses were found to be colonized by ESBL-producing *E. coli*. Two of the *S. aureus* isolates (0.9% of all

horses) were MRSA (*mec*A positive, *mec*C negative; *spa* types t011 and t091). The four most common species of CoNS found in the subset of nasal samples were *Staphylococcus sciuri* (9/20), *Staphylococcus succinus* (9/20), *Staphylococcus vitulinus* (10/20) and *Staphylococcus xylosus* (15/20).
Conclusion: Compared to livestock, horses outside of equine clinics were less frequently colonized with MRSA. Carriage rates were similar to that among humans in the same regions. However, prevalence of ESBL-producing *E. coli* was higher, which warrants further studies.

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311/ZOP
Antimicrobial resistance in zoonotic bacteria obtained from companion animals
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Introduction: Antimicrobial multidrug-resistant microorganisms (MDRO) are an increasing public health problem. Companion animals, often treated like "family members", may act as a source for the transmission of MDRO to their human owners. While the occurrence of MDRO among humans and livestock is well characterized, data for companion animals are rare.
Intention: This study aims to elucidate the prevalence of colonization with methicillin-resistant (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) as well as extended beta-lactamase (ESBL) producing *Enterobacteriaceae* (ESBL-E) among cats and dogs.
Method: Nasal, buccal and perianal swabs were collected from cats (n = 74) and dogs (n = 193) in two small animal practices and an animal shelter located in the northwestern part of Germany. All samples were first enriched (24 h, 37°C) and then cultured on solid chromogenic selective media (24 h, 37°C). Moreover, a subgroup of 16 specimens (13 dogs, 3 cats) was intended for analysis of the occurrence of coagulase-negative staphylococci (CoNS). Identification of bacterial isolates was based on MALDI-TOF mass spectrometry. Susceptibility testing was done by VITEK. MRSA and ESBL-E were detected phenotypically followed by molecular confirmation and genotyping.
Result: Amongst the 193 dogs, 20 (10.4%) carried *S. aureus*. The majority of *S. aureus*-positive dogs revealed colonization only in the nose (11/20), 5/20 showed *S. aureus* colonization only on the oral mucosa. Another 4/20 dogs were positive in both sites. 5/20 *S. aureus* isolates were MRSA (2.6% of all dogs) belonging to CC398-associated *spa* types t034 (3/5), t108 (1/5) and t011 (1/5). All isolates harbored the *mecA* gene. Amongst the 74 cats, six (8.1%) were identified as *S. aureus* carriers. Two animals revealed nasal and two buccal *S. aureus* colonization*;* two were positive at both body sites. One of six *S. aureus* isolates was MRSA (1.4% of all cats). The MRSA isolate was associated with *mec*C and *spa* type t843. The most prevalent canine CoNS were *Staphylococcus intermedius* (4/13), *Staphylococcus pseudintermedius* (3/13), *Staphylococcus xylosus* (3/13) and *Staphylococcus equorum* (3/13). In cats, primarily *Staphylococcus felis* (3/3) was identified. Eight dogs (4.1%) and none of the cats carried ESBL-producing *E. coli*.
Conclusion: Both cats and dogs carried MDRO. The study supports the necessity of a "One Health" approach to address the dynamic interactions between humans, animals, environment and pathogens.

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312/ZOP
Shiga toxin glycosphingolipid receptors and their occurrence in membrane microdomains of human kidney epithelial cells
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Introduction: Shiga toxins (Stxs) of enterohemorrhagic *Escherichia coli* (EHEC) are implicated in watery diarrhea, bloody diarrhea, and potentially lethal hemolytic-uremic syndrome (HUS). Stxs display an AB_5 molecular configuration comprising a catalytically active A-subunit and a B-pentamer, the latter being responsible for binding to glycosphingolipid (GSL) receptors and retrograde subcellular traffic of the holotoxin. Globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer) represent the high and less efficient GSL receptors, respectively, expressed on human endothelial cells. Injury of microvascular endothelial cells is considered to be key in the development of severe clinical complications, while involvement of Stxs in damaging epithelial cells is largely unknown.
Objective: This study is aimed at determining 1) the Stx GSL receptors of human kidney epithelial cells, 2) association of GSLs with *lipid rafts*, which has turned out to be the prerequisite for cellular internalization of Stx, and 3) Stx-caused cytotoxic action towards renal epithelial cells.
Materials and Methods: Lipids were isolated from kidney epithelial cell lines. Phospholipids and triglycerides were saponified and neutral GSLs were purified by anion exchange chromatography. Thin-layer chromatography (TLC) overlay assays using polyclonal anti-Gb3Cer and anti-Gb4Cer antibodies were performed to detect Stx GSL receptors. Mass spectrometry in combination with TLC overlay assays was carried out for structural analysis of GSLs. Detergent-resistant membranes (DRMs) were employed to assess cellular *lipid raft* composition.
Result: We demonstrate here that human kidney epithelial cells harbor both globo-series GSL receptors namely Gb3Cer and Gb4Cer. In addition, we provide data on structural diversity of Stx GSL receptors obtained by means of electrospray ionization mass spectrometry (ESI-MS). In detail, investigations revealed considerable heterogeneity of the lipid anchor (ceramide) of Stx GSL receptors caused by variable chain lengths of the fatty acid moiety with chain lengths ranging from C16 up to C24. Moreover, GSLs co-localize with *lipid raft* markers in DRMs, indicating association of Stx GSL receptors with plasma membrane microdomains. Finally, Stx was found to cause cellular injury in epithelial cell cultures.
Conclusion: Our findings support the hypothesis of a functional role of microdomain organization of Stx GSL receptors in kidney epithelial cells.

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313/ZOP
Parallel colonization of *S. aureus* and MRSA in pigs
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Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) colonize healthy pigs and pig-workers. Regarding the possibility of zoonotic spread of these bacteria in the both sectors, health care system and animal production, the question arises

whether there are concepts of natural protection that minimizes the risk of MRSA transmission. Only few studies exist regarding the competition between MSSA and MRSA in the anterior nares and in the rectum of pigs (Dall´ Antonia et al., 2005).
Therefore in the present study 155 pigs from 4 farms were screened for MRSA and MSSA for the occurrence of co-colonization in their anterior nares and intrarectal at the time of sampling. Additionally two types of swabs were tested: i) with Amies transport medium containing charcoal and ii) with a Nylon® Flocked Swab.
The results were evaluated in view of a possible protective impact of MSSA against MRSA. The distribution of the MRSA *spa*-types across the 4 farms was homogeneous. No pigs carried both, MSSA and MRSA, at the time of sampling. Nevertheless, there were pigs with nasal colonization with MRSA or MSSA. No ESBL-E was found in the anterior nares. Most pigs with double *S. aureus* detection (nasal and intrarectal) were MRSA-positive. The *spa*-types of the strains differed only in one pig. The statistical analysis regarding the co-colonization is still ongoing, however the relative frequencies of MRSA and MSSA detection indicate that MRSA and MSSA seem to be mutually exclusive.
These results might support the hypothesis that MRSA and MSSA compete for colonization space, and provide an estimate of the extent to which MSSA interferes with MRSA colonization. Further studies in both the health care and animal production sector should estimate the interfering potential of *S. aureus* strains for the dynamics of colonization and infection.

Reference
[1] Dall´ Antonia, M., Coen, P.G., Wilks, M., Whiley, A., Millar, M. (2005) Competition between methicillin-sensitive and -resistant *Staphylococcus aureus* in the anterior nares. Journal of Hospital Infection *61*, 62-67.

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314/ZOP
Poultry as a source of *mcr-1* positive, colistin resistant *Escherichia coli*
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Recent findings of a new plasmid mediated mechanism of resistance to colistin (MCR-1) suggest that use of colistin as a last resort antibiotic is seriously threatened. Since the first identification of the *mcr-1* gene in food animals and human patients in 2015, there is a worldwide concern about its actual distribution in human and animal populations [1]. We aimed to identify the *mcr-1* gene in *Escherichia coli* isolates from poultry and determine its genomic context.
E. coli isolates (n=340) obtained from extraintestinal sites of diseased chicken (broiler, n=170; laying hen, n=143) and turkey (n=8) in Germany (n=281), Belgium (n=20), The Netherlands (n=19), and Hungary (n=20) between 1999 and 2012 were screened for the presence of the *mcr-1* gene by PCR [1]. Multi locus sequence types (STs) and resistance genes were extracted from whole genome sequences of *mcr-1*-positive isolates. MIC data were determined with the VITEK2 system.
Seven *E. coli* isolates (Germany, n=6; Hungary, n=1) from 5 chickens and 2 turkeys proved *mcr-1* positive. They belonged to phylogenetic groups B2, D, and ABD and to sequence types, such as ST69 and ST131, which are frequently associated with extraintestinal infections in humans. According to EUCAST definition 5 isolates showed resistance to colistin (MICs 8 mg/L to >16 mg/L); the remaining 2 isolates were susceptible. Genes for ESBL/AmpC-type β-lactamases or acquired carbapenemases were not present. The earliest strains were isolated from turkeys in Germany in 2007 and 2008, respectively. Strain IHIT31034 (ABD-ST1842) harboured the *mcr-1* gene on an IncX4 plasmid of approx. 33 kb in size. In strain IHIT31035, the *mcr-1* gene was located on an IncHI2 plasmid associated with insertion sequence IS*Apl1*. Both plasmids revealed substantial similarities with plasmids previously described for isolates from humans and livestock animals [2-4].
Initial data of plasmid backbones and genomic characteristics of *E. coli* from poultry carrying the *mcr-1* gene suggest that the colistin resistance gene has been spread by horizontal gene transfer and recombination among various plasmids and *E. coli* lineages. Sequence types observed include those of increased extraintestinal virulence in poultry, which have previously been suggested to be of zoonotic relevance.

References
[1] Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, et al. (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16: 161-168.
[2] Zhi C, Lv L, Yu LF, Doi Y, Liu JH (2016) Dissemination of the *mcr-1* colistin resistance gene. Lancet Infect Dis 16: 292-293.
[3] Li A, Yang Y, Miao M, Chavda KD, Mediavilla JR, et al. (2016) Complete sequences of *mcr-1*-harboring plasmids from extended spectrum beta-lactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae (CPE). Antimicrob Agents Chemother.
[4] Falgenhauer L, Waezsada SE, Yao Y, Imirzalioglu C, Kasbohrer A, et al. (2016) Colistin resistance gene *mcr-1* in extended-spectrum beta-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. Lancet Infect Dis 16: 282-283.

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315/ZOP
Colistin resistance gene *mcr-1* and its genomic context in clinical bacterial isolates from cats, dogs, and horses
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Since its first description in November 2015, the plasmid-encoded colistin resistance gene *mcr-1* has been mainly reported in *Escherichia coli* isolates from livestock animals and humans but only exceptionally from pets [1-3]. As companion animals are a likely source of zoonotic and multidrug resistant bacterial pathogens, we aimed to find out whether *mcr-1* occurs among isolates of different bacterial species from cats, dogs and horses, predominantly from Germany.
Acinetobacter baumannii (n=40), *Acinetobacter* spp. (n=15), *E. coli* (n=582), *Enterobacter* spp. (n=9), *Klebsiella* spp. (n=134), and *Pseudomonas aeruginosa* (n=60), obtained in our diagnostic laboratory from clinical samples of cats (n=203), dogs (n=495) and horses (n=142) in the years 1998-2015 were screened for the presence of the *mcr-1* gene by PCR [1]. Multi locus sequence types (STs), resistance genes and genetic environment of the colistin

resistance gene were extracted from whole genome sequences of *mcr-1*-positive isolates. MIC data were determined with the VITEK2 system. Plasmid location of *mcr-1* was verified by CeuI digestion of whole cell DNA and southern blot hybridization.
The *mcr-1* gene was solely found in *E. coli* and in none of the other bacterial species tested. *Mcr-1* positive *E. coli* (ST10, ST23, ST354, ST359 and ST2301) were obtained from enteritis, cystitis and wound infection in two cats and three dogs from Germany (n=4) and Italy (n=1). The isolates revealed MICs between 4 and 8 mg/L for polymyxin B, expressed ESBLs CTX-M-1, CTX-M-15, SHV-12, and TEM-123 and were additionally resistant to several other antimicrobial substances. *Mcr-1* genes were located on IncX4- and IncHI2-plasmids showing similarities with plasmids recently described for isolates from humans and pigs [1, 3, 4].
Our findings demonstrate the complexity in the epidemiology of plasmid-mediated colistin resistance suggesting companion animals as important players. As polymyxins play a very limited role in the treatment of dogs and cats, the factors that contribute to the emergence of colistin-resistant strains in these animals still need to be discerned.

References
[1] Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, et al. (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16: 161-168.
[2] Zhang X-F, Doi Y, Huang X, Li H-Y, Zhong L-L, Zeng K-J, et al. (2016) Possible transmission of *mcr-1*-harboring *Escherichia coli* between companion animals and human. Emerg Infect Dis. DOI: 10.3201/eid2209.160464.
[3] Skov RL, Monnet DL (2016) Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. Eurosurveillance, Vol. 21(9).
[4] Li A, Yang Y, Miao M, Chavda KD, Mediavilla JR, et al. (2016) Complete sequences of *mcr-1*-harboring plasmids from extended spectrum beta-lactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae (CPE). Antimicrob Agents Chemother. DOI: 10.1128/AAC.00550-16.

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316/ZOP

Enterohemorrhagic *Escherichia coli* (EHEC) of O serogroups O26 and O111 share a common atypical enteropathogenic *Escherichia coli* (aEPEC) ancestor

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A variety of non-O157 EHEC have emerged as serious causes of hemolytic uremic syndrome (HUS) and diarrhea worldwide. The most important non-O157 O serogroups causing one third of the EHEC infections in Germany are O26, O103, O111 and O145.
In a previous study we observed cluster formation of O26 and O111 EHEC in one single sequence type complex, STC29. STC29 also harbors aEPEC of the same O serogroups, which differ from EHEC merely in absence of *stx*-converting bacteriophages. This suggests an ongoing microevolutionary scenario of bidirectional-conversion, in which the phage-encoded *stx*-gene is transferred between aEPEC and EHEC [1].
In this study we aim to develop a microevolutionary model of aEPEC and EHEC strain conversion of STC29 strains by whole genome sequencing (WGS), concentrating on epidemiologically highly important EHEC lineages of O serogroups O26 and O111. WGS of 99 selected strains (aEPEC (n=20), EHEC (n=79) of human (n=47) and bovine (n=58) origin) was performed, analyzing the maximum common genome (MCG) for single nucleotide polymorphisms (SNPs) as well as the presence of virulence associated genes (VAGs) and the occupation of insertion sites for mobile genetic elements in those strains.
The resulting minimum spanning tree of the MCG-based SNP-analysis revealed three distinct clusters. Cluster 1 harbored O111 strains also designated as ST16 with MLST. Interestingly, the distinct Cluster 2 included only O26 aEPEC strains of ST29, while the more heterogeneous Cluster 3 combined EHEC as well as aEPEC strains of O serogroup O26 that were only roughly separated into strains of ST29 and ST21.
The analysis of the presences or absence of accessory VAGs confirmed the results of the SNP-analysis, and suggests a parallel evolution of the MCG of those strains and the acquisition of virulence genes. Furthermore, the analysis of insertion sites for mobile genetic elements resulted in a similar relation of the analyzed strains. Our cumulative results of MLST, SNP-analysis of the MCG, the presence of VAGs and occupation of insertion sites led to the development of a microevolutionary model of EHEC of O serogroups O26 and O111, which developed as two distinct lineages from a common aEPEC ancestor of ST29 by lysogenic conversion with *stx*-converting bacteriophages.
None of the analyses revealed a separated grouping of strains based on the host species they had been isolated from. Hence, these strains do not appear to harbor host-specific genomic alterations, neither within the MCG nor in the acquired VAGs, and therefore do not appear to emerge by adaptation to a specific niche, supporting the zoonotic nature of aEPEC and EHEC of these STC29 strains.
In conclusion, EHEC belonging to STC29 of O serogroups O26 and O111 originate from a common ancestor; further aEPEC and EHEC of those serogroups share a common phylogeny and are *bona fide* zoonotic agents.

Reference
[1] Eichhorn, I., et al., *Highly virulent non-O157 EHEC serotypes reflect a similar phylogenetic lineage, giving new insights into the evolution of EHEC.* Appl Environ Microbiol, 2015.

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317/ZOP

Borrelia plasmid assembly - a pain in the neck

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Background: Bacteria of the *Borrelia* (*B.*) *burgdorferi* sensu lato species complex are the causative agents of Lyme borreliosis. The complex currently consists of about 20 genospecies including the human pathogenic species *B. burgdorferi* sensu stricto. Compared to other bacteria, the genome of Borrelia are very complex comprising a linear main chromosome (appr. 900 kbp) and up to >20 linear and circular plasmids (in total appr. 600 kbp). Plasmid-encoded genes are important for the host- pathogen and vector-pathogen interaction and for human pathogenicity.
Next generation sequencing has offered enormous opportunities for whole genome sequencing of pathogenic bacteria. However, proper plasmid assembly may not be achieved using NGS methods that are based on short sequence reads. In the study, we have compared various methods including plasmid enrichment procedures, library construction, and sequencing to see what works best.
Materials and Methods: We used *B. burgdorferi* sensu stricto strain B31 for comparative analyses of all methods (proof-of-

principle) and sequences available in GenBank (B31) as reference. For plasmid enrichment different commercial plasmid purification kits were compared. The best plasmid fractions were sequenced using an Illumina MiSeq (Nextera library prep). Two further *B. burgdorferi* s.s. (PAli, PAbe) were included in additional analyses: three Illumina library preparation methods (Nextera, TruSeq, Mate pair) were analyzed in combination with different assembly methods (read mapping, de novo assembly). In addition, SMRT technology (Pacific Biosciences) was used and compared to Illumina assemblies.

Result: The plasmid prep resulted in enrichment of plasmids (linear and circular) and depletion of the main chromosome. Read mapping using Illumina raw reads of plasmid enriched preparations led to improved reconstruction of most plasmids. Different library preparation methods did not show differences in plasmid assembly. Full length plasmids were obtained for B31 and PAli with SMRT technology.

Conclusion: Using plasmid enrichment and read mapping of Illumina sequences, encouraging results were generated at moderate costs. SMRT technology gave the best results in terms of plasmid assembly but was more costly and required much higher amounts of DNA than Illumina technology for library construction.

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