ABSTRACTBAND

71. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) e. V.

978-3-948023-01-0

Abstracts

ORAL PRESENTATIONS

Monday, 25 February 2019

Tuesday, 26 February 2019

Wednesday, 27 February 2019

POSTER PRESENTATIONS

Monday, 25 February 2019 • 19.00-21.00

Postersession 01

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KEY

Fachgruppe (FG), Ständige Arbeitsgemeinschaft (StAG)

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

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Diagnostische und Klinische Mikrobiologie

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Diagnostische Verfahren in der Mikrobiologie

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Food Microbiology and Food Hygiene (FG LM) ..... LMV, LMP
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Gastrointestinale Infektionen

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Mikrobiota, Probiota und Wirt

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Nationale Referenzzentren und Konsiliarlaboratorien

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

Workshop 01
Virulence Principles in Respiratory Tract Infections (FG MP)
25. Febr. 2019 • 13:30-15:00

001/MPV

***Staphylococcus aureus* and influenza virus: interaction of pathogens can be analysed in an human alveolus-on-a-chip model**

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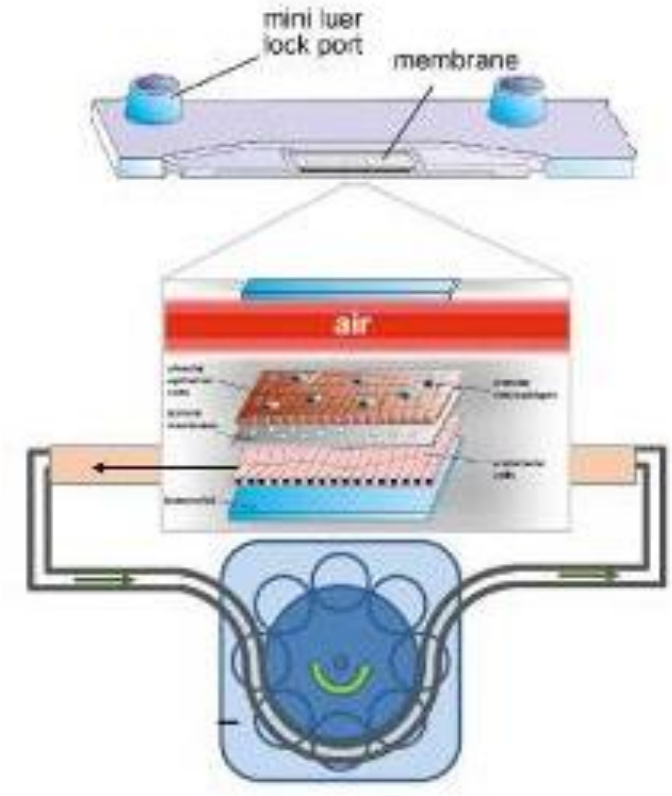
Question: Postinfluenza models of *Staphylococcus aureus* pneumonia demonstrate the severe outcome of a coinfection associated with substantial morbidity and mortality, in particular in the immunocompromised host. To date, investigations concerning microbial infections of the lung are usually carried out in animal models. However, lung anatomy and physiology as well as composition of the immune system differ significantly between rodents and humans. To investigate cell interactions between epithelial, endothelial and immune cells after influenza virus/ *S. aureus* coinfection, we established a human alveolus-model generating a reactive tissue-tissue interface between the vascular endothelium and the airway-facing epithelium. Alterations in the immune system, which are present in immunocompromised host, can be examined as well.
Methods: MOTiF biochips were seeded with human endothelial cells on the vascular site and with epithelial cells and macrophages on the airway site (Figure 1). This organoid was cultured for up to 14 days with a robust and stable air-liquid interphase under dynamic flow conditions. Barrier integrity was proven by transepithelial electrical resistance (TEER) measurements and permeability assays. Expression and localization of cell-type specific markers and functional proteins was proven by immunofluorescence. Viral and bacterial infection was performed through airway up to 8 hours, depending on multiplicity of infection (MOI).
Results: Dynamic conditions for maintaining air-liquid-interface allow a stable barrier with high transepithelial resistance and an intact vascularity. We provide evidence for an increase of barrier integrity after **Introduction** of macrophages proven by TEER measurement and permeability tests. Our data indicate a stable surfactant production of alveolar epithelial cells type II. Subsequent infection has been successfully established and pathogenicity factors can be investigated.
Conclusions: We established a functional, biochip-based human *in vitro* alveolus model that is suitable for investigation of complex co-infections and immune functions. Separated airway and vascular chambers allow infections with pathogens from the airway site. Thereby inducing an immune response, it is possible to observe migration of immune cells from the vascular site to the infection to study species-specific mechanism of pathogens.

Figure 1



Presentation on Monday, February 25, 2019 from 13:30 – 13:40 in room Hörsaal 104.

002/MPV

Changes in nuclease activity of *Staphylococcus aureus* as a potential mechanism to escape neutrophil extracellular trap-(NET)-mediated killing during persistence in the airways of cystic fibrosis patients

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In cystic fibrosis (CF), patients suffer from a decreased lifespan related to chronic lung infections predominantly caused by *S. aureus*. To erase and limit propagation of *S. aureus* in the lungs of the CF host, neutrophils are capable to form "neutrophil extracellular traps" (NETs) consisting of antimicrobial peptides bound to extracellular DNA structures. A mechanism that *S. aureus* uses to avoid NET-mediated killing (NETosis) is the secretion of nuclease, which degrades host DNA thereby allowing S. aureus to escape. This study aims to elucidate mechanisms of the adaptation of *S. aureus* in relation to NETosis.
Therefore, nuclease activity of *S. aureus* isolates from a longitudinal CF study was determined using DNA agar, a DNA-degradation assay and a FRET-based fluorescence assay. Transcription of *S. aureus nuc1* and *nuc2* expression was assessed by qRT-PCR. For visualization of *S. aureus* in association with NETs in CF airways, expectorated sputum was stained with NET-specific probes and analyzed via fluorescence microscopy. Testing the impact of nuclease activity onto bacterial survival, NETosis assays were performed using *S. aureus* CF isolates with differential nuclease activity.
In CF sputa, *S. aureus* was found tightly enclosed in NETs. Comparing clonal sequential *S. aureus* isolates (n=112) from one single CF patient, increased nuclease activity of long-term persisting isolates was observed. In contrast, a more heterogeneous picture was seen in 29 strain pairs (early, late) from different CF patients with late isolates being more active (n=6), less active (n=11) or not changed (n=12). Further transcriptional analysis revealed that nuclease activity was related to the expression of *nuc1* rather than to *nuc2*. Higher survival rates of *S. aureus* isolates during NETosis were observed for isolates with high nuclease activity compared to low nuclease activity.

Preliminary results indicate a time-dependent adaptation of *S. aureus* nuclease activity during persistence in the CF airways at least for for some patients. Further experiments are ongoing to determine the impact of nuclease activity of clinical *S. aureus* isolates with differential nuclease activity on the formation and detachment of biofilm formation as another important function of nuclease.

Presentation on Monday, February 25, 2019 from 13:45 – 13:55 in room Hörsaal 104.

003/MPV

Establishing Colonization of Mice with *Streptococcus pneumoniae* to study the natural route of Influenza A virus mediated co-infections of the lower respiratory tract

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Introduction: *Streptococcus pneumoniae* is a typical colonizer of the human upper respiratory tract (URT). About 20–50% of healthy children and 8–30% of healthy adults are asymptomatically colonized. Influenza A viruses (IAV) have been identified as important pathogens that can pave the way for pneumococcal spread from the URT to the lungs causing severe medical conditions, including pneumonia. The exact mechanisms behind these bacterial and viral co-infections are still not well understood.
Objectives: Here, we aim to establish a natural route of bacterial and viral co-infections by colonizing the mice with a non-invasive pneumococcal strain before introducing the IAV to the host.
Materials & methods: First, C57BL/6J mice were colonized with *S. pneumoniae* 19F for seven consecutive days or infected with human-derived H1N1. Clinical conditions, weight, microbiological and immunological parameters were monitored over the whole period of infections. Second, based on the results of the single infections/colonization co-infections were performed.
Results: Nasopharyngeal pneumococcal colonization of mice resulted in a systemic and local inflammation during the first two days. All monitored values normalized by day four. Constant bacterial counts were recovered from nasopharynx over a period of seven days. Viral infections were characterized by weight loss and a shift in neutrophils/leukocytes counts systemically at day seven p.i. In addition, viral RNA was recovered from lungs of the animals. Viral infections of mice harboring pneumococci in the nasopharyngeal cavity were performed seven days post colonization. Initial monitoring of co-infected animals revealed no clinical signs of disease during the first seven days. However, initial bacterial spread to the lungs and elevated neutrophil counts in the blood stream were detected seven days post viral application.
Conclusions: Although the co-infected animals did not show critical clinical signs of pneumonia, the proposed model serves as a starting point for future experimental directions, which will include studies on synergistic effects of the pathogens in the host, systemic and local immune responses, tissue pathology, and microbiome composition.

Presentation on Monday, February 25, 2019 from 14:00 – 14:10 in room Hörsaal 104.

004/MPV

Characterizing the role of CERT in *Chlamydia psittaci* infections of epithelial cells

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Introduction
Chlamydia psittaci is an obligate intracellular bacterium that causes infections in humans and animals. As all Chlamydiaceae, *C. psittaci* has a biphasic cycle of development that takes place inside the eukaryotic host cell within a membrane-bound vacuole, the inclusion. *Chlamydia trachomatis* is known to recruit the cellular ceramide transporter CERT to their inclusions and have been shown to take up sphingomyelin. How *C. psittaci* obtains this important lipid has not been addressed.
Goals: The goal of our study is to decipher the role of the cellular ceramide transporter CERT in *C. psittaci*-infections of human cells.
Material and Methods: Localization of CERT was studied in *C. psittaci* infected and control cells by immunofluorescence using specific antibodies against CERT and by ectopic expression of CERT fused to eGFP. CERT knockout cells were generated by CRISPR/Cas9. The function of CERT for *C. psittaci* development and lipid uptake was analyzed in these cells and compared to control cells.
Results: We found that *C. psittaci* recruits the ceramide transport protein CERT to its inclusion. CRISPR/Cas9-mediated knockout of CERT showed CERT is a crucial factor for *C. psittaci* infections affecting inclusion growth and infectious progeny formation. Interestingly, in CERT-knockout cells, the uptake of fluorescently labeled sphingolipids into bacteria inside the inclusion was boosted.
Conclusion: We identified the non-vesicular transport protein CERT as a crucial factor for infections with this *C. psittaci*. CERT affects different stages of the infection and unexpectedly restricts sphingolipid uptake into *C. psittaci* inclusions suggesting a function beyond sphingolipid uptake. Interestingly, the recruitment of CERT is conserved between different *Chlamydia* species but its functions seem to be species-specific.

Presentation on Monday, February 25, 2019 from 14:15 – 14:25 in room Hörsaal 104.

005/MPV

Hypoxia-induced citrate limitation results in *C. burnetii* containment in macrophages

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Introduction: Coxiella burnetii is the causative agent of the zoonotic disease Q fever. Apart from acute Q fever, around 2-5% of *C. burnetii* infected humans will develop chronic Q fever, which mainly manifests as endocarditis years after exposure to the pathogen. Usually, humans get infected through the inhalation of contaminated aerosols, often discharged from infected ruminants. Alveolar macrophages are the first line of defense against inhaled *C. burnetii*. They replicate within a unique lysosome-derived vacuole, called the coxiella containing vacuole. Details about how *C. burnetii* are fought by host macrophages and how they escape the immune system and persist for years inside the host are not fully established yet.
Materials and methods: Murine bone marrow-derived macrophages (MΦs) were infected with *C. burnetii* under normoxia or hypoxia. Samples for western blots, DNA/RNA isolation and citrate measurements were taken.
Results: Our experiments revealed that under normoxic conditions, *C. burnetii* replicate in macrophages and fail to induce robust accumulation of hypoxia-inducible factor 1α (HIF1α). Exposure to hypoxia, in contrast, stabilizes HIF1α which is essential for inhibiting *C. burnetii* replication. This was proven in murine MΦs and human (PBMC) derived macrophages. Mechanistically, HIF1α impairs the activity of signal transducer and activator of transcription 3 (STAT3), reduces the intracellular citrate level and thereby prevents *C. burnetii* replication. Interestingly, hypoxic MΦs also restrict intracellular replication of *Legionella pneumophila*, another intracellular pathogen. However, while HIF1α mediated limitation of citrate prevents *C. burnetii* replication under hypoxia, this seems not to be the case in the context of *L. pneumophila* infection. Furthermore, viable *C. burnetii* induce less HIF1α stabilization than heat-killed or a T4SS-defective mutant, suggesting that *C. burnetii* manipulate HIF1α in a

T4SS-dependent manner. Molecular details and consequences of this activity are under investigation.
Conclusion: Our data suggest that regulation of citrate levels by HIF1α represents a novel principle of nutritional pathogen-containment which might be *C. burnetii* specific.

Presentation on Monday, February 25, 2019 from 14:30 – 14:40 in room Hörsaal 104.

006/MPV
How to get rid of O_2? Detoxification in *Mycoplasma pneumoniae*
C. Blötz*[1], L. Krüger[1], A. Kahle[1], J. Stülke[1]
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As a human pathogen, *Mycoplasma pneumoniae* can cause severe illness. The close association with lung epithelial cells allows the organism to live with one of the smallest genomes (688 ORFs). This is reflected by a limited repertoire of enzymes, and by the reduced capabilities of the organisms" proteome. All life on earth is constantly exposed to reactive oxygen species (ROS). These compounds are highly reactive and can heavily damage macromolecules like DNA, proteins, and lipids. To minimize ROS induced damage, every cell needs to keep the balance between oxidants and antioxidants. Antioxidants can be enzymes like catalase, superoxide dismutase, and peroxiredoxins (Prx). Since *M. pneumoniae* lacks catalase and superoxide dismutase, this work was focused on the identification and characterization of Prx in *M. pneumoniae*. Prx are antioxidant enzymes, that degrade ROS via catalytic cysteine residues, and that are subsequently reduced via the thioredoxin system. Sequence alignments identified MPN625 and MPN668 as potential candidates. Both Prx candidates contain two conserved cysteine residues. In this work, their enzymatic activity against peroxides was demonstrated. The formation of homodimers is characteristic for Prx, and in this study it was shown, that MPN625 and MPN668 exist in this form. Furthermore, binding studies revealed two ligand binding sites of MPN625 and MPN668, which is consistent with homodimer formation. For the first time, clean deletions were performed and the genes encoding the Prx were successfully deleted from the genome of *M. pneumoniae*. This revealed no growth defect of the Δ*prx* single mutants in glucose and glycerol containing media, as well as no altered cytotoxicity against human bronchial epithelial cells (HBECs). However, the single mutants were less sensitive to peroxide stress than the WT. In agreement with expression studies, we suggest that MPN625 and MPN668 can take over the function of each other. Oxidative stress occurs, when the balance between ROS and antioxidants is shifted towards ROS. In this case, it is of major importance, that antioxidant enzymes are regulated in a way, that they can quickly respond to prevent oxidative stress. In this study, the trigger enzyme GlpQ and the regulator Spx were revealed to control expression of the two Prx. Thus, MPN625 and MPN668 are the functional Prx of the minimal bacterium *M. pneumoniae*. Accordingly, these antioxidant enzymes have been renamed PrxA (MPN625) and PrxB (MPN668).

Presentation on Monday, February 25, 2019 from 14:45 – 14:55 in room Hörsaal 104.

Workshop 02
Eukaryotic Pathogens incl. DMykG Lecture (FG EK)
25. Febr. 2019 • 13:30-15:00

007/EKV
Alternative models to study antifungal susceptibility and virulence of pathogenic molds
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Abstract has not been submitted.

008/EKV
The *Candida albicans* peptide toxin Candidalysin causes cytolysis and activates the NLRP3 inflammasome in mononuclear phagocytes
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Clearance of invading microbes requires phagocytes of the innate immune system. However, successful pathogens have evolved sophisticated strategies to evade killing by these immune cells. The opportunistic human fungal pathogen *Candida albicans* is efficiently phagocytosed by macrophages but causes inflammasome activation, damages the host cells, and escapes after hypha formation. Previous studies suggest that macrophage lysis by *C. albicans* results from early inflammasome-dependent cell death (pyroptosis) followed by late damage due to glucose depletion and membrane piercing by growing hyphae.
We are interested in the role of Candidalysin, a cytolytic peptide toxin encoded by the hypha-associated gene *ECE1*. We have previously shown that Candidalysin directly damages epithelial membranes and triggers an epithelial danger response. Here we describe that Candidalysin is a central trigger for NLRP3 inflammasome-dependent caspase-1 activation via potassium efflux in human macrophages, murine macrophages and murine dendritic cells. In addition, Candidalysin induces inflammasome-independent cytolysis in these immune cells upon infection with *C. albicans*. This suggests that Candidalysin-induced cell lysis is a third mechanism of *C. albicans*-mediated phagocyte damage in addition to damage caused by pyroptosis and by the growth of glucose-consuming hyphae.
Candidalysin is one of eight short peptides encoded by *ECE1* and released from the Ece1 polyprotein after processing. While a major contribution of Candidalysin to fungal pathogenicity and anti-fungal immune response is clear, the function of the non-Candidalysin Ece1 peptides remains obscure. Future studies will, therefore, characterize the potential role of non-Candidalysin Ece1 peptides in fungal biology and host interaction.

Presentation on Monday, February 25, 2019 from 14:00 – 14:10 in room Hörsaal 007.

009/EKV

Redox proteomic analysis reveals oxidative modifications of proteins by increased levels of intracellular reactive oxygen species during hypoxia adaptation of the human-pathogenic mould *Aspergillus fumigatus*

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Question: The filamentous fungus *Aspergillus fumigatus* is a ubiquitously distributed, opportunistic pathogen, which causes diseases ranging from allergic responses, to chronic, but also life-threatening, invasive infections. Patients at risk of developing an invasive aspergillosis comprise those with blood malignancies, allogenic bone marrow as well as solid organ transplants, and chronic lung diseases. At the site of infection, *A. fumigatus* faces abrupt declines in oxygen concentrations. An increasing number of studies have demonstrated that elevated levels of reactive oxygen species (ROS) are formed under hypoxic growth conditions. Hypoxic ROS is able to introduce protein thiol modifications that may either impair protein functions or modulate hypoxia adaptation by activating hypoxic genes. Here, we aimed to study ROS formation in *A. fumigatus* under low oxygen levels and to identify proteins that undergo redox-dependent post-translational modifications under these conditions.
Methods: We characterized the proteomic response of *A. fumigatus* to a short period of hypoxia by LC-MS/MS-based detection of relative changes in protein abundances and redox-dependent cysteine modifications. The study was supplemented by further characterisation of a redox-sensitive protein using molecular biology techniques.
Results: The oxidant-sensing probe dichlorodihydrofluorescein diacetate revealed increased amounts of intracellular ROS in *A. fumigatus* exposed to hypoxia. Moreover, nuclear accumulation of the oxidative stress response regulator AfYap1 was observed under hypoxic growth conditions. The highest number (n=18) of proteins with significant increased levels of oxidatively modified thiols groups was detected after one hour of hypoxic cultivations. These included proteins with important roles in maintaining redox balance and protein folding, such as the thioredoxin Asp f 29 and the protein disulphide-isomerase PdiA. Our data also showed that the mitochondrial respiratory complex IV assembly protein Coa6 was significantly oxidised by hypoxic ROS. Deletion of the corresponding gene resulted in a complete absence of hypoxic growth in *A. fumigatus*.
Conclusions: Our redox proteomics study defined important cellular pathways that are targets of hypoxia-induced ROS. We further showed that Coa6 is an essential factor of respiration under hypoxia in *A. fumigatus.*

Presentation on Monday, February 25, 2019 from 14:15 – 14:20 in room Hörsaal 007.

010/EKV

Identification of azole tolerance-affecting transcription factors and ABC transporters in *Aspergillus fumigatus*.

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Aspergillus fumigatus is an airborne fungal pathogen and the primary cause of invasive aspergillosis. This severe infection is associated with high mortality rates and is a major threat for immunocompromised patients. Invasive aspergillosis is typically treated with azole antifungals. However, the emergence of azole resistance is challenging this therapeutic approach. The only currently known mechanisms that mediate azole resistance in *A. fumigatus* are related to mutations in the azole target structure CYP51 and its promoter. Mitochondrial dysfunctions can affect the azole susceptibility of molds. We have previously shown that disruption of mitochondrial fission and fusion increases the azole tolerance of *A. fumigatus*. To further explore the mechanism behind this altered azole susceptibility, we analyzed the transcriptome of the fission/fusion mutant. We identified multiple efflux pumps and transcription factors that are differentially expressed in the fission/fusion mutant. The promoters of several candidate ATP-binding cassette (ABC) transporters and transcription factors were subsequently replaced with a conditional doxycycline-inducible promoter system. Phenotypic characterization of the conditional mutants revealed the ability of five ABC transporters and seven transcription factors to affect the azole susceptibility of *A. fumigatus*. The identified genes could be involved in non-CYP51-dependent azole resistance mechanisms.

Presentation on Monday, February 25, 2019 from 14:30 – 14:40 in room Hörsaal 007.

011/EKV

Phenotypic, genomic, and proteomic characteristics of biofilm-forming *Candida parapsilosis* lineages.

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The human pathogenic yeast *Candida parapsilosis* has gained significant importance over the past decades as one of the principal causes of fungal bloodstream infections. Its capacity to adhere to host cells and clinically used abiotic surfaces (e.g. prostheses or catheters) is crucial during colonization processes and the establishment of infections. Isolates of *C. parapsilosis* are known to be able to switch between several different colony morphologies *in vitro*, which are correlated with different cell shapes and altered cell surface properties.
Prompted by a set of clinical specimen from a single patient that yielded stable smooth- as well as crepe-morphology lineages, we investigated the differences between five lineages and characterized the phenotypic spectrum as well as underlying mechanisms in >300 different-morphology clinical isolates.
In contrast to the initial assumption that the five different isolates were switched forms of an otherwise clonal strain, we confirmed by genome sequencing that the patient was colonized by at least three distinct lineages. Detailed statistical analysis placed the lineages distantly across the population of *C. parapsilosis*. Interestingly the Candidemia-associated blood culture isolate was of smooth morphology and matched with a nasal isolate of similar morphology; however the BC-isolate had undergone significant genomic rearrangements.
Next, we analyzed biofilm formation capacity and differences in morphotypes among several hundred clinical isolates which showed a multitude of phenotypic combinations, but no correlation with site of isolation became evident. Drug susceptibility profiles were altered between groups of biofilm-forming vs. non-forming isolates, and differences in colony morphology correlated with biofilm formation capacity and agar invasion, but not necessarily with virulence in a galleria mellonella infection model.
In addition to a core cell wall proteome consisting of carbohydrate-active enzymes, a set of adhesins was detected in the cell walls of selected hyperbiofilm-forming isolates by mass spectrometry. Downstream analyses showed distinct correlations of phenotypes such as altered virulence or morphology with adhesin incorporation.
Incorporation of specific adhesin proteins into the cell wall of *C. parapsilosis* is highly associated with colony morphology, which

governs important clinical parameters such as biofilm formation capacity and at least partially alters drug susceptibility.

Presentation on Monday, February 25, 2019 from 14:45 – 14:55 in room Hörsaal 007.

Workshop 03
Diagnostic Microbiology (StAG DV)
25. Febr. 2019 • 13:30-15:00

012/DKMV

Clostridium difficile (Hall and O'Toole 1935) Prévot 1938 (Approved Lists 1980), _Peptoclostridium difficile_ Yutin and Galperin 2013 and _Clostridioides difficile_ (Hall and O'Toole 1935) Lawson _et al._ 2016, three names but one taxon and the problem of which one to use.

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An important pathogen is an anaerobic, Gram-positive, spore forming rod that has been known since 1938 as *Clostridium difficile*. With the realisation that the taxonomy of the genus *Clostridium* is in need of extensive revision various changes have been made in the nomenclature of organisms that were previously included in that genus. *Clostridium difficile* is no exception, with Yutin and Galperin proposing a new genus *Peptoclostridium* in 2013 based on genome analysis, resulting in the creation of the name *Peptoclostridium difficile.* However, the name was not validly published based on the Rules of the International Code of Nomenclature of Prokaryotes, leaving the way open for Lawson *et al*. to propose an alternative name, *Clostridioides difficile* initially in the journal Anaerobe and was validly published based on the Rules of the International Code of Nomenclature of Prokaryotes in 2016. One now encounters three names in the literature or databases, *Clostridium difficile, Peptoclostridium difficile* and *Clostridioides difficile*. It is not uncommon to find different names being used in different articles in the same volume of a single scientific journal. Some authors appear to be aware that these three names refer to the same taxon, while in other instances it is unclear whether authors are aware of the two most recent changes. The European Centre for Disease Prevention and Control primarily uses the name *Clostridium difficile* without reference to the others. The Robert Koch Institute uses *Clostridium difficile*, but with reference to *Clostridioides difficile* and the CDC, Atlanta, USA primarily uses *Clostridium difficile*, although the name *Clostridioides difficile* also appears on their website. Given the importance of this organism in the clinical environment clarity is needed in the use of names as well as documenting the inter-relationship between the three. The Rules of the International Code of Nomenclature of Prokaryotes provides the mechanisms by which one can link different names, but is a text with which few are familiar and even fewer implement. Properly understanding the way names are created and applied in a changing field of knowledge and classification is a key element to making sure that information can be correctly and efficiently transferred between staff in the medial or research environment as well as between different organisations or different databases that will prevent misunderstandings or lead to potentially life threatening situations.

Presentation on Monday, February 25, 2019 from 13:30 – 13:40 in room Hörsaal 005.

013/DKMV

Rapid Sepsityper®: from identification to susceptibility testing

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Question

Sepsis is a syndrome burdened worldwide by high morbidityand mortality. Survival rate in case of not appropriate antibiotic treatment significantly decreases hour by hour, hence the rapid identification of the causative agent is crucial for the patients" outcome.

The Rapid Sepsityper® (Bruker Daltonik) allows bacterial identification by MALDI-TOF MS directly from positive blood culture bottles in 10-15 minutes. It represents a shortened version of the conventional protocol, i.e. MALDI identification directly from the bacterial pellet by direct spotting (without ethanol/formic acid extraction).

In this study, we evaluated the implementation of the Rapid Sepsityper into the routine practice , and the possibility to use the samebacterial pellet to carry out susceptibility testing (RUO method), in order to shorten the time to report.

Methods

From 12/05/2018 to 31/08/2018, the routine identification of positive blood cultures was performed by Rapid Sepsityper for n=1546 samples (corresponding to n=1165 bacteriaemic patients).

Result of MALDI identification was compared *in primis* with result of Gram staining, and then with the result of the plate subculture.

For n=769 samples, antibiotic susceptibility testing (Microscan Walkaway, Beckman, Colistrip, Merlin, disc-diffusion for ceftazidime/avibactam, Liofilchem) was performed using the same bacterial pellet as for species identification.

Results

Reliable identification at species level was obtained in 1293/1480 (87.4%) of monomicrobial samples, and in 26/66 (39.4%) of polymicrobial samples. Failed identifications were restricted in most of cases to coagulase-negative staphylococci, corynebacteria, viridans streptococci and yeasts.

For polymicrobial samples, in 21% of cases both bacteria were identified, in 18.4% one was identified.

For 722/769 (93.8%) samples, susceptibility testing using the pellet was successful, for n=47 (6.1%) it was repeated, as bacterial growth was insufficient (mainly coagulase-negative staphylococci).

Conclusions

Implementation of the Rapid Sepsityper into routine showed a very good efficiency, greater than 95% for the bacterial families of major clinical relevance, such as Gram-negative bacilli, *S. aureus*, enterococci and haemolytic streptococci.

The bacterial pellet obtained by the Rapid Sepsityper could be suitable to perform antibiotic susceptibility testing, enabling simplification of the routine workflow, andshorten the reporting time.

Presentation on Monday, February 25, 2019 from 13:45 – 13:55 in room Hörsaal 005.

014/DKMV

Comparison of Fourier-Transform-Infrared (FTIR) Spectroscopy, Whole Genome Sequencing and PFGE for Strain Typing in an Outbreak Investigation

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Introduction:

Pulsed-field gel electrophoresis (PFGE) is one of the well-established methods for subtyping of bacterial strains in investigations of (possible) outbreaks but often performed only in specialised reference laboratories.

With decreasing costs, more general approaches like whole genome sequencing (WGS) become accessible. Further, Fourier-Transform-Infrared (FTIR) spectroscopy has recently been presented as an easy-to-apply method for strain typing in microbiology routine settings.

Objectives:

We have evaluated the applicability of FTIR spectroscopy and WGS for strain typing with a number of strains from an outbreak to

assess the handling, complexity and discriminatory power of these technologies.

Materials and Methods:

N=17 strains collected between May 2016 and October 2017 from an outbreak with OXA-48-positive *K. pneumoniae* were analysed by FTIR and WGS.

FTIR spectroscopy was performed with at least 3 technical replicates after overnight culture on an IR Biotyper system (Bruker Daltonik, Germany).

For WGS, DNA of the strains was prepared using the Nextera XT DNA Library Prep Kit followed by sequencing on a MiSeq-Instrument (Illumina, San Diego, USA).

IR Biotyper software and ASA3P (Universität Gießen, Germany) were used for data analysis. PFGE typing had been conducted before at the National Reference Laboratory for multidrug-resistant gram-negative bacteria (Director: Prof. S. Gatermann).

Results:

From the N=17 strains, PFGE had found n=13 being related to the outbreak and n=4 individual strains.

This clustering could also be seen in the WGS data.

FTIR grouped 11 of the 13 strains from the outbreak cluster together. The 2 remaining strains were seen as individual strains as well as the 4 other individual strains.

Summary:

PFGE and WGS showed 13 strains as being related to the outbreak. FTIR showed similar results with standard algorithms but classified two more strains as being individual.

FTIR results could be obtained after one overnight culture while WGS analysis was available after about 1-2 weeks.

Presentation on Monday, February 25, 2019 from 14:00 – 14:10 in room Hörsaal 005.

015/DKMV

Using phage-coated magnetic beads to capture low amounts of viable pathogenic bacteria

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Background:

Infections caused by multi-drug resistant bacteria are one of the greatest threats for human health. However, the time gap between the initial signs of an infection and targeted antibiotic therapy depends on the availability of pure bacterial cultures for antimicrobial susceptibility testing. Therefore, innovative approaches regarding the rapid separation of bacterial cells from patient samples are urgently needed.

Objective:

The objective of this study was to test the capacity of phages coupled with magnetic beads for specifically capturing viable multi-drug resistant bacterial cells.

Methods:

Newly isolated and characterized phages were treated with UV-radiation in order to obtain non-infective DNA-free phage ghosts. Inactive phages were covalently linked with different magnetic beads. Capture experiments were performed with bacteria spiked in LB medium or in blood samples at defined concentrations. The capture performance and verification of sustained growth capacity was analyzed by counting CFU and via liquid growth assays.

Results:

With increasing UV-doses the number of resulting phage ghosts gradually increased under maintenance of phage integrity and binding capacity. Phages could equally well be bound to tosylactivated Dynabeads and Dynabeads coated in Epoxy groups, each with a size of 4.5 µm, or to MACS MicroBeads with around 50 nm in size. Using phage-coated Dynabeads, capture efficiencies of up to 27% could be achieved when bacteria were spiked at concentrations between 10^5 and 10^1 CFU/mL. However, the capture efficiency could be increased up to 50% by using MicroBeads, even when the number of bacteria was only 10^1 CFU/ml. Further tests showed a reduced but still reasonable effectiveness under more challenging conditions, as about 17% of $2*10^2$ CFU/mL *Acinetobacter baumannii* cells could be purified from spiked blood samples in viable form using phage KARL-1. Moreover, the functionality of this approach could also be confirmed with different phages using *Staphylococcus aureus* or *Pseudomonas aeruginosa* as host.

Conclusion:

Even low numbers of bacterial cells, as they typically occur in clinical samples, such as from sepsis patients, can be extracted in viable form within a very short time using magnetized phages. Those bacteria are then readily available for antimicrobial susceptibility testing. This system may have the potential to significantly contribute to an accelerated diagnosis of life-threatening infectious diseases.

Presentation on Monday, February 25, 2019 from 14:15 – 14:20 in room Hörsaal 005.

016/DKMV

Mass Spectrometry-based Phyloproteomics of *Clostridioides difficile* as alternate Typing Method to Ribotyping is able to differentiate the Ribotype 027

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Introduction

Clostridioides difficile, a Gram-positive spore-forming bacterium, is the leading cause of nosocomial diarrhea worldwide and therefore a substantial burden to the healthcare system. Over the past decade, the hypervirulent *C. difficile* PCR-ribotype (RT) 027 emerged rapidly all over the world what was associated with both, increased severity and mortality rates.

Objective

To prevent disease spread, it is of great importance to identify epidemic strains such as RT027 as fast as possible. Commonly used diagnostic methods, e.g. multi locus sequence typing (MLST) or PCR-ribotyping, are time-consuming, wherefore the aim of the study was to develop a typing scheme based on Mass-spectrometry-based-Phyloproteomics (MSPP), a fast, inexpensive, and reliable method.

Methods

To establish a *C. coli*-MSPP scheme, we analyzed and evaluated 77 ribotyped strains representative for five MLST clades by mass spectrometry. MLST, based on whole genome sequences, and PCR-ribotyping were used as further reference methods.

Results

We were able to associate nine biomarkers with their encoding genes and include them in our *C. difficile* MSPP scheme. The most important finding was that one of the clades only contained isolates of the hypervirulent RT027.

Conclusion

In this study, the MSPP principle that was previously used for subtyping of several organisms by our group, was now successfully applied to *C. difficile*. Using this technique, we are able to distinguish RT027 isolates from non-RT027 isolates what makes it fast and valuable alternative to currently used diagnostic tools.

Presentation on Monday, February 25, 2019 from 14:30 – 14:40 in room Hörsaal 005.

017/DKMV

A metagenomics tool to investigate spirochete diversity in different anatomical and ecological sites

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Spirochetes, a morphologically distinct phylum of bacteria, range from pathogenic (e.g., *Treponema pallidum*) to symbiotic (e.g., *Sphaerochaeta coccoides)* to free-living (e.g., *Exilispira thermophile*) organisms. The ability of spirochetes to inhabit vastly different anatomical and ecological sites is remarkable and indicates the diversity of bacteria that make up this phylum. To investigate the evolution and diversity of spirochetes in biological samples, we developed a metagenomics sequencing approach that enables the characterization of spirochetes from different ecological sites. *In-silico*, we determined that by sequencing different regions of the 16S rRNA gene (e.g., variable region 4), a variety of spirochetes can be detected in biological samples. The *in-silico* results indicate that the resolution of the different species of *Treponema* is especially good as more than 20 species (97% similarity threshold) can be differentiated using a single variable region. To validate the *in-silico* results, we created a bacterial mock community by mixing together 16 species of known spirochetes (e.g. *Treponema pallidu*m). We discuss the results of the validation experiments and present the areas of applications of the metagenomics techniques. To show the applicability of the NGS tool for clinical samples, we present data from a recent investigation of oral spirochetes of captive and wild non-human primates (NHPs). We discuss the benefits and limitations of the metagenomics approach in identifying novel species and uncovering the diversity of spirochetes. Our understanding of the biology of spirochete will develop as we understand more about the diversity of species and their ability to inhabit and adapt to different ecological landscapes.

Presentation on Monday, February 25, 2019 from 14:45 – 14:55 in room Hörsaal 005.

Workshop 04
Molecular Epidemiology of Infectious Diseases (StAG RK, FG MS)
25. Febr. 2019 • 13:30-15:00

018/MSV

Molecular epidemiology of measles virus in respect to the elimination goal: Fair or fail?

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Measles virus is a highly contagious paramyxovirus transmitted by droplet infection. Contraction of measles virus results in maculo-papular rash, high fever and malaise. Approximately one of 1,000 infections will lead to death. An attenuated life vaccines against measles was developed in the 1960s by John F. Enders. Descendants of this vaccine are still in use and have led to a dramatic decrease of the global incidence and the number of fatal measles cases. Since measles vaccine evokes a robust immune response which is supposed to last life-long and measles virus infects only human beings, measles is one of the few viral pathogens that have been targeted by WHO for global elimination.

As Germany and other European countries are nearing elimination, a high-quality surveillance and the verification of the elimination process become more and more important. Surveillance is based on two principles: firstly the notification of the disease by clinicians and of laboratory results indicative of an infection to the local health authority and secondly the molecular surveillance enabling us to study transmission of single virus variants. The elimination process is considered to be successful, when the incidence has dropped to a value 1 measles per inhabitant (corresponding to only 80 annual measles cases in Germany!). This can be accomplished only, when the vaccination rates are further improved and stabilized on a high level. As a second criterion, transmission chains of one certain virus type must not circulate longer than a 12 months period in the country. Molecular classification according to the WHO protocol uses a 450 nt fragment from the N-gene region of the measles virus genome. Analyzing this data set, measles virus circulation has demonstrated during the last years, that variants were detected for more than 12 months in Germany. Thus, virus circulation is still considered to be endemic. On a happier note, the situation looks differently, when a second, larger sequence window is used. Using this data set, the situation in Germany may be not as bad as we thought.

To further improve the situation for Germany, all patients should be checked for missing vaccinations according to the STIKO recommendations and throat swabs and/or urine samples from suspected measles cases should be sent to the National Reference Center Measles, Mumps, Rubella in Berlin.

Presentation on Monday, February 25, 2019 from 13:30 – 13:40 in room Hörsaal 004.

019/MSV

Ultraplexing – a method for more efficient bacterial whole-genome sequencing

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Background: Accurate and comprehensive whole-genome sequences are a prerequisite to study bacterial phenotype/genotype associations. The current gold-standard is the combination of short- (e.g. Illumina technology) and long-read sequencing data (e.g. Oxford Nanopore technology, ONT). Generating long-read data with ONT for a high number of samples is expensive, since a maximum of 12 samples can be multiplexed by barcoding, which does not efficiently use flow-cell capacity.

Aim: We aimed to overcome the limits of ONT barcoding to generate cost efficient hybrid assemblies. We developed a new bioinformatic tool, called ultraplexer, to match non-barcoded long-reads to corresponding bar-coded short reads based on k-mer frequencies. This allows to more efficiently use flow-cell capacity.

Material and Methods: The performance of the ultraplexer, was evaluated by two simulated and one experimental data set, each containing long- and short-read sequencing data. Datasets were simulated for ten different bacterial species with relevance in clinical microbiology and for five sets of 10-50 *S. aureus* genomes (NCBI RefSeq Database). The experimental dataset contained 10 *S. aureus* isolates. Following read allocation by ultraplexing, a hybrid assembly was performed with Unicycler. For each experiment we assessed the accuracy at the level of correctly classified reads and at the level of assemblies (contigs; mean base-pair accuracy; coverage).

Results: Using the simulated dataset of ten different bacteria species, 100% of the reads were classified correctly. The assemblies had one contig, a base-pair accuracy of 13.58 single nucleotide polymorphisms (SNPs) per megabase (mb) and completely covered the original genome. When using the simulated set of ten *S.aureus* genomes, 96% of the reads (mean) were classified correctly. Each assembly had one contig, a base-pair accuracy of 12.85 SNPs per mb, and completely covered the original genome. In the datasets with up to 50 different *S. aureus* genomes the number of correct classified reads was about 40%, and yielded a single contig in 97%. Base-pair accuracy was 12.44 SNPs per mb with a coverage of nearly 100%. The assemblies of the experimental dataset had 1.8 contigs (mean) and the longest contig reached an average length of 2.84mb.

Conclusion: We developed a method (ultraplexing), that allows more efficient sequencing for hybrid assemblies and maximizes ONT flow-cell capacity. Thus sequencing is more speedy and less expensive.

Presentation on Monday, February 25, 2019 from 13:45 – 13:55 in room Hörsaal 004.

020/RKV

Recurring invasive meningococcal infections – quantifying the risk

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Introduction
Invasive meningococcal disease (IMD) is a rare condition with a high case fatality rate. While most affected patients appear to suffer from only a single episode in their life span, there is anecdotal evidence for recurrent infection [1]. The incidence of recurring IMD, however, has not been quantified. The National Reference Laboratory (NRL) for meningococci has analyzed 5,822 cases of meningococcal disease in the past 16 years, which offers the opportunity to retrospectively quantify the risk of recurrent infection.
Aim of the study
To assess the risk of recurring invasive meningococcal infections in Germany.
Methods
Patients living in Germany with IMD that were registered by the NRL between 2002 and 2017 as part of the laboratory surveillance program were analyzed. IMD was assumed for cases, where *Neisseria meningitidis* was detected by culture or PCR from blood or cerebrospinal fluid. A recurring IMD was defined as the detection of *N. meningitidis* in a following sample from the same patient after an interval of at least 30 days. Patient identity was assessed by comparison of month of birth, sex, and county of living. In some cases, identity was reported beforehand by senders.
Results
Out of the 5,583 patients with an average observation period of 9.14 years, 13 suffered a second episode and one a third IMD episode. Assuming an average lethality of 9.6 % [2], the risk of a recurring IMD was 30.3 per 100,000 person years of survivors of the first episode compared to an average general incidence of IMD of 0.9 per 100,000 in the observation period (Source: https://survstat.rki.de/). The median interval from the first to the second episode was 1.64 years. Rare serogroups (Y: 21 %, W: 14 %, W: 7 %, Z: 3 %, E: 3 %, non-groupable: 3 %) were more common in patients with recurring IMD. The same strain has not been observed more than once in a patient.
Discussion
Surviving IMD patients are at a more than 30-fold risk of IMD compared to the general population. Increased risk might be caused by undiagnosed complement deficiencies. The study most likely underestimates the risk of recurrent infection. Therefore, a joint analysis of statutory notification data will be initiated with the RKI. The high risk of re-infection argues for vaccination of IMD patients following survival of disease.
References

1. Hildenhagen, O. et al., Dtsch Med Wochenschr, 1985. **110**(39): p. 1498-501.
2. Hellenbrand, W., Epidemiologisches Bulletin, 2016. **43**: p. 471-84.

Presentation on Monday, February 25, 2019 from 14:00 – 14:10 in room Hörsaal 004.

021/RKV

Development of a specific and sensitive ELISA as an in-vitro diagnostic tool for the detection of Bartonella henselae antibodies in human serum

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Question: Bartonella henselae causes cat scratch disease and several other clinical entities. Infections with B. henselae are frequently occurring, however, the infection is only rarely diagnosed mainly due to a lack of knowledge in the medical community. Microscopic immunofluorescence assays (IFA) are widely used for serodiagnostics of B. henselae-infections, but are laborious, time consuming and interpretation is subjective. An easy and reliable method for the serological diagnosis of B. henselae infections is needed to overcome the shortcomings of the current IFA. Here, we report the development of an ELISA detecting human anti-B. henselae antibodies from serum samples.
Methods: By separating the water-insoluble fraction of B. henselae Houston-1 via ion exchange chromatography, 16 subfractions were generated and tested for immunoreactivity via line blotting. One particular fraction (#24) was selected and spotted on ELISA-plates using an industrial production platform.
Results: By the use of well-characterized human sera from the strictly quality-controlled serum library of the German National Consiliary Laboratory for Bartonella infections, sensitivity of this ELISA was 100% for molecular proven infections and 76% for clinically suspected infections at a specificity of 93%, respectively.
Conclusions: This ELISA is therefore a reliable high-throughput method allowing serodiagnostics of B. henselae infections.

Presentation on Monday, February 25, 2019 from 14:15 – 14:20 in room Hörsaal 004.

022/MSV

NGS based molecular typing of *Staphylococcus haemolyticus* - replacing PFGE to increase resolution

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Background: *Staphylococcus haemolyticus* (*S. haemolyticus*) is a colonizer of skin and mucous membranes, associated with infections in immunocompromised patients and premature infants. Epidemiological investigations of infection clusters are predominantly realized via macrorestriction analysis by pulsed-field gel electrophoresis (PFGE). Since macrorestriction analysis suffers from several disadvantages, this study aims at validating a whole genome sequencing (WGS) based approach.
Methods: In total 131 *S. haemolyticu*s isolates from Germany (n=118) and from abroad (n=13), spanning a ten year period (2007-2017), were included. Besides unrelated isolates, isolates from suspected outbreaks and from a long-term-colonization study at a single hospital were comprised. All strains were subjected to resistance testing using broth microdilution, SmaI-macrorestriction analysis (BioNumerics) and WGS (Illumina technology). Genome data was subjected to in house pipelines for quality control and sequence reconstruction. SeqSphere+ was used to generate an *ad hoc* core genome multilocus sequence typing (cgMLST) scheme. Clustering results were compared to those obtained from macrorestriction analysis, focusing on discriminatory power, reproducibility and cluster concordance.
Results: Multidrug resistance was expressed by 88.6 % of all isolates. Macrorestriction analysis resulted in the identification of eleven clusters containing 82.4 % of all isolates. Remaining "singletons" were predominantly sensitive isolates. Reproducibility of PFGE was low and clusters were partly not supported by epidemiological metadata indicating the need for alternative typing strategies.
Outlook: Ongoing work is focusing on the comparison of typing results, the validation of the cgMLST scheme and a deeper analysis of genome data with respect to antibiotic resistance and virulence associated genes.

Presentation on Monday, February 25, 2019 from 14:30 – 14:40 in room Hörsaal 004.

023/MSV

Spectrum of mutations in antibiotics resistance and pathoadaptive loci of *Pseudomonas aeruginosa* isolates identified with amplicon sequencing

S. Fischer*[1], L. Greipel[1], N. Cramer[1], J. Klockgether[1], B. Tümmler[1]
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Pseudomonas aeruginosa is an important causative agent of nosocominal infections and is the pathogen which contributes most to the shortened life expectancy in cystic fibrosis (CF) patients. In this project we analyzed mutations of antibiotics resistance and pathoadaptive loci of CF isolates compared to non-CF isolates using fast and cost effective Illumnia amplicon sequencing of 37 loci (17 antibiotics resistance, 20 pathoadaptive loci) in more than 500 isolates to investigate potential differences comparing the habitats. Major differences in the number of amino acid sequence changing variants were found in the investigated loci comparing CF and non-CF isolates ranging from only few amino acid changes (LepA) up to a high number of changes including an accumulation of early stop codons (AlgG, AlgU). Additionally, a correlation of the mutations with the structure of the AlgG and MexZ locus was identified showing evidence for differential functionality in the habitats. These potential function changing isolate shall be tested *in vivo* to test for function modification.

Presentation on Monday, February 25, 2019 from 14:45 – 14:55 in room Hörsaal 004.

Workshop 05
Diagnostic Stewardship - "Meet the needs of your partners!" (FG DKM, FG QD)
25. Febr. 2019 • 17:00-18:00

024/DKMV

Survival of pneumococci in three different commercial collection and transport systems (swabs) – do we miss pneumococcal disease due to flaws in transport conditions?

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Question: *Streptococcus pneumoniae* is a major pathogen for pneumonia, septicemia and meningitis. Identification can be done directly from patient samples or from cultures. However, susceptibility testing is only possible from cultures. Therefore a prerequisite for susceptibility testing of pneumococci is the arrival of viable pneumococci in the laboratory. We studied the culturability of pneumococci stored for up to 48h in different commercial collection and transport swabs. Additionally we investigated the influence of inoculated bacterial mass, temperature and strain on recovery.

Materials and Methods: We chose three different collection and transport systems with liquid transport agar: deltaSwab (deltalab), Sigma Transwab (MWE) and eSwab (Copan). *S. pneumoniae* ATCC49619 was chosen for initial studies on the influence of time and inoculum. To mimic patient samples (PS) a McFarland 0.5 solution was prepared and serially diluted. Swabs were inoculated with 100µl of the dilutions and stored at 4°C or room temperature (RT). Culture controls were done at 0, 24 and 48h. To mimic the situation of sending strains to reference laboratories (RS) we used 20 different pneumococcal strains from our collection and inoculated the swabs with the bacterial mass from a complete 90mm agar plate grown o/n at 35°C, 5%CO2. Swabs were stored at 4°C or RT. Culture controls were done after 0, 24 and 48h.

Results: The serially diluted ATCC 49619 could be recovered from the sigma Transwab down to a dilution of 1:100 and from the eSwab down to a dilution of 1:10. From the delta swab pneumococci could neither be recovered after 24 nor after 48h. Recovery from RS swabs was sufficient for all swabs and all storage types after 24h. However, after 48h of storage the amount of bacteria was already much reduced in the deltaSwab if stored at RT. For all swabs recovered amounts of bacteria were high if swabs were stored at 4°C.

Conclusion: The three transport systems differed markedly in their recovery rate for pneumococci. Surprisingly recovery of pneumococci from swabs, which were stored at 4°C was higher than recovery from swabs which were stored at RT. Different strains did not show markedly different recovery rates. Transport longer than 48h cannot be recommended. A comparison of the agar recipes did not reveal striking differences in composition of the transport agar. The reason for the strikingly different results remains unknown.

Presentation on Monday, February 25, 2019 from 17:00 – 17:08 in room Hörsaal 005.

025/DKMV

Diagnostic performance of serum (1→3)-β-D-glucan and mannan for early diagnosis and follow-up in patients with candidemia

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Introduction: *Candida* spp. account for approx. 10% of bloodstream infections in ICUs and are associated with a in-hospital mortality rate of 30%. Fungal biomarkers, like (1→3)-β-D-glucan (BDG) or mannan (Mn), have proven to be useful in the rapid diagnosis of candidemia. However, data on the serum levels of these biomarkers before and after the diagnosis of candidemia are scarce.

Goals: To determine if the diagnosis of candidemia can be established earlier by measurement of serum BDG and Mn and to examine if the levels of these biomarkers are reflecting treatment response.

Methods: The study was performed at the University Hospital Erlangen between August 2015 and May 2017. All serum samples from patients with culture-proven candidemia were collected. The sera were obtained from Clinical Chemistry which stores all samples for 7 days. This enabled us to get access to sera from the 4 to 5 days prior to blood culture sampling (day 0). For follow-up purposes, all serum samples after day 0 were collected until death or discharge. Furthermore, clinical data were acquired.

BDG and Mn levels were measured with the Fungitell assay and the Platelia *Candida* Ag Plus assay according to the manufacturers" instructions.

Results: In total, 707 serum samples from 88 candidemia episodes were analyzed. *C. albicans* was the most common pathogen (48%) followed by *C. glabrata* (30%) and *C. parapsilosis* (9%). In-hospital mortality was 40% and 44% of the patients with a fatal outcome died within 7 days after day 0.

The median BDG and Mn levels at day 0 were 229 pg/ml and 32 pg/ml, respectively. Using the manufacturers´ cut-offs the sensitivity of BDG and Mn at this time point was 73% and 33%, respectively.

The median time span from day 0 to blood culture positivity was 2 days. In contrast, BDG and Mn levels were elevated up to 6 days before day 0 in 43% and 30% of patients, respectively. During follow-up, decreasing BDG and Mn levels were associated with survival at the end of antimycotic therapy in 70% and 86% of patients, respectively. Furthermore, increasing BDG and Mn levels were associated with death in 63% and 60% of patients, respectively.

Summary: Serum BDG has superior sensitivity in comparison to Mn for the diagnosis of candidemia. Biomarker positivity often precedes culture positivity and enables earlier diagnosis and initiation of therapy. Decreasing biomarker levels during follow-up suggest a favorable outcome whereas increasing levels do not predict death.

Presentation on Monday, February 25, 2019 from 17:10 – 17:18 in room Hörsaal 005.

026/DKMP

Detection and identification of pathogenic dermatophytes using multiplex real-time PCR assays

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[2]*PathoNostics B.V., Maastricht, Netherlands*

Introduction: Fungal infections of nails, skin and hair caused by dermatophytes such as *Trichophyton* (T.) spp., *Microsporum* (M.) spp. or *Epidermophyton* (E.) spp. are one of the most common human infections in the world. As the epidemiology varies between different dermatophyte species a specific diagnosis plays an important role to ensure a targeted therapy. In order to evaluate the DermaGenius multiplex real-time PCR assays (PathoNostics), real-time PCR results were compared to routine diagnostic methods including culture detection, microscopy and a standard PCR-ELISA assay.

Methods: After isolating DNA of 31 clinically isolated skin specimens and 18 cultures, dermatophytes were identified macroscopically, microscopically and molecularbiologically as well as analysed using the DermaGenius assay. Sanger sequencing of both, the internal transcribed spacer (ITS) region of the ribosomal DNA and the translation elongation factor alpha (*TEF1α*) gene was used when routine diagnostics could not give a definite result.

Results: In total, all (n = 49) clinical specimens and cultures could be detected with the DermaGenius assay. Out of 49 samples, the DermaGenius assay could identify 76% correctly by their specific melting temperature. Fourteen samples were detected but not differentiated including *T. soudanens*e (n = 1) identified as *T. rubrum*, *T. erinacei* (n = 2) as *T. benhamiae*, *T. equinum* (n = 1) as *T. tonsurans* and *M. ferrugineum* (n = 1) as *M. canis*. Although different variants of *T. mentagrophytes* (n = 4), *T. quinckeanum* (n = 2) and *T. schoenleinii* (n = 1) species were detected by real-time PCR, they all showed similar melting temperatures to either *T. interdigitale* or *T. mentagrophytes*.

Conclusions: The DermaGenius multiplex real-time PCR assays (PathoNostics) are able to identify the most frequently isolated clinically prevalent dermatophytes and are suitable for routine molecular diagnostic laboratories as they enable high samples throughput with limited hands-on time. However, the identification and differentiation of less common dermatophytes as well as variants of *T. mentagrophytes* is still complicated since they are phylogenetically very closely related. After taking the anamnesis of patients or a fungal culture into consideration, only sequencing enables an identification of dermatophytes that could not be detected during routine diagnostics.

Presentation on Monday, February 25, 2019 from 17:20 – 17:28 in room Hörsaal 005.

027/DKMV

Clinical presentation and secondary prevention of skin infection in patients with Panton-Valentine leukocidin (PVL)-producing *S. aureus:* A cohort study on the effectiveness of decolonization in an outpatient setting.

L. G. Hanitsch[1], R. Krüger[1], P. A. Hoppe[1], D. Humme[1], A. Pokrywka[1], M. Niebank[1], M. Stegemann[1], A. Kola[1], R. Leistner*[1]

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Introduction/aim. Recurrent skin or soft tissue infections (SSTI) are often associated with Panton-Valentine leukocidin producing strains of *S. aureus* (PVL-SA). In order to prevent reinfection and spreading, decolonization measures are required in addition to treatment of active infections. Even though most PVL-SA patients are treated as outpatients, there are only a few studies assessing the effectiveness of outpatient decolonization in PVL-SA patients.

Material and methods. We assessed the results of decolonization for PVL-SA by a retrospective review of patient files and personal interviews. Successful decolonization was defined as the absence of any further SSTI for at least 6 months after the completion of the last decolonization treatment. Clinical and demographical data were assessed.

Results. Our cohort consisted of 67 index patients with recurrent skin abscesses due to PVL-SA. Additional 48 symptomatic and 40 asymptomatic contacts were identified by PVL-SA screening (n=155). Contact screening revealed household transmission of PVL-SA in 65% of cases.

92/97 PVL-SA-positive and symptomatic patients, were available for complete follow-up > 6 months. The mean follow-up was 15 months. After completion of the first decolonization 47% were free of symptoms. Repeated decolonization increased the rate continuously to 100% after a maximum of five decolonization treatments. Successful eradication after first decolonization was significantly higher in single households compared to households with $\geq$ 2 members ($p < 0{,}001$).

Conclusion. Decolonization is a successful preventive measure for reducing the risk for PVL-SA SSTI in the outpatient setting. Special attention should be given to patients with many close physical contacts as these settings could pose relevant decolonization hurdles.

Presentation on Monday, February 25, 2019 from 17:30 – 17:38 in room Hörsaal 005.

028/KMV

Prevalence of tuberculosis in homeless persons in Münster, Germany

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Introduction: The incidence of tuberculosis (TB) in Germany is increasing again since 2013. Homeless persons are a risk group for infectious diseases in general and TB in particular. Among individuals with latent TB infection (LTBI), homeless persons run a higher risk to develop active TB compared to the general population. Specifically, the prevalence of TB and LTBI in homeless persons in Germany is largely unknown.

Aim: We aimed to assess the prevalence of TB/LTBI in homeless persons and to identify risk factors for TB/LTBI.

Material & Methods: A total of 150 homeless persons were recruited in Münster, Germany, between October 2017 and July 2018. Participants were screened for exposure to *Mycobacterium tuberculosis* complex using T-SPOT®TB (Oxford Immunotec, UK), an Interferon-gamma release assay (IGRA). IGRA-positive participants were additionally screened for active pulmonary TB by analysing three sputa using a line-probe assay (GenoType® MTBDRplus, Hain, Germany), microscopy, culture (solid and liquid media) and chest X-ray (Figure 1). Risk factors for LTBI/TB were assessed using a standardized questionnaire.

Results: Of 142 evaluable IGRA results, 21 (15%) were positive and 2 (1%) were borderline. The IGRA-negative group included more likely German citizens (63% vs. 35%, p=0.01), persons born in Germany (57% vs. 22%, p<0.001) or persons residing mainly in Germany during the past five years (82% vs. 67%, p=0.005) compared to the IGRA-positive group.

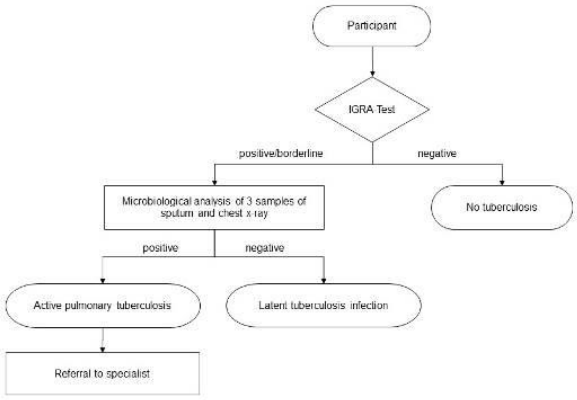
Participants also had more likely a negative IGRA if they were citizens of a low-TB-incidence country according to WHO (52% vs. 26%, p=0.01), were born in a low-incidence country (70% vs. 30%, p<0.0005).Cough for more than three weeks was also associated with a positive IGRA result (24% vs. 44%, p=0.05). All IGRA-positive participants were screened for active TB by microbiological analysis of sputa and chest X-ray; no case was found.

Summary: The prevalence of LTBI (diagnosed by a positive/borderline IGRA) was 16% in our population of homeless persons; no active TB was detected. Treatment of LTBI can be

considered in homeless persons with positive IGRA. The highest risk for LTBI was found in persons from high-incidence countries.

Figure 1: Diagnostic procedures to determine tuberculosis (TB) infections in homeless persons. All Participants were tested by Interferon-gamma release assay (IGRA). Patients with borderline/positive IGRA results were screened for active pulmonary TB.

Figure 1



Presentation on Monday, February 25, 2019 from 17:40 – 17:48 in room Hörsaal 005.

029/DKMV

A host gene signature for diagnosis and risk stratification of acute infection and sepsis at hospital admission: HostDxTM Sepsis

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Background: Acute infections and sepsis are leading causes of morbidity and mortality worldwide. In Germany and other countries, the number of patients visiting emergency departments has increased substantially in recent years. Diagnostic procedures to evaluate patients with acute infections and suspected sepsis are inaccurate or slow. Analysis of host-response signatures using RNA expression has been described for diagnosis and risk stratification. We here describe the development of HostDx™ Sepsis, a 30-host-gene PCR test that identifies i) the presence of an infection, ii) the type of infection (viral or bacterial), and iii) the severity of the infection using whole blood. The HostDx Sepsis test is being developed as a cartridge-based, sample-to-answer, quantitative assay with turn-around time of less than 30 minutes.
Methods: To identify gene signatures specific for presence, type and severity of infection, we analyzed publicly available microarray and NGS gene expression data sets from cohorts of children and adults with community- and hospital-acquired infection and sepsis. Gene sets to distinguish between infections and non-infectious inflammation, between viral and bacterial infections, and that can predict the severity of infection were discovered and validated in 38 independent cohorts (total N=2452) to establish clinical performance.
Results: Validation performance for the presence of any bacterial infection in a hospital population showed a 94% sensitivity and 60% specificity (99% negative predictive value at 15% prevalence). The mean area under the receiver operator characteristics curve (AUROC) was 0.88 for prediction of 30-day mortality, markedly improving AUROCs for laboratory parameters and/or clinical scores including lactate and SOFA. To demonstrate proof of feasibility, gene signatures distinguishing between viral and bacterial infections were successfully converted to rapid multiplex PCR assay formats, with correlation of 0.95 to a NanoString® reference standard (digital detection and quantification of unique transcripts).
Discussion & Conclusions: Emergency physicians currently rely on a battery of tests with low accuracy to diagnose acute infections and sepsis. The host-response signatures described demonstrated high diagnostic and prognostic accuracy in numerous independent cohorts. As a rapid triage assay HostDx Sepsis will allow for improved decision making for antibiotics, downstream testing, and level-of-care decisions.

Presentation on Monday, February 25, 2019 from 17:50 – 18:00 in room Hörsaal 005.

Workshop 06
Pathogenesis of Gastrointestinal Infections (FG MP, FG GI)
26. Febr. 2019 • 08:30-10:00

030/GIV

Quantity matters: Co-occurrence of multiple enteric pathogens can predict acute diarrhoea in African infants – a prospective case control study

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Introduction: Diarrhoea is the second leading cause of mortality in children <5 years. In low-income countries, many children suffer from multiple episodes per year and the highest incidence is found in children ≤12 months. However, epidemiologic data on enteric pathogens in African infants is scarce.
Aim: The aim of this prospective case-control study was to analyse the causes of acute diarrhoea in Ghanaian children aged ≤12 months.
Methods: Ghanaian children aged 0-12 months who visited the outpatient department (OPD) of a rural Ghanaian hospital in 2014 and 2015 with acute diarrhoea were assigned to the case group. Children of the same age with other conditions but diarrhoea were assigned to the control group. Clinical and demographic data were assessed. Stool samples were analysed for 18 pathogens by multiplex polymerase chain reaction (PCR) at the University Medical Center Hamburg-Eppendorf. To assess the association of diarrhoea with different variables such as positive PCR and the cycle threshold (Ct) value, univariate analyses were performed. A binary logistic regression was performed to analyse the association of different pathogens with positive PCR and diarrhoea, adjusted for variables that were significant in the univariate analyses.
Results: A total of 204 patients at a median age of 7 months (range, 0.5-12 months) were included in the study. While 107 patients (52%) suffered from acute diarrhoea, 97 were treated as controls without diarrhoea (48%). In univariate analyses, diarrhoea was significantly associated with female sex (p=0.048), a higher number of pathogens (median 3 versus 2, p=0.001) and a positive PCR result for Rotavirus (p=0.011), Enterotoxigenic Escherichia coli (p=0.005), Giardia lamblia (p=0.036) and Cryptosporidium (p=0.044). A significant association between a lower Ct value and presence of acute diarrhoea was observed for Cryptosporidium (p=0.015). Adjusting for gender and the number of pathogens, Rotavirus was the only pathogen associated with presence of diarrhoea (p=0.034).
Summary: Enteric pathogens were highly prevalent in Ghanaian children 0-12 months visiting the OPD, independently from acute diarrhoea. Acquisition of enteric pathogens occurs very early in life. Rotavirus seems to be the most important pathogen. A significant association of the number of stool pathogens in children with acute diarrhoea suggests that not a single pathogen but rather co-occurrence of multiple pathogens can lead to diarrhoea.

Presentation on Tuesday, February 26, 2019 from 8:30 – 8:40 in room Hörsaal 104.

031/GIV

Genome comparison of sorbitol-fermenting and non sorbitol-fermenting EHEC O157 – what`s the difference?

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a major enteric pathogen capable of causing bloody diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome (HUS). It is linked to sporadic incidents as well as large outbreaks of gastrointestinal disease all over the world. Key virulence traits implicated in human disease are intimate attachment of the bacteria to epithelial cells mediated by the locus of enterocyte effacement (LEE region) and the production of Shiga toxins able to inhibit protein synthesis in host cells. A phenotypic feature of classic O157:H7 is the inability to rapidly ferment sorbitol, a characteristic which is also used for diagnostic purposes. However, in addition to these non sorbitol-fermenting (NSF) O157 strains, a sorbitol-fermenting, nonmotile O157 variant (SF O157:H-) has emerged as an important cause of gastroenteritis. SF O157 infections have been linked with a higher rate of progression to HUS suggesting that this variant might be more virulent than classical O157:H7 strains. SF O157 shares the two main virulence factors, the LEE region and a gene encoding Shiga toxin, with its NSF relative, but genetic differences between these two groups have been also described for chromosomal as well as plasmid-located regions. While several complete genome sequences of classical O157:H7 strains have been published since 2001, no whole genome sequence of an SF O157 strain is available so far. In this study, we aimed for a detailed genomic comparison of an SF O157 strain with classical, NSF O157. For this purpose, we selected an SF O157 strain isolated from a HUS patient in the course of an SF O157 outbreak event for whole genome sequencing. We performed long-read sequencing using the Oxford Nanopore MinION technology and short-read sequencing using an Illumina MiSeq instrument. A de novo assembly yielded two circular contigs – a chromosome of 5.5 Mb and a plasmid of 121 kb. The obtained plasmid sequence resembles earlier plasmid descriptions. The chromosome sequence is characterized by the presence of a high number of prophages as has been also described for classical O157:H7 strains. The comparative analysis between the genomes of the here analyzed SF O157 isolate and a classical NSF O157:H7 strain will reveal common and differentiating features of the two variants, potentially enabling a better understanding of the underlying pathogenicity.

Presentation on Tuesday, February 26, 2019 from 8:45 – 8:55 in room Hörsaal 104.

032/GIV

Novel insights into the *Helicobacter pylori*-leukocyte interaction in the in murine stomach via Multiphoton intravital microscopy (MP-IVM)

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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. The infection with this Gram-negative, microaerophilic, spiral-shaped bacterium causes a chronic bacterial gastritis that may lead to peptic ulcer disease, duodenal ulcer, MALT (mucosa-associated lymphoid tissue) lymphoma or gastric cancer.[1] *H. pylori* is supposed to be acquired in the early childhood and the transmission probably occurs via the oral-oral or fecal-oral route within families.[1],[2] Despite a strong immune response upon infection, *H. pylori* is able to persistently colonize the human gastric mucosa. Therefore *H. pylori* employs various strategies that either help to avoid a proper recognition by the human immune system via pattern recognition receptors (PRR) or that lead to immune tolerance via the induction of regulatory T-cells that suppress an *H. pylori* specific memory T-cell response.[3],[4] Besides mounting evidence that *H. pylori* actively modulates the human immune system to its own benefit the direct interaction of *H. pylori* with immune cells *in vivo* is not well understood.

Within this study, we aimed for a deeper understanding of the *H. pylori*-leukocyte interaction *in vivo*. In our recently established murine stomach model, we use multiphoton intravital microcopy (MP-IVM) to study the inflammatory processes during the acute phase and chronic phase of an *H. pylori* infection. Different mouse reporter strains where a subset of leukocytes express GFP (*e.g*. neutrophils, macrophages) were infected with *H. pylori* PMSS1 wt mRFP[5] for different durations (24h up to 6 weeks). Recent advances within this model are shown.

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[2] X. Didelot, S. Nell, I. Yang, S. Woltemate, S. van der Merwe, S. Suerbaum, *Proc Natl Acad Sci U S A* **2013**, *110*, 13880-13885.
[3] I. C. Arnold, I. Hitzler, A. Muller, *Frontiers in cellular and infection microbiology* **2012**, *2*, 10.
[4] M. Oertli, M. Sundquist, I. Hitzler, D. B. Engler, I. C. Arnold, S. Reuter, J. Maxeiner, M. Hansson, C. Taube, M. Quiding-Jarbrink, A. Muller, *J Clin Invest* **2012**, *122*, 1082-1096.
[5] D. Keilberg, Y. Zavros, B. Shepherd, N. R. Salama, K. M. Ottemann, *MBio* **2016**, *7*.

Presentation on Tuesday, February 26, 2019 from 9:00 – 9:10 in room Hörsaal 104.

033/MPV

Proteome profiling by label-free mass spectrometry reveals differentiated response of *Campylobacter jejuni* 81-176 to sublethal concentrations of bile acids

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Introduction: *Campylobacter jejuni* is the leading cause of bacterial gastroenteritis among human beings worldwide. In the human gut, *C. jejuni* mainly colonizes the jejunum. Bile acids are one of the major constituents of intestinal fluid and inhibit microbial growth. They are grouped into (i) primary bile acids comprising of cholic acid (CA), chenodeoxycholic acid (CDCA), taurocholic acid (TCA), glycocholic (GCA), glycochenodeoxycholic (GCDCA), and taurochenodeoxycholic acid (TCDCA); (ii) secondary bile acids comprising of deoxycholic acid (DCA), and, lithocholic acid (LCA); and (iii) tertiary bile acids, mainly, ursodeoxycholic acid (UDCA). Accordingly, *C. jejuni* is confronted with these bile acids that are primarily toxic to bacteria after ingestion. However, little is known about the adaptation of *C. jejuni* to the resulting stress under physiological conditions.

Objective

The aim of this study was to investigate the proteomic response in *C. jejuni* strain 81-176 to sublethal concentrations of CA, DCA, LCA, TCA, CDCA, UDCA and GCA.

Materials and Methods: Label-free mass spectrometry (SWATH-MS) was used to characterize the adaptation of *C. jejuni* to sublethal concentrations of seven bile acids.

Results: The proteomic analysis revealed that bile acids with the lowest IC50 (DCA, CDCA and GCA) induced the most significant proteome changes. Overall we found a down-regulation of all basic biosynthetic pathways and a general decrease of the transcription machinery. Concurrently, an induction of factors involved in detoxification of reactive oxygen species, protein folding, and bile acid exporting efflux pumps was detected. Exposure to DCA, CDCA and GCA resulted in increased expression of components of the more energy-efficient aerobic respiration pathway, while the

anaerobic branches of the electron transport chain were down-expressed.
Conclusion: Our results show that *C. jejuni* has a differentiated system of adaptation to bile acid stresses.
The findings enhance the understanding of the pathogenesis of campylobacteriosis, especially for survival of *C. jejuni* in the human intestine, and may provide clues to future medical treatment.

Presentation on Tuesday, February 26, 2019 from 9:15 – 9:25 in room Hörsaal 104.

034/MPV

The Many-Faced Pathogen - Intracellular Phenotypes of *Salmonella* Paratyphi A

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The typhoidal *Salmonella enterica* serovar Paratyphi A (SPA) is a human-restricted pathogen. Key differences between typhoidal and non-typhoidal *Salmonella* (TS and NTS) regarding their specific virulence mechanisms remain unknown. After successful invasion or phagocytic uptake, *Salmonella* resides in a membrane-bound compartment, the *Salmonella*-containing vacuole (SCV). The subsequent intracellular lifestyle depends on the translocation of effector proteins via a type 3 secretion system (T3SS) which is encoded on the *Salmonella* pathogenicity island 2 (SPI2). During the intracellular lifestyle, host cell membranes are manipulated by effector proteins of the SPI2-T3SS and *Salmonella*-induced filaments (SIFs) are formed. Loss of gene function through pseudogene formation is a unique characteristic of TS that may affect various virulence functions. Effectors of different SPIs are pseudogenes in TS. We are investigating if the observations that were made with the broad-host serovar Typhimurium (STM) are applicable to explain the pathogenesis of TS.
To analyze the intracellular lifestyle of SPA, live-cell imaging and immunohistochemistry approaches with different cell lines are mainly used. A 3D and 2D infection model with human organoids is currently being established.
We were able to provide insights into the virulence profile of SPA in unravelling unknown intracellular phenotypes in comparison with STM. SPA resides in an SCV and shows SIF-biogenesis occasionally. The SPI2-T3SS of SPA is functional in the translocation of effector proteins. With live-cell imaging approaches, intracellular movement in the host cell cytosol by SPA could be observed for the first time. This process is mediated neither by the SPI2-T3SS nor the recruitment of actin but rather by the intracellular expression of flagella.
Our results provide insights into the virulence profile of SPA in unravelling unknown intracellular phenotypes. The SPI2-T3SS in SPA is functional; however, its role for the intracellular lifestyle is still unclear. The purpose of the cytosolic motility of SPA is currently under investigation. Is intracellular flagella-dependent motility of SPA crucial for evasion of the autophagosome machinery? Does SPA utilizes this process for cell-to-cell spread and is it essential for systemic spread in the host? Furthermore, we are establishing an *ex vivo* organoid model for more *in vivo* relevant research and perform ultrastructural analyses of intracellular SPA.

Figure 1

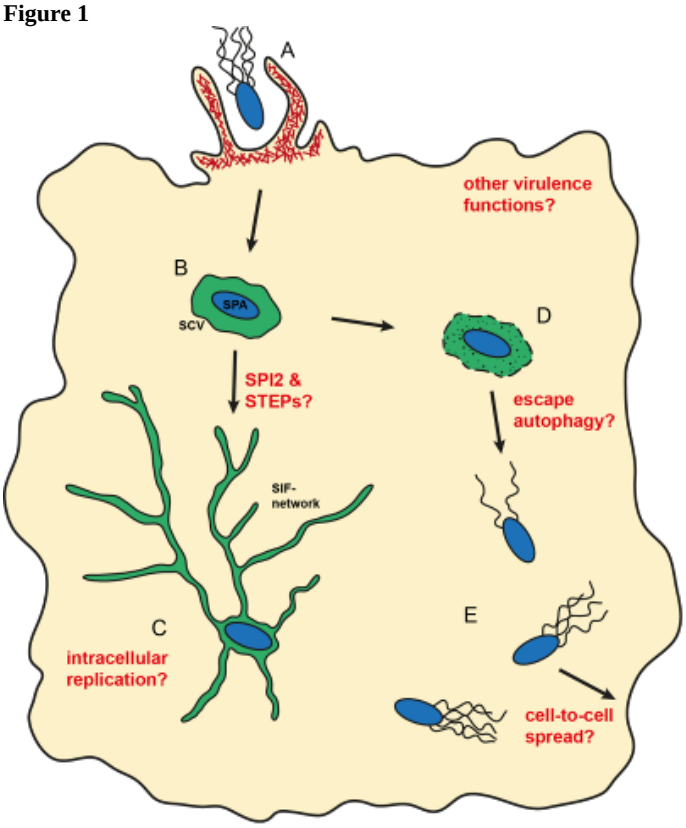

Figure 1: Model of the intracellular lifestyle of SPA. After invasion (A), SPA is present in a *Salmonella*-containing vacuole (SCV) (B). Either it induces the formation of SIFs in later stages of infection (C) or the SCV membrane gets lysed (D) and the expression of flagella is induced (E). Subsequent to flagella expression, SPA is capable of movement in the host cell cytosol. It is also possible that SPA remains dormant in stages B or D.

Presentation on Tuesday, February 26, 2019 from 9:30 – 9:40 in room Hörsaal 104.

035/MPV

RNA-seq and BIOLOG phenotype microarrays reveal profound effects of Shiga toxin phage carriage in *E. coli* K-12 MG1655

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Introduction
Shiga toxins (Stx) are encoded on temperate phages, which can infect and lysogenize susceptible bacteria, thus either transforming commensal strains into pathogens or increasing the virulence of already pathogenic bacterial hosts. In order to learn more about the impact of Stx phage on the bacterial host, we performed RNA-seq analysis with naive *E. coli* K-12 MG1655 and lysogens carrying Stx2-encoding phage from the 2011 *E. coli* O104:H4 outbreak strain and the O157:H7 strain PA8.
Methods
RNA-seq was performed with total RNA from naive and Stx2-converted MG1655 cells grown in LB at 37°C to mid-log phase. Directional cDNA libraries were sequenced on Illumina platform. Raw data were processed using READemption and differences in gene expression were determined by DESeq2. The ability of the strains to utilize carbon sources was tested in a microplate Tecan reader in growth experiments with minimal medium and using the BIOLOG PM1 MicroPlate™ Carbon sources.
Results
On average 14 million reads were sequenced per library and at least 94% of them could be mapped to the reference genomes. DESeq2 analysis revealed 63 and 94 upregulated and 70 and 72 downregulated genes in O104:H4 and PA8 lysogens, respectively, in comparison to naïve *E. coli* K-12 MG1655. Multiple genes involved in the transport and metabolism of galactose, L-lactate, ribose and sialic acid were found to be downregulated in the

lysogens. Indeed, growth experiments in minimal medium supplemented with these compounds as sole carbon sources revealed moderate to severe growth defects of the lysogens when compared to *E. coli* K-12 MG1655. Phenotype microarray experiments using the Biolog PM1 plate confirmed the growth defects of the lysogens with L-lactate and ribose and revealed a significant decrease in the cell respiration with 52 additional substrates among them all tested amino acids.

Conclusions

Our study revealed a profound impact of the Stx2-encoding phage carriage on *E. coli* K-12 MG1655 host gene expression and in particular on its carbon source utilization. The phage factors responsible for the observed phenotypes are currently under investigation.

Presentation on Tuesday, February 26, 2019 from 9:45 – 9:55 in room Hörsaal 104.

Workshop 07
Interaction of pathogens with immune cells (FG EK, FG II)
26. Febr. 2019 • 08:30-10:00

036/EKV

Candida albicans CRASP11 activates dendritic cells and induces inflammatory immune response

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Introduction

The human organism is continuously challenged by an arsenal of microorganisms. To detect and clear these invading microbes and to maintain homeostasis it is fundamental to recognize the intruders. Therefore, the human host utilizes dendritic cells (DC) that express pattern-recognition-receptors (PRR) which bind highly conserved microbial-associated-molecular-patterns (MAMPS). Dectin-1 and dectin-2 are mainly responsible for detection of fungal pathogens, like *Candida albicans*. The fungus colonizes mucosal surfaces in most humans without causing any symptoms. However, in immunocompromised individuals candida can cause superficial and life-threatening systemic infections. In this project, we characterize how candida by secreting immune modulatory CRASP11 interacts with the human immune system.

Objectives

Aim of this project is to characterize general features how *Candida albicans* by secreting CRASP11 interacts with human dendritic cells via the PRRs dectin-1 and dectin-2 and how CRASP11 alters the dendritic cell function.

Methods

C. albicans protein CRASP11 is expressed in *E. coli* and purified by affinity chromatography. Binding was analyzed by Biolayer Interferometry, Confocal Microscopy, ELISA and Western Blot Approach. For functional studies dendritic cells were generated from Buffy Coat and activation and function was assayed with Cytokine Measurements and Flow Cytometry.

Results

We identified *Candida albicans* CRASP11 as a new ligand for both dectin-1 and dectin-2 on human dendritic cells. CRASP11 is exposed on the surface of *C. albicans* and is secreted into the medium. CRASP11 binds dectin-1 and dectin-2 on monocyte-derived dendritic cells. CRASP11 binds dectin1 with a KD of 12 nM and dectin-2 with a KD of 200 nM. By binding dectin-1 and dectin-2 CRASP11 activates dendritic cells and induces the expression of activation and maturation marker CD83 and co-stimulatory CD86. CRASP11 and culture supernatant derived from intact *C. albicans* cells induce secretion of pro-inflammatory IL-1beta, IL-12, IL-6 and TNF-a. Supernatant from a CRASP11 knock out candida strain did not induce inflammatory cytokines and dendritic cell activation.

Conclusion

Candida albicans CRASP11 binds both dectin-1 and dectin-2 with high affinities on the surface of human dendritic cells. Additionally, CRASP11 induces dendritic cell activation and pro-inflammatory immune response.

Presentation on Tuesday, February 26, 2019 from 8:30 – 8:40 in room Hörsaal 007.

037/EKV

Monocytes of blood donors with chronic toxoplasmosis differ phenotypically from those of naïve controls

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Toxoplasma gondii is a common parasite of humans world-wide and can lead to severe disease predominantly but not exclusively in individuals with defective or immature immune responses. Immunocompetent hosts mount a vigorous cell-mediated immune response during infection, which does however not clear the infection thus enabling the parasite to persist presumably for the hosts" life. Remarkably, mice with chronic toxoplasmosis show increased resistance to heterologous pathogens possibly by elevated numbers of inflammatory monocytes. The impact of chronic *T. gondii* infection on human-derived monocytes is largely unknown.

Here, we determined phenotypes of monocytes from healthy blood donors either chronically infected with *T. gondii* or *T. gondii*-negative, and we unravelled monocyte responses after parasite infection *in vitro*.

CD14+ monocytes from *T. gondii*-seropositive individuals expressed significantly less CD16 as compared to those from seronegative controls. Furthermore, the percentages of CD62L+ and CD64+ monocytes were decreased or increased, respectively, in individuals with chronic toxoplasmosis as compared to controls. However, chronic toxoplasmosis was not associated with a significant shift in the distribution of classical, intermediate and non-classical monocyte subpopulations. *In vitro* infection of monocytes from both seropositive and seronegative blood donors with *T. gondii* led to an expansion of CD14 single positive classical monocytes and a decrease of CD14/CD16 double positive monocytes. Furthermore, the percentages of CCR2+ monocytes strongly decreased after infection. Only monocytes from chronically infected individuals but not those from naïve controls dose-dependently up-regulated MHC class II expression following *in vitro* infection. Finally, expression of IL-12 mRNA increased after infection with *T. gondii* particularly in cells from chronically-infected individuals, but to a lesser extent also in those from seronegative controls.

Together, these results reveal that chronic toxoplasmosis in humans may exert long-term effects on the phenotype of monocytes, i.e. cells of the innate immune system. These alterations may also have important implications for the function of these cells.

Presentation on Tuesday, February 26, 2019 from 8:45 – 8:55 in room Hörsaal 007.

038/EKV

Skin Models as test systems for antifungal innate immunity

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Research efforts in the last decades have shed light on mechanistic interactions of fungal pathogens with host organisms down to the molecular level. Although considerable progress has been made in the last decades, the function of the individual components of epithelial barriers with regard to host-microbial interaction is not fully understood.

The fungal pathogen *Candida albicans* colonizes basically all human epithelial surfaces including the skin. Under certain conditions, such as immunosuppression, invasion of the normally protected epithelia occurs. In natural skin these infections are only superficial but not much is known about defense mechanisms against *C. albicans* in subepithelial layers such as the dermis.

Using immune cell-supplemented 3D skin models we could previously define a new role for fibroblasts in the dermis and identify a minimal set of cell types for skin protection against *C. albicans* invasion (1). Dual RNA-Seq of individual host cell populations and *C. albicans* revealed that dermal invasion is directly impeded by dermal fibroblasts. They are able to integrate signals from the pathogen and CD4+ T cells and shift towards an antimicrobial phenotype with broad specificity that is dependent on TLR2 and IL-1β. By modulating these responses directly, we could show that immune modulators can be employed to activate or suppress the epidermal defense mechanisms during *C. albicans* infection. These results highlight a central function of dermal fibroblasts for skin protection opening new possibilities for treatment of infectious diseases.

1. Kühbacher A, Henkel H, Stevens P, Grumaz C, Finkelmeier D, Burger-Kentischer A, Sohn K, Rupp S. (2017) Dermal Fibroblasts Play a Central Role in Skin Model Protection against C. albicans Invasion. J Infect Dis. 215: 1742-1752.

Presentation on Tuesday, February 26, 2019 from 9:00 – 9:10 in room Hörsaal 007.

039/IIV

CD101 controls bacterial replication

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Introduction

CD101 is an Immunoglobulin-like transmembrane glycoprotein preferentially expressed by multiple myeloid and lymphoid cell populations in the gut. Its reduced expression has been recently associated with a decreased control of bacterial infections and simultaneously clinical exacerbations of immune mediated disorders including inflammatory bowel disease (IBD)[1-3].

Aims

Thus, we elucidated in this study the mechanisms by which CD101 interferes with distinct bacteria and mediates protection from IBD.

Material and methods

The expression of CD101, bacterial replication and dissemination as well as the extent of intestinal inflammation was assessed in the dextran sodium sulfate (DSS) and *Salmonella*-induced mouse models of acute and chronic colitis using conventional and conditional CD101-knockout mice crossed to the respective Cre-deleter strains. Bacterial elimination and cellular metabolism were assessed using gentamicin protection assays and high performance liquid chromatography. The expression of CD101 and the composition of the intestinal microbiota in patients with mild, moderate and severe Crohn´s disease and ulcerative colitis were compared to age- and sex-matched control individuals

Results

Different antimicrobial metabolites accumulated in CD101-expressing myeloid cells. Accordingly, various gram-negative bacteria including several *Enterobacteriaceae* were more efficiently eliminated *in vitro*. CD101-expressing mice inhibited the replication of *Salmonella* significantly better than their CD101-deficient counterparts and exhibited decreased bacterial translocation and systemic spreading. Most importantly, in IBD patients a reduced CD101-expression on peripheral and intestinal CD11b+ myeloid cells and CD4+ T lymphocytes correlated with an enhanced intestinal permeability and disease activity.

Summary

CD101 exhibits a protective role in murine enterocolitis as well as in human IBD. Our data suggest that CD101 does not only exhibit anti-inflammatory effects, but also that CD101 expression on myeloid cells is directly associated with an improved elimination of intracellular bacteria. How certain bacterial species (that might be missing in IBD patients) promote the function of CD101 and how CD101 restricts bacterial replication is part of our ongoing analyses.

Literature

[1]Schey, R., et al. *Mucosal Immunol* **9**:1205-1217.

[2]Mohammed, J. P., et al. *J Immunol* **187**:337-349.

[3]Rainbow, D. B., et al. *J Immunol* **187**:325-336.

Presentation on Tuesday, February 26, 2019 from 9:15 – 9:25 in room Hörsaal 007.

040/IIV

Neutrophils as targets for host-directed therapy against tuberculosis

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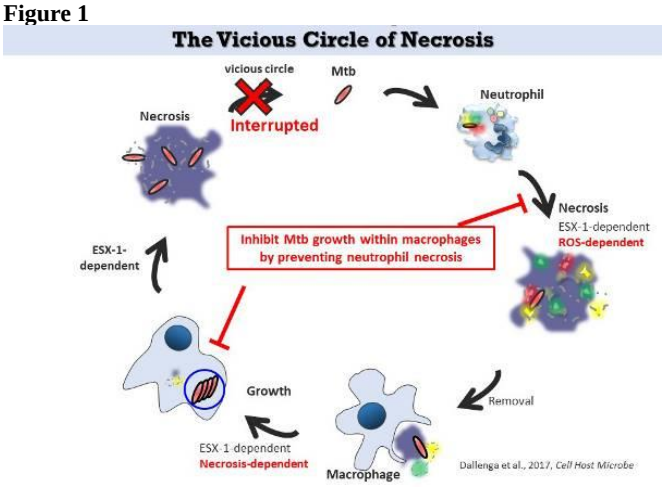
With multi drug-resistant *M. tuberculosis* variants on the rise, novel approaches to tackle the global tuberculosis epidemic are needed. Neutrophils represent the main infected cell population in lungs of patients with active tuberculosis. Here we show that *M. tuberculosis* complex strains differentially induce necrosis of human neutrophils in an ESX-1-dependent manner. Necrosis was a prerequisite for mycobacterial growth in human macrophages as well as dendritic cells after subsequent removal of infected, necrotic neutrophils. After identification of reactive oxygen species (ROS) as drivers of necrosis, we were able to prevent necrosis by pharmacological inhibition of myeloperoxidase. Thereby, we restored the capability of phagocytosing macrophages to control mycobacterial growth, an intervention that we recently successfully applied also *in vivo* in mice. This highlights neutrophil-related molecules as putative targets for host-directed therapy. Moreover, we analyze neutrophil signatures in patient"s sputum to access disease severity and treatment success and to establish point-of-care diagnostics. Taken together, host cell necrosis represents the starting point for a vicious circle leading to subsequent uptake of infected necrotic cells by other phagocytes, mycobacterial growth therein and, again, induction of host cell necrosis, a scenario that is very likely to take place in patients. Interruption of this vicious circle by inhibition of necrosis and subsequent restoration of the anti-mycobacterial functions represent an intriguing approach for host-directed therapy.

Figure 1



Presentation on Tuesday, February 26, 2019 from 9:30 – 9:40 in room Hörsaal 007.

041/IIV

Innate Lymphoid Cells in the female genital tract and their role in chlamydial infection

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Chlamydia trachomatis is the most common bacterial agent of sexually transmitted disease. In women, *C. trachomatis*-infection can cause pelvic inflammatory disease, which may result in scarring and thereby infertility. A mouse model of human *C. trachomatis* infection has been established, where infection with *C. muridarum* in female mice causes comparable symptoms and sequelae. Previous *in vivo* studies have found that the immune response to primary *C. muridarum*-infection involves the infiltration of neutrophils and monocytes starting 1 to 2 days post-infection (dpi). After about 7 dpi, T cells accumulate at the site of infection.
ILCs play an important role in tissue defense, repair and homeostasis. They are present in epithelial tissues but little is known about their distribution in the female genital tract and their potential role in genital infections. To better understand the role of ILCs in genital tract infections - with respect to immune response, bacterial clearance and tissue damage - we infected mice with *C. muridarum.* Genital tracts of naïve and infected mice were analyzed for the distribution of the ILC subsets during infection and cytokine production in response to the pathogen. The results show that in naïve wt mice cNK cells represent the most prominent cell population within the ILCs in the genital tract. ILC1 and ILC2 cells are also detectable although their total cell numbers in naïve wt mice are much lower than the cNK cell number. ILC3s are barely detectable and may not play an important role during chlamydial infection. Four days post infection the total cNK cell number increases 5.5-fold indicating that they might play a role at early stages of chlamydial infection. At later stages the numbers of cNK cells decrease and reach baseline levels at 14 dpi. Interestingly, at later time points of infection the total number of cNK cells in the genital tract increases again. Functionally, cNK cells show transient production of IFNg starting before their numerical expansion. ILC1 numbers remain stable initially. Intriguingly, until 30 dpi the total cell number of ILC1 cell increases about 10-fold. The number of ILC2 cells does not vary significantly during chlamydial infection at any time. These results identify composition of ILCs in the murine genital tract and a response to chlamydial infection. They suggest that ILCs may partake in anti-bacterial defence and tissue damage, possibly through interaction with myeloid cells.

Presentation on Tuesday, February 26, 2019 from 9:45 – 9:55 in room Hörsaal 007.

Workshop 08
Foodborne Pathogens (FG ZO, FG LM, StAG KM)
26. Febr. 2019 • 08:30-10:00

042/ZOV

Murine fecal microbiota transplantation lowers intestinal *Campylobacter jejuni* loads and pro-inflammatory immune responses in secondary abiotic mice

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Background and objectives: Conventional mice are protected from *Campylobacter jejuni* infection by their distinct host specific gut microbiota composition. We here addressed whether peroral application of murine gut microbiota might be a therapeutic measure for lowering intestinal *C. jejuni* loads in vertebrates.
Materials and methods: Secondary abiotic mice generated by broad-spectrum antibiotic treatment were perorally infected with viable *C. jejuni*. One week later mice were stably colonized with 109 *C. jejuni* per g feces and subjected to oral murine fecal microbiota transplantation (FMT) on three consecutive days by gavage.
Results: Until two weeks post FMT, mean intestinal *C. jejuni* loads declined by approximately 5 log orders of magnitude. Remarkably, following FMT mice displayed less distinct large intestinal apoptotic and T cell responses that were accompanied by lower pro-inflammatory mediator concentrations in colonic *ex vivo* biopsies as compared to *C. jejuni* colonized mice without FMT.
Conclusion: Murine fecal microbiota transplantation might be considered an effective measure to lower intestinal *C. jejuni* loads in colonized/infected vertebrates including farm animals.

Presentation on Tuesday, February 26, 2019 from 8:30 – 8:40 in room Hörsaal 005.

043/MSV

How should we compare the Antimicrobial Resistance (AMR) data between different populations in Germany? – *Escherichia coli* data from humans and different animals

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[2]*Bundesinstitut für Risikobewertung, Epidemiologie, Zoonosen und Antibiotikaresistenz, Berlin, Germany*
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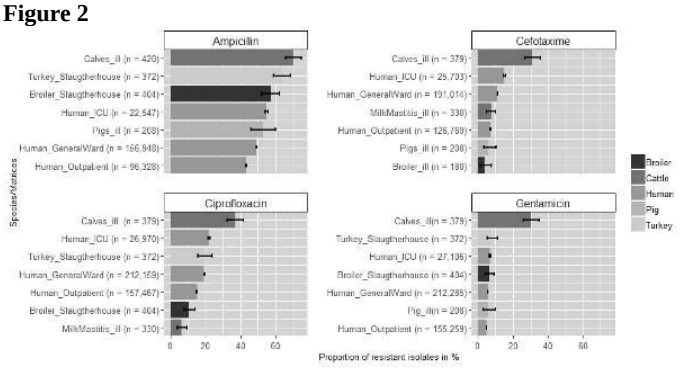
Background: German One Health Initiative (GOHI) has been initiated by four German federal institutions aiming to strengthen cooperation and information exchange between human, veterinary und environmental medicines.
Goals: Since data on resistance of *E. coli* are available for both, human and veterinary medicine, we use *Escherichia coli* (*E. coli*) data from three national surveillance and monitoring systems for AMR in humans and different animal population to describe and analyze the differences among those systems.
Materials and methods: We used *E. coli* data from 2014 to 2016 from *Antimicrobial Resistance Surveillance*–Network (ARS, Robert Koch Institute), *Zoonosis Monitoring* (German Federal Institute for Risk Assessment) and *GERM-Vet* (Federal Office of Consumer Protection and Food Safety) and compared the proportion of resistant isolates to common antimicrobial substances (ampicillin, ciprofloxacin, cefotaxime and gentamicin) in human clinical data (outpatient, general ward and ICU) with clinical and non-clinical data (slaughterhouse) from different animals by using the human EUCAST clinical breakpoints.
Results: There are substantial differences in data collection systems; therefore a certain degree of harmonization is needed. Proportion of resistant isolates differs between species and antimicrobials. Resistance against all four antibiotics is highest among ill calves (Ampicillin 70%, cefotaxime 30%, ciprofloxacin 38%, and gentamicin 30%). Low cefotaxime resistance in E. coli is to be found in commensal isolates from farm animals (<10%).
Conclusion: Both, human and animal populations show a wide variety of resistance proportions, with difference between populations and antibiotics. Thus, the interpretation of the results must be handled with caution. More detailed analyses are necessary including different types of antimicrobial substances and combinations of resistance (resistance patterns).

Figure 1

Variable	*Antibiotika-Resistenz-Surveillance* (ARS)	Zoonosis Monitoring (ZoMo)	GERM-Vet
Data	Human (clinic)	Animal (non-clinic)	Animal (clinic)
System	Surveillance	Monitoring	Monitoring
Participation	Voluntary	Mandatory	Voluntary
Population	‚one species'	‚more species'	‚more species'
Antimicrobial Susceptibility Testing (AST) panel	Not standardized	Standardized Panel	Standardized Panel
AST methods	Mostly microdilution	Bouillon - Microdilution	Bouillon - Microdilution
AST Results	'susceptible', 'intermediary', 'resistant' (or MIC)	Minimum Inhibitory Concentration (MIC)	Minimum Inhibitory Concentration (MIC)
Breakpoints	EUCAST / CLSI	EUCAST-ECOFF	CLSI

Figure 2



Presentation on Tuesday, February 26, 2019 from 8:45 – 8:55 in room Hörsaal 005.

044/LMV

Detection and isolation of virulent *Helicobacter pylori* from apparently health livestock and ability to get transmitted through milk

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Helicobacter pylori is the most important etiological agent of chronic gastritis and it is linked to the increase in the cases of human gastric cancer worldwide. The exact reservoir of *H. pylori* is not known, and the animals are implicated as a reservoir of infection. Therefore, the aims of the present study were to determine the occurrence of *H. pylori* in livestock and investigate whether the animal isolates can be transmitted through contaminated milk via the oral route causing gastric infection. Feces and milk samples from apparently healthy cattle, buffaloes, and sheep were examined by nested PCR, and the PCR positive samples were further subjected to bacterial culture followed by partial 16s sequencing of the isolates. The bacterial virulence genotype was characterized. Twenty-nine percent of the animals showed the presence of *H. pylori*, mainly the virulent *cagA+vacA+s1a m1 i1* genotype, which is known to be associated with serious diseases in humans.
Helicobacter pylori has been characterized for a long time in terms of spiral viable culturable form (SVCF), whereas, the existence of a coccoid viable non-culturable form (CVNCF) was demonstrated several years ago. The transformation from the default SVCF to CVNCF can occur under adverse conditions to facilitate long-term bacterial survival. Accordingly, UHT (ultra-high temperature) milk was inoculated with the SVCF of the fecal animal strain with the virulent *cagA+vacA+s1a m1 i1* genotype and was incubated at different temperatures for 1 to 30 days. The inoculated strain remained viable for up to 10 days at 4 °C. Increasing period of storage and or temperature led to a decrease in the number of the SVCF and occurrence of the CVNCF. The infectivity of the survived forms was determined by feeding healthy groups of laboratory mice with the contaminated UHT milk containing SVCF or CVNCF for 40 days. The gastric mucosa of the two mouse groups showed similar levels of *H. pylori* load. This highlights that *H. pylori* can persist in contaminated milk by entering a non-culturable state, which can induce gastric infection.

Presentation on Tuesday, February 26, 2019 from 9:00 – 9:10 in room Hörsaal 005.

045/KMV

Antibiotic therapy affects *Staphylococcus aureus* clonality during persistence in the airways of cystic fibrosis patients

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Introduction: Cystic fibrosis (CF) is a life limiting genetic disease, which especially affects the lungs of CF patients with chronic bacterial infection of the airways leading to lung insufficiency and early death. *Staphylococcus aureus* is one of the earliest pathogens, which can be isolated from the airways of already of CF infants and can persist for extended periods.
Objectives: It is not known, if there is an association between the occurrence of one or several *S. aureus* clones within the airways and lung function, age, gender or frequency of antibiotic treatment.
Methods: *S. aureus* isolates were collected during a prospective 21-month multicenter study from 195 CF patients from 16 CF centers in Germany and 1 center in Austria (Junge et al., PlosOne 2016). *Spa* sequence typing was performed by amplification of the variable region of protein A by PCR with ensuing sequencing. *Spa* types were assigned with the Ridom StaphType software. Additionally, age, gender, percentage of visits with antibiotics, percentage of visits with exacerbation, mean lung function (FEV1% predicted) and co-infection with *Aspergillus fumigatus* or *Stenotrophomonas maltophilia* were assessed.
Results: From 1963 specimens, 3963 different *S. aureus* isolates were cultured with a mean number of isolates of 21 per patient (range of 1-83): 1171 isolates from nasal cultures (mean 6 per patient, range 0-26), 1619 isolates from throat swabs (mean 8, range 0-32), 1103 isolates from sputa (mean 6, range 0-51) and 70 isolates from nasal lavage (only from one of the 17 centers, mean 0.3; range 0-16). 270 different *spa* types were assigned. Patients were distinguished according to special clonal groups: 1. only one clone, 2. related clones, 3. dominant clones and 4. prevalent clones.
There was a higher probability for patients' isolates belonging to group 2 "related clones", if the patients were older (p=0.033). The more often patients were treated with antibiotics, the higher was the probability for the patients to belong to group 1 "only one clone" (p=0.003), group 3 "dominant clone" (p=0.020) and group 4 "no prevalent clone" (p=0.005).
Conclusion: Our study revealed that antibiotic therapy had a strong impact on the *S. aureus* clones, which were cultured from the airways. From patients with more cycles of antibiotic treatment mostly single clones, which dominated and which did not belong to the prevalent clones were isolated indicating that these clones better resisted antibiotic therapy.

Presentation on Tuesday, February 26, 2019 from 9:15 – 9:25 in room Hörsaal 005.

046/LMV

Antimicrobial susceptibility and genetic diversity of *Listeria monocytogenes* from German food production facilities

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Listeriosis, caused by *Listeria monocytogenes (Lm)*, is one of the most severe foodborne diseases. Contamination of food products during processing is recognized as a major transmission pathway. Biocides are widely applied as disinfectants to prevent bacterial contamination during food production, storage or handling. There is concern that the widespread use of biocides may contribute to the selection and maintenance of antimicrobial resistant bacteria.
In order to deepen the knowledge on biocide and antibiotic susceptibility of *Lm*, 93 *Lm* isolates from German food production facilities sampled between 2008 and 2016 were characterized regarding i) biocide susceptibility for active substances frequently used in food production, ii) associations between reduced susceptibility to biocides and antibiotic resistance, and iii) genetic diversity.
Minimum inhibitory and bactericidal concentrations of biocides were determined by broth microdilution. Antibiotic susceptibility testing was performed using the Micronaut-S Listeria system (Merlin Diagnostika, Germany). Whole genome sequencing was carried out on an Illumina MiSeq sequencing platform. Genetic characterization included core genome analyses, multilocus

sequence typing and detection of genes related to biocide tolerance and antibiotic resistance as well as virulence factors.
15 *Lm* isolates were tolerant to benzalkonium chloride (BAC) and 13 of these isolates contained known genes contributing to biocide tolerance. Antibiotic resistance was found in 100 % (daptomycin), 76 % (tigecycline) and 8 % (meropenem) of the isolates, but was not associated with the occurrence of biocide resistance. The *Lm* isolates belonged to 23 MLST clonal complexes. The stress survival islets SSI1 and SSI2 were identified in 43 % (n=40) and 9 % (n=8) of the isolates, respectively. The *Listeria* pathogenicity island 1 was found in all isolates. Most of the BAC tolerant strains harbored internalin A (*inlA*) genes with premature stop codons or deletions (n=9).
Our study demonstrates a high genetic diversity in the *Lm* isolates including genotypes that are frequently involved in human listeriosis. Although tolerance to BAC was observed in several isolates, no association between biocide tolerance and antibiotic resistance was detected.

Presentation on Tuesday, February 26, 2019 from 9:30 – 9:40 in room Hörsaal 005.

047/KMV

Rapid and easy detection of carbapenemases in *Enterobacterales* in the routine microbiology laboratory using a new algorithm

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Question
The aim of this study was to evaluate the performance of five different carbapenemase tests and to develop an algorithm for carbapenemase detection which can easily be applied in the routine microbiology laboratory
Methods
The new immunochromatographic assays NG CARBA 5 and RESIST4 O.K.N.V., the colorimetric test β-CARBA, the carbapenemase-inactivation method (CIM) and the Cepheid Xpert Carba-R genotypic assay were challenged with a collection of 189 molecularly characterized clinical isolates with decreased susceptibility to at least one carbapenem; 146 isolates were carbapenemase producers (KPC (n=13), GES (n=2), IMI (n=9), NDM (n=29), VIM (n=49), IMP (n=9), OXA-48-like (n=36), OXA-58 (n=2)). All tests were performed from Mueller Hinton agar. For the CIM test a new protocol was developed which used zinc supplement for improved detection of MBLs (zCIM).
Results
The overall sensitivity/specificity values for carbapenemase detection were 85.6%/100% for RESIST4 O.K.N.V., 89%/100% for CARBA 5, 87.7%/100% for Xpert Carba-R, 72.6%/100% for β-CARBA and 97.3%/97.7% for the zCIM test. All KPC and OXA-48-like carbapenemases were correctly detected by all methods. Sensitivity/specificity values for NDM and VIM exceeded 90% in all tests except for β-CARBA. For IMP, sensitivity was 100% for β-CARBA and zCIM, 55.6% for CARBA 5 and 44.4% for Xpert Carba R. IMI and GES were only detected by the zCIM test (10/11; 90.9%), while OXA-58 was detected by β-CARBA and zCIM tests (2/2; 100%).
Conclusions: Our study shows that, except for the β-CARBA test, all methods show excellent sensitivity/specificity results for detection of the four most frequent carbapenemases among a collection of well characterized clinical CPE isolates. For optimized detection, we propose the following algorithm: First an immunochromatographic test or the Xpert Carba-R are used, which will detect the majority of all carbapenemases present in Germany (KPC, VIM, NDM and OXA-48-like). In case of a negative first test and a high suspicion of a carbapenemase, the zCIM is employed as a second test and will also detect the rare carbapenemases, e.g. GES or IMI. The combined use of CARBA 5 and zCIM will detect 99.3% of all carbapenemases and represents a rapid, simple and inexpensive strategy for the accurate detection of CPE in any conventional diagnostic microbiology laboratory.

Presentation on Tuesday, February 26, 2019 from 9:45 – 9:55 in room Hörsaal 005.

Workshop 10
News from Microbial Detection, Identification, Antimicrobial Susceptibility Testing and Quality Management (StAG DV, FG DKM)
26. Febr. 2019 • 13:15-14:45

048/DKMV

Evaluation of the GenoType CM *direct* assay for the direct detection of *Mycobacterium* species in patient specimens

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[2]*MVZ Labor Limbach, Studienabteilung, Heidelberg, Germany*
[3]*MVZ Labor Limbach, Molekulare Diagnostik, Heidelberg, Germany*

Introduction:
For the direct detection of TB-bacteria in clinical specimens, multiple molecular assays are available and performance data usually well documented. In contrast to this, no commercial PCR assays are available for the rapid and direct detection of non-tuberculous mycobacteria (NTM) in clinical specimens. We performed an extensive evaluation of the new commercially available GenoType CM *direct* assay for the direct detection of 15 *Mycobacterium* species, including *M. tuberculosis* complex directly in patient specimens.
Material and Methods:
Approx. 700 specimens were analyzed using the GenoType CM *direct* assay and compared to mycobacterial culture in liquid BACTEC MGIT medium and on solid culture media. Additionally, smear microscopy was taken into account. After decontamination of the specimens with the NALC-method, 500 µl of the final suspension was used for manual DNA isolation with the GenoLyse kit. The assay is based on the Hain strip technology.
Results:
Positive GenoType CM *direct* results were obtained in 46 specimens of which 20 specimens were smear positive, 25 smear negative, and for one specimen no smear data were available. Results included the following species: *M. tuberculosis* complex (n=15), *M. intracellulare* complex (n=10), *M. avium* (n=9), *M. kansasii* (n=2), *M. malmoense* (n=2), *M. xenopi* (n=1), *M. abscessus* (n=3), *M. chelonae* (n=1), and *M. celatum* (n=3). Positive cultures, but negative GenoType assays were seen in 12 specimens, all of them smear negative (*M. tuberculosis* complex (n=8), *M. intracellulare* complex (n=1), *M. avium* (n=2), and *M. gordonae* (n=1). Unspecific cross hybridization could quite often be detected in negative samples.
Conclusions:
The GenoType CM *direct* assay is a commercial assay for the direct detection of the most relevant mycobacterial species directly in patient specimens. It could be shown that this is a reliable method for the rapid detection and identification of mycobacteria in smear positive clinical specimens enabling rapid and unique results in smear positive specimens with negative TB-PCR. Although the sensitivity in smear negative specimens is lower than in smear positive specimens, positive results could be obtained in a large number of specimens with a negative smear microscopy. Yet, due to the unclear clinical relevance of detection of DNA from NTM, using PCR assays without cultures for screening of NTM is not recommended.

Presentation on Tuesday, February 26, 2019 from 13:15 – 13:25 in room Hörsaal 005.

049/DKMV

Polymyxin susceptibility testing with the VITEK® 2 in comparison to the broth microdilution method

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Question: Colistin (CS) and polymyxin B (PB) are last-line antibiotics for the treatment of MDR Gram-negative (Gr-) infections. A CLSI-EUCAST working group has recommended the broth microdilution (BMD) method according to the standard ISO 20776-1 for susceptibility testing (ST) of CS. BMD, however, is rarely used in routine laboratories. Polymyxin ST has been evaluated in semi-automated systems with conflicting results. The objective of this study was to evaluate the VITEK® 2 for testing the susceptibility of Gr- pathogens to CS and PB.
Methods: The study comprised 64 were Enterobacterales strains and 39 non-fermenting bacteria. 63 strains were collected during various resistance surveillance studies in Germany, and 40 strains were provided by the German National Reference Laboratory for Multidrug-Resistant Gram-negative Bacteria (Bochum, Germany). BMD MICs of CS and PB were determined according to the ISO standard on panels prepared in-house. ST of CS and PB with the VITEK® 2 was carried out according to the manufacturers" instructions (bioMérieux, Marcy l"Etoile, France). VITEK® 2 and BMD ST were done in parallel. Two trials (A and B) were performed. MICs of CS were interpreted by EUCAST breakpoints (BP; S, MICs ≤2 mg/l; R, MICs >2 mg/l). BP of PB for *Acinetobacter* spp. and *P. aeruginosa* were those set by the CLSI (S, MICs ≤2 mg/l; R, MICs ≥4 mg/l). For the purpose of the study, these BP we also applied to Enterobacterales. Categorical agreement (CA), very major errors (VMEs) and major errors (MEs) were calculated.
Results: When the BMD method was used, 41/64 (64.0%) of the Enterobacterales strains and 11/39 (28.2%) of the non-fermenting bacteria were CS-resistant. The VITEK® 2 tested all CS-susceptible strains (n=51) correctly, but failed to detect 16 CS-resistant strains in either trial. Five CS-resistant strains in trial A and six in trial B, were detected solely by PB. The CA with BMD for VITEK® 2 was appr. 80% with CS (77% for Enterobacterales isolates; 82-85% for non-fermenting bacteria) and about 85% for PB (84% for Enterobacterales isolates; 87-90% for non-fermenting bacteria). There were no MEs (false resistance), but the rate of VMEs (false susceptibility) was about 20% with CS and 12-15% with PB. Large differences in the VME rates between species, however, were evident (Tab. 1).
Conclusion: Using the VITEK® 2, we observed marked differences among species concerning the correct discrimination of CS-resistant and -susceptible isolates.

Figure 1

Table 1: Agreement between results obtained by BMD and VITEK® 2 for specific Gram-negative organisms achieved with colistin and polymyxin B

Organism (No. of strains)	Trial	Test system	Colistin				Polymyxin B			
			Result nS	Result nR	%CA	%VME	Result nS	Result nR	%CA	%VME
Acinetobacter spp. (18)	A	BMD	14	4	94.4	5.6	14	4	94.4	5.6
		VITEK® 2	15	3			15	3		
	B	BMD	14	4	94.4	5.6	15	3	100	0
		VITEK® 2	15	3			15	3		
P. aeruginosa (18)	A	BMD	11	7	72.2	27.8	13	5	77.7	22.2
		VITEK® 2	16	2			16	2		
	B	BMD	11	7	66.7	33.4	14	4	77.8	16.7*
		VITEK® 2	17	1			16	2		
E. coli (9)	A	BMD	6	3	100	0	6	3	100	0
		VITEK® 2	6	3			6	3		
	B	BMD	6	3	100	0	6	3	100	0
		VITEK® 2	6	3			6	3		
E. cloacae complex (15)	A	BMD	4	11	26.7	73.3	4	11	53.4	46.6
		VITEK® 2	15	0			11	4		
	B	BMD	4	11	26.7	73.3	4	11	53.4	46.6
		VITEK® 2	15	0			11	4		
K. pneumoniae (30)	A	BMD	6	24	90.0	6.7*	6	24	93.3	6.7
		VITEK® 2	8	21			8	22		
	B	BMD	6	24	90.0	6.7*	6	24	93.3	3.3*
		VITEK® 2	8	21			7	22		
All (103)§	A	BMD	51	52	79.6	19.4*	52	51	85.4	14.6
		VITEK® 2	71	31			67	36		
	B	BMD	51	52	78.6	20.4*	55	48	86.4	11.7*
		VITEK® 2	72	30			66	35		

*The VITEK® 2 did not produce a result in all cases. §Data from species with low numbers of tested strains is not presented. nS, number of susceptible strains; nR, number of resistant strains.

Presentation on Tuesday, February 26, 2019 from 13:30 – 13:40 in room Hörsaal 005.

050/DKMV

Fourier-transform infrared (FTIR) spectroscopy for species identification and strain typing of clinical *Enterobacter cloacae* complex isolates

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Background: *Enterobacter (E.) cloacae* complex has emerged as an important pathogen frequently involved in nosocomial infections and outbreaks. Fast and reliable strain typing methods are crucial for surveillance and outbreak analysis to detect pathogen reservoirs and transmission routes. Fourier-transform infrared (FTIR) spectroscopy is a spectrum-based technique which can be used for identification and strain typing of bacterial isolates.
Objective: The aim of the study was to evaluate FTIR spectroscopy for species identification and strain typing of clinical *E. cloacae* complex isolates. Whole genome sequencing (WGS) was used as the reference method.
Material and Methods: *E. cloacae* complex isolates (n = 239) were recovered from weekly microbiological patient screenings on a neonatal unit. All isolates were analyzed by FTIR spectroscopy on an IR Biotyper system. The first isolate of every patient as well as isolates that showed low similarity to other isolates were analyzed by WGS (n = 53) followed by multi-locus sequence type (ST) extraction, SNP-based phylogenetic analysis, and average nucleotide identity (ANI) analysis for species attribution.
Results: WGS analysis showed that the strains belonged to 6 different species within the *E. cloacae* complex comprising 17 sequence types, each of which represented a phylogenetic cluster. Clustering of FTIR spectra of sequenced isolates based on their Euclidean distance showed low concordance with the species (Adjusted Rand Index [ARI] = 0.300) and acceptable concordance with the ST (ARI = 0.717). Training of an artificial neural network (ANN) with all FTIR spectra and cross-validation of the ANN markedly improved the grouping of the isolates with respect to species and ST. However, the discriminatory power of the ANN was reduced, when confronted with spectra from species or ST not contained in the training data. The number of putative transmission events based on the room occupancy data were drastically reduced when typing results were included in the analysis.
Conclusions: FTIR spectroscopy is a promising method for species identification and strain typing of clinical *E. cloacae* complex isolates. Discriminatory power can be significantly improved by implementing an ANN for spectrum analysis. Due to its low costs and fast turnaround times, FTIR presents a valuable tool for real-time surveillance, which can be complemented by subsequent WGS if high-resolution typing is required.

Presentation on Tuesday, February 26, 2019 from 13:45 – 13:55 in room Hörsaal 005.

051/KMV

Sepsis caused by enterobacteria: application of MALDI-TOF MS for the rapid detection of ESβL/AmpC and carbapenemases

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Question
Enterobacteria are common causative agents of sepsis. β-lactam antibiotics are widely used in empiric therapy, but the spread of cephalosporinase- (ESβL, AmpC) and carbapenemase-producers threats the effectiveness of the treatment. Early detection is crucial for the clinical outcome. Laboratory methods currently available are either costly, or slow, and not applicable directly to positive blood cultures.
In this study we developed a"full MALDI based" approach for the rapid detection of cephalosporinase- and carbapenemase-producing enterobacteria directly from positive blood culture bottles, applying a combination of the most recent applications of the MALDI Biotyper system (Bruker Daltonik),species identification, subtyping for KPC-producing *Klebsiella pneumoniae*, followed by

the evaluation of the carbapenemase- and cephalosporinase-production by hydrolysis assays.

Methods

N=92 blood cultures positive for enterobacteria (different genera and species) were included. The bacterial pellet obtained by the Sepsityper kit was used for the species identification, and for the simultaneous subtyping of KPC-producing *K. pneumoniae* by the MALDI Biotyper system. The residual pellet was used to investigate the carbapenemase- and cephalosporinase production by MBT STAR-Carba and MBT STAR-Cepha hydrolysis assays. The results of the new approach were compared with phenotypical reference tests (synergy test with inhibitors).

Results

92/92 isolates were identified at species level at high confidence level, 11/12 (91.3%) K. *pneumoniae* KPC+ strains were detected by MALDI subtyping.

STAR-Carba assay resulted positive for 16/16 carbapenemase-producing (n=12 *K. pneumoniae* KPC+, n=1 *E. coli* KPC+, n=3 *K. pneumoniae* MβL+), and negative for the remaining n=76 strains.

STAR-Cepha assay resulted positive for 16/16 ESβL-producing strains, 3/3 AmpC-producing strains, and for all the carbapenemase-producers but 1 *K. pneumoniae* MβL+, but negative for the remaining n=57 strains (*wild-type*, penicillinase- or constitutive AmpCs-producers).

Conclusions

The "full MALDI based" approach proved to be reliable and accurate to detect the most relevant enterobacterial resistances against β-lactam antibiotics. Moreover it is very rapid, enabling to deliver a conclusive result after 30 min-2 h starting from the positive blood culture bottle. The ease of use and the analysis of all assays on the same platform make this approach suitable for the implementation into routine workflow.

Presentation on Tuesday, February 26, 2019 from 14:00 – 14:10 in room Hörsaal 005.

052/DKMV

Influence of Decontamination Techniques on *Brucella* Identification by MALDI-TOF MS and NGS

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Question: MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) and NGS (next generation sequencing) have gained increasing importance for rapid bacterial species identification in routine diagnostic laboratories. As mass spectrometry and DNA sequencing devices are usually located outside of biosafety level 3 laboratories, complete inactivation of highly pathogenic bacteria is indispensable. However, altered mass spectra and NGS profiles are expected depending on the inactivation technique applied. We assessed the impact of various inactivation techniques on bacterial identification by MALDI-TOF MS and NGS.

Methods: Ethanol (EtOH), 2-propanol (2-Prop), trifluoroacetic acid (TFA), heat, hydrogen peroxide (H2O2), peracetic acid (PAA), formaldehyde (FA), glutaraldehyde (GA) and sodium hypochlorite (NaClO) were applied to kill *Brucella*. After inactivation, samples were prepared for mass spectrometry using ethanol/formic acid extraction and analyzed on a Bruker Microflex™ LT. Whole genome sequencing was performed on an Illumina MiSeq™.

Results: Hierarchical analysis of mass spectra showed two major clusters containing EtOH, 2-Prop, heat and TFA on the one hand and H_2O_2 and PAA on the other hand. The use of GA und NaClO resulted in mass spectra of insufficient quality, which did not match these clusters. Using Bruker Daltonics' as well as our in-house database, *Brucella* was reliably identified at genus level following EtOH, 2-Prop, heat and TFA treatment.

Treatment with EtOH, heat and TFA had no negative impact on NGS-based *Brucella* identification. DNA quantity and NGS quality were generally lower for samples treated with FA and NaClO. Nevertheless, mapping of sequence reads against respective *Brucella* reference sequences covered >98% of the genome regardless of the inactivation technique applied. This allowed successful 16S rRNA classification at genus level as well as MLST-based species identification.

Conclusion: Choosing a suitable inactivation technique is crucial for successful identification of highly pathogenic bacteria by MALDI-TOF MS, as it may significantly influence quality and information content of mass spectra. Therefore, methods used to create reference databases and those applied in routine diagnostics should be identical and must be carefully validated to allow reliable identification. In contrast, NGS based analyses are less prone to misidentification independent of the inactivation technique applied.

Presentation on Tuesday, February 26, 2019 from 14:15 – 14:25 in room Hörsaal 005.

053/DKMV

Antimicrobial kINPen09 and MiniMIP plasma treatment of *Candida albicans* biofilms: A comparison.

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Introduction: The major form of microbial life is the biofilm, where cells live in dense communities and can survive lethal conditions for planktonic cells. Biofilms are microbial communities that adhere to surfaces, produce continuously toxins and enzymes. They also contaminate food, which is an increasing industrial problem. An innovative method for combating biofilms is the treatment with non-thermal atmospheric pressure plasmas. The choice of plasma source is particularly important. For this purpose, two atmospheric pressure plasmas based on different physical principles were investigated for their ability to combat *Candida albicans* biofilms.

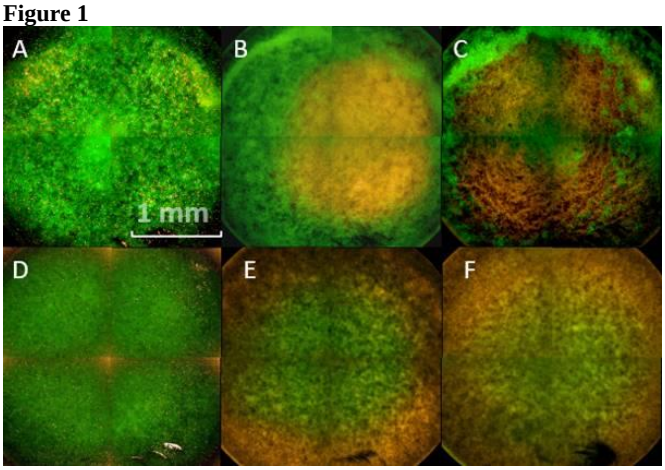
Targets: Microbial communities represent an increasing problem in medicine but also in the food industry. An efficient and rapid removal of biofilms becomes therefore increasingly important. With the aid of the kINPen09 and the MiniMIP plasma devices, decisive new findings on the effects of plasma on *C. albicans* biofilms could be obtained.

Methods: *C.albicans* biofilms were cultivated in 96-well plates for 24 h and treated with a radiofrequency-plasma jet kINPen®09 or a microwave-induced plasma torch MiniMIP for different times. After treatment, thermal images were taken and the proliferation, vitality and viability of the cells were investigated by using colony forming unit (CFU), fluorescence- and XTT- assay. To visualize the influence of plasma fluorescence microscopy, confocal laser scanning and atomic force microscopy were performed. Optical emission spectroscopy (OES) was used to illustrate the differences in plasma composition.

Results: Microbiological assays are shown in Table.1. Fluorescence microscopy is shown in Fig. 1. AFM shows different morphological changes of the cells between both plasma devices. By OES, differences in the plasma gas composition of the two plasma devices were detected.

Summary: This study shows that depending on the respective issue it is important which type of plasma source should be used. It was shown that the MiniMIP plasma caused stronger inactivation effects within a shorter plasma treatment time than the kINPen®09 plasma. Additionally, it had been shown that there are not only differences in the three-dimensional impact on the biofilms but also on the cell morphology and the composition of the plasma gas itself due to the type of plasma generation.

Figure 1



Presentation on Tuesday, February 26, 2019 from 14:30 – 14:40 in room Hörsaal 005.

Workshop 11
Multi Drug Resistance: When, Who and Where does that take us? (FG PR, StAG HY)
26. Febr. 2019 • 15:15-16:45

054/PRV

Report of the National Reference Laboratory for Multidrug-Resistant Gram-negative Bacteria on Carbapenemases in Germany in 2018

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Question: Multidrug-resistance in *Enterobacterales*, *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 still challenging for the microbiological laboratory.

Material/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 and WGS methods allow the detection of still unknown β-lactamases.

Results: A total of 5238 isolates were investigated for carbapenemases in the National Reference Laboratory in 2018 until October 11th. Specimen sources were mostly rectal swabs (24.1 %), urinary (15.9 %) and respiratory samples (11.9 %). Carbapenemases were found in 1455 *Enterobacterales* strains, 407 of *A. baumannii* and 372 of *P. aeruginosa*. The most frequent carbapenemases in *Enterobacterales* were OXA-48 (n = 447), VIM-1 (n = 262), NDM-1 (n = 156), KPC-2 (n = 142), NDM-5 (n = 99), KPC-3 (n = 60), OXA-244 (n = 57), OXA-181 (n = 55), OXA-232 (n = 34), NDM-7 (n = 13) and VIM-4 (n = 12). GIM-1, OXA-162, VIM-2, IMI-1, IMI-2, GES-5, and and others were found in less 10 isolates each. In *P. aeruginosa,* VIM-2 was the most frequent carbapenemase (n = 252), followed by GIM-1 (n = 29), VIM-1 (n = 18), IMP-7 (n = 18) and NDM-1 (n = 14). VIM-4, GES-5, VIM-11, IMP-13, VIM-17, IMP-1, IMP-28 and others were found in less than 10 isolates each. OXA-23 was the most frequent carbapenemase in *A. baumannii* (n = 3013), followed by OXA-72 (n = 60) and NDM-1 (n = 16). GIM-1, OXA-143, OXA-58 and others were found in less than 10 isolates each.

Conclusions: A variety of different carbapenemases is detected in Germany. The molecular epidemiology in Germany differs significantly from observations made in other countries like Greece, Italy or the USA with a predominance of OXA-48. Compared to previous years, variants of OXA-48 are again on the rise, together with variants of NDM and VIM.

Presentation on Tuesday, February 26, 2019 from 15:15 – 15:25 in room Hörsaal 010.

055/PRV

The importance of adjusting for Enterococcus species when assessing the burden of vancomycin resistance. A cohort study including over 1,000 cases of enterococcal bloodstream infection.

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Question
Infections caused by vancomycin-resistant enterococci (VRE) are on the rise worldwide. Few studies have tried to estimate the mortality burden as well as the financial burden of those infections and found that VRE are associated with increased mortality and higher hospital costs. However, it is unclear whether these worse outcomes are attributable to vancomycin resistance only or whether the enterococcal species (*Enterococcus faecium* or *Enterococcus faecalis*) play an important role. We therefore aimed to determine the burden of enterococci infections attributable to vancomycin resistance and pathogen species (*E. faecium* and *E. faecalis)* in cases of bloodstream infection (BSI).
Methods
We conducted a retrospective cohort study on patients with BSI caused by *Enterococcus faecium* or *Enterococcus faecalis* between 2008 and 2015 in three tertiary care hospitals. We used univariate and multivariable regression analyses to compare risk factors for in-hospital mortality and length of stay. We calculated total hospital costs
Results
Overall, we identified 1,160 consecutive cases of BSI caused by enterococci: 596 (51.4%) cases of *E. faecium* BSI and 564 (48.6%) cases of *E. faecalis* BSI. 103 cases of *E. faecium* BSI (17.3%) and 1 case of *E. faecalis* BSI (0.2%) were infected by vancomycin-resistant isolates. Multivariable analyses revealed (i) that in addition to different underlying diseases *E. faecium* was an independent risk factor for in-hospital mortality and prolonged hospital stay and (ii) that vancomycin-resistance did not further increase the risk for the described outcomes among *E. faecium*-isolates. However, the overall hospital costs were significantly higher in vancomycin resistant *Enterococcus faecium*-BSI cases as compared to vancomycin susceptible *Enterococcus faecium*- and *Enterococcus faecalis*-BSI cases
Conclusions
Our data indicates that in-hospital mortality and infection-attributed hospital stay in enterococci BSI might rather be influenced by Enterococcus species and underlying diseases than by vancomycin resistance. Future studies should consider adjusting for Enterococcus species in addition to vancomycin resistance in order to provide a conservative estimate for the burden of VRE infections.

Presentation on Tuesday, February 26, 2019 from 15:30 – 15:40 in room Hörsaal 010.

056/PRV

Molecular surveillance of carbapenemase-producing *Pseudomonas aeruginosa* at three medical centres in Cologne, Germany

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Objectives:
P. aeruginosa is a leading nosocomial pathogen. Resistance to carbapenems is either mediated via efflux pumps, loss of porin or carbapenemases. Carbapenemase-producing *P. aeruginosa* (CPP) strains are known to cause outbreaks and harbour a genetic reservoir. However, little surveillance data is available at the local level. The study was conducted in three medical centres in Cologne (one tertiary and one secondary care centre and one children hospital, 1500 beds).

Methods:
Identification and susceptibility testing were performed with VITEK 2 system (bioMérieux). *P. aeruginosa* non-susceptible to piperacillin, ceftazidime, cefepime, imipenem, meropenem and ciprofloxacin (4MRGN according to the German classification) isolated from clinical and screening specimens from 2015 to 2017 were analysed. A two-step algorithm to detect carbapenemases was performed (phenotypic tests followed by PCR and sequencing). In case of contradictory results, isolates were sent to the German National Reference Centre. Inhibition zone diameters were determined with imipenem alone and in combination with (a) 930 mg EDTA or (b) 4000 mg Cloxacillin (difference of (a) ≥ 5 mm or (b) < 6 mm resp. was considered to be indicative of a carbapenemase). Subsequently CDT-positive isolates were further analysed by PCR and sequencing. CPP isolates were further genotyped by RAPD.

Results:
Seventy first 4MRGN-*P. aeruginosa* isolates were available for further analysis, of which 21 were CPP as follows: $bla_{\text{VIM-1}}$ (n=2), $bla_{\text{VIM-2}}$ (n=17), bla_{IMP} (n=1) and bla_{NDM}/bla_{GES} (n=1). 15 CPP were hospital-acquired (specimen collected more than two days after admission), mostly from intensive care units (80%) and nearly all (except one) from the tertiary care centre. RAPD typing revealed two different clusters of VIM-2-producing *P. aeruginosa* containing 13 and 2 isolates each. However, using conventional epidemiology, we were only able to confirm three patient-to-patient transmissions and one room-to-patient transmission.

Conclusion:
These data give insight into the epidemiology of CPP in three centres in Germany over a period of three years. Carbapenemases are a relevant resistance mechanism in 4MRGN-*P. aeruginosa*, VIM-2 being the most common carbapenemase. Genetically related strains seem to be endemic in the region. The results support the need for a local molecular surveillance system.

Presentation on Tuesday, February 26, 2019 from 15:45 – 15:55 in room Hörsaal 010.

057/PRV

Plasmid-mediated transmission of KPC-2 carbapenemase in Enterobacteriaceae in critically ill patients

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Background: Carbapenem-resistant Enterobacteriaceae (CRE) cause health care-associated infections worldwide that are of severe concern due to limited treatment options.

Objectives: We characterised outbreak isolates of KPC-2-producing CRE in critically ill patients. Transmission of a promiscuous plasmid across different genera of bacteria was likely the cause of emergence of CRE pathogens in two hospitals.

Materials and methods: Genetic relationship of the isolates was analyzed by rep-PCR (DiversiLab) and pulsed-field gel electrophoresis (PFGE) using XbaI-restricted whole genomic DNA. Transfer of carbapenem resistance was tested in liquid mating assays. Resistance genes, plasmid content and plasmid sizes of clinical isolates and transconjugants were determined by PCR and S1-nuclease restriction plus PFGE at first, and in further details by whole genome sequencing (WGS, Illumina, MiSeq) and plasmid reconstruction (de novo assembly).

Results: Eleven isolates (8 *Citrobacter freundii*, 2 *Klebsiella oxytoca*, 1 *Escherichia coli*) were obtained from seven patients within the six months of the outbreak in 2016. One patient developed a CRE infection while the other six patients were CRE colonized. Six of the seven patients carried the same *C. freundii* clone; one *K. oxytoca* clone was found in two patients and one patient carried *E. coli* and *C. freundii*. Further analyses confirmed the presence of a conjugative, *bla*KPC-2-carrying 70 kb-IncN plasmid in all isolates of *C. freundii* and *E. coli* and an 80 kb-IncN plasmid in the *K. oxytoca* clone. The transconjugants harboured either the 70 kb or 80 kb plasmid with *bla*KPC-2, embedded within transposon variant Tn*4401g*, and further β-lactamase genes (*bla*OXA-1 and *bla*TEM-1) and in part PMQR genes *aac(6")Ib-cr* and *qnrB2*. WGS and downstream bioinformatics analyses of all plasmid sequences showed an almost perfect match in comparison with a *bla*KPC-2-carrying plasmid of a large outbreak in another German hospital two years earlier [1]. Differences in plasmid sizes and open reading frames point to the presence of inserted mobile genetic elements.

Conclusions: There are only a few outbreak reports worldwide on the transmission of *bla*KPC-2-carrying plasmids across different bacterial genera. Our data suggest a supra regional spread of *bla*KPC-2-carrying IncN-plasmids in Germany harbouring the Tn*4401g* isoform.

Reference:
[1] Yao Y et al. Genome Announc. 2014 Nov 13;2(6). pii: e01157-14. doi: 10.1128/genomeA.01157-14.

Presentation on Tuesday, February 26, 2019 from 16:00 – 16:10 in room Hörsaal 010.

058/HYV

Quantifying the contribution of the aqueous environment in the hospital for colonization of immunocompromised patients with *Pseudomonas aeruginosa* using whole-genome sequencing

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Objectives

Pseudomonas (P.) aeruginosa is an important pathogen of nosocomial infections with high mortality rates especially in immunocompromised patients. Infections with this pathogen are often preceded by colonization of the patient"s mucosal surfaces. *P. aeruginosa* can be found in the aqueous environment of the patient in the hospital (e.g. in sinks, shower drains, and toilets), but the contribution of these reservoirs to colonization is only partially understood. Our study aims at quantifying *P. aeruginosa* colonization with isolates originating from the hospital environment and thereby determining the risk for colonization by exposure to sanitary facilities in the hospital.

Methods

Weekly rectal screening swabs were taken from patients in the hematology/oncology department as well as weekly specimens from the patients' environment. Patients were retrospectively identified, in which *P. aeruginosa* was found after preceding negative results, thereby indicating new colonization. Whole-genome sequencing (WGS) and phylogenetic SNP analysis was used to compare these patient isolates to all environmental isolates that were found in the patients" bathrooms in the preceding three months before occupancy by the respective patient.

Results

During an 11 month period, 52 patients were identified that became colonized with *P. aeruginosa*. Occupancy data identified 239 environmental isolates that represented candidates for transmission. WGS and phylogenetic analysis grouped all isolates into 27 clusters (comprising 2-54 isolates) and 24 singletons. 24 (46.2 %) patient isolates clustered with one or more environmental isolate. However, adding room occupancy data to the SNP analysis, a transmission from the environment was shown only in 6 (11.5 %) cases. Interestingly, 4 of these 6 isolates exhibited a multi-resistant phenotype characterized by the expression of a metallo-betalactamase.

Conclusions

Our study indicates that the sanitary installations play a minor role as a source of *P. aeruginosa* transmission and subsequent colonization of immunocompromised patients. Other transmission routes or selection within the patient seem to be more important for colonization with *P. aeruginosa* in this cohort. However, it is worrisome that transmission capability seems to be higher in certain multidrug-resistant clones.

Presentation on Tuesday, February 26, 2019 from 16:15 – 16:25 in room Hörsaal 010.

059/PRV

Thinking Outside the Box: Potential Association of Carbapenem Resistance with Nurse Density in Europe – A 30 Country Observational Study

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Background: Antimicrobial resistance (AMR) is one of the biggest contemporary public health threats. Estimations reveal a worldwide increase in resistance, which will cause higher rates of therapy failure, mortality and growing healthcare expenditure.

Objectives: The aim was to investigate potential associations between healthcare workforce density and AMR, with a focus on carbapenem resistant gramnegative species (CRGN).

Methods: A 30 European country database observational study was conducted. The six-year prevalence of CRGN (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter* spp.) in addition to methicillin resistant *Staphylococcus aureus* (MRSA) was determined based on 708,892 invasive isolates from the EARS-net surveillance program [1]. Bivariate correlation and multivariate regression analysis (log-linear model) were performed to identify associations between CRGN and healthcare workforce (density of nurses and of physicians), in addition to factors previously shown to be associated with AMR.

Results: Differences in CRGN prevalence were found across the 30 countries and regional patterns were identified. Our data revealed an association of nurse density with CRGN, in contrast to physician density with CRGN or nurse density with MRSA. We obtained a multivariate model of selected factors that explained ca. 83% of total CRGN variance with nurse density being a significant contributor to variance explanation. This contribution was higher than the contribution of outpatient antimicrobial use. The model was used to estimate CRGN prevalence in Switzerland and Turkey (both not included in the database used) and showed relative accuracy as compared to CRGN prevalence data in these two countries [2].

Conclusion: This study contributes new knowledge to the AMR debate by identifying a potential association between nurse density and CRGN. Including nurses in future AMR policies might open new opportunities for interventions to respond to the spread of carbapenem resistance in healthcare facilities. This is of special importance since nurse aspects are largely missing in the EU Commission's action plan on AMR [3].

[1] ECDC Surveillance Atlas of Infectious Diseases, 2016.

[2] Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR), WHO European Region, 2016.

[3] A European One Health Action Plan against Antimicrobial Resistance (AMR), 2017.

Presentation on Tuesday, February 26, 2019 from 16:30 – 16:40 in room Hörsaal 010.

Workshop 12
Host – Pathogen Interactions (FG MP)
26. Febr. 2019 • 15:15-16:45

060/MPV

Identification of host-pathogen responses in avian and murine whole blood model

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Blood stream infections belong to the most severe forms of infections and are commonly associated with high mortality rates. Although these infections are common in certain clinical settings, the interactions between circulating immune cells and pathogens in peripheral blood are not fully understood.

Within the scope of an interdisciplinary research project on combating infectious blood stream diseases of humans and animals, murine and avian whole blood infection assays have been established to identify the interplay of peripheral immune cells with divergent groups of microbes like *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*

Using flow cytometry, microbiology and microscopy, leukocyte numbers, cell-pathogen contacts and pathogen survival were determined *ex vivo* in peripheral blood of two mouse strains BALB/c, C57Bl/6 and two chicken lines differing in egg-laying performance.

Preliminary results demonstrated differences between two mouse strains with respect to the stability of granulocytes and their interactions with C. *albicans*, with greater granulocytes stability in BALB/c mice. Less killing of *C. albicans* in murine blood was observed as compared to avian blood. Chicken-line dependent differences regarding pathogen survival, fate of immune cells and

interactions between different pathogens and immune cells were noticed. Comparing different leukocyte subsets, the bacterial pathogens were found to be most commonly associated with monocytes while *C. albicans* associated to both monocytes and granulocytes.
Thus, we identified both species and strain specific differences in the interaction of peripheral leukocytes with model pathogens. While the underlying mechanisms yet remain to be determined, these results highlight the importance of host phylogeny and genetics for the interaction with pathogens, with possible consequences for the transferability of results obtained in a distinct host strain to general populations or other species.

Presentation on Tuesday, February 26, 2019 from 15:15 – 15:25 in room Hörsaal 104.

061/MPV

Calcium-dependent ceramide release in response to *N. meningitidis* infection

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Introduction: *Neisseria meningitidis* (Nm) is a major cause of bacterial meningitis and sepsis. A critical step in the pathogenesis of meningococcal meningitis is the interaction of the pathogen with endothelial cells forming the blood-brain barrier. Recent published data proved that *N. meningitidis* can activate the enzyme acid sphingomyelinase (ASM) (1), a lipid hydrolase that cleaves sphingomyelin into ceramide, in brain endothelial cells.
Aim: The aim of this study was to identify the contribution of meningococcal factors that activate the ASM/ceramide system. We hypothesized that the meningococcal pilus induced Ca^{2+} flux in human brain endothelial cells might trigger lysosomal exocytosis, which in turn results in translocation of the ASM to the plasma membrane of the infected host cell.
Methods: Human brain microvascular endothelial cells (HBMEC) were exposed to a highly piliated *N. meningitidis* isolate (Nm 8013), an isogenic nonpiliated PilE-deficient mutant or purified pili. Surface display of ASM, ceramide and LAMP1 was quantified by flow cytometry. ASM surface activity was analyzed using a commercial available ASM activity assay kit. Cytosolic Ca^{2+} concentrations were determined using the Fluo-8™ calcium indicator. Ceramides were visualized by *d*STORM.
Results: Here we demonstrate that *N. meningitidis* strain 8013 was effective at inducing ceramides on HBMEC, whereas the isogenic pilus-deficient meningococcal mutant *Nm* 8013Δ*pilE* failed. Because they could be abrogated by amitriptyline, increased surface ceramide amounts reflected increased ASM activity of the translocated enzyme. In addition to live, piliated bacteria, treatment of HBMEC with purified pili also triggered transient surface display and activation of the enzyme followed by ceramide release. In parallel, we observed that purified pili induced transient increases in cytosolic Ca^{2+} levels in infected cells and triggered lysosomal exocytosis as detected by exposure of LAMP1. Pretreatment of cells with 2-APB, a IP_3 receptor blocker, showed a decrease of ASM as well as LAMP1 surface levels and ceramide amount on infected HBMEC.
Summary: These results demonstrate that the meningococcal pilus contributes to activation of the ASM/ceramide system and indicate that pilus–induced translocation of ASM to the plasma membrane is mediated by exocytosis of lysosomes, which is dependent on intracellular Ca^{2+} release.

References
(1) Simonis *et al PLoS Pathogens,* 10:e1004160 (2014).

Presentation on Tuesday, February 26, 2019 from 15:30 – 15:40 in room Hörsaal 104.

062/MPV

scSLAM-seq reveals early events of infection with unprecedented temporal resolution

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Question. Over the last years, single-cell RNA-seq (scRNA-seq) has emerged as a powerful tool to profile cellular physiology and identities. Applied to infection, we pioneered the use of scRNA-seq to investigate the heterogeneity in the host response of mouse bone-marrow-derived macrophages (BMDM) to *Salmonella* [1]. However scRNA-seq, only measures the total RNA abundances in a single-cell and inherently precludes to look at fast transcriptomics changes happening at the onset of infection. Regulatory changes happening at early time points of infection are currently unknown due to the long half-life of mammalian host mRNAs.
Methods. In this abstract, we pioneer a new single-cell sequencing approach to directly quantify and analyze not only the total RNA levels but also the real-time kinetics of transcriptional activity for thousands of genes in individual cells. We demonstrate the power of our approach by studying the earliest host response fibroblasts to lytic cytomegalovirus (CMV) infection and BMDMs to *Salmonella*.
Results. We demonstrate that scSLAM-seq provides high quality RNA profiles for "total", "new" and "old" RNA (>4,000 genes per cell). This enables to attribute trajectories of transcriptional responses to individual cells and provides a temporal dimension to scRNA-seq. Thereby, scSLAM-seq recovers the earliest virus and bacteria-induced changes in transcriptional activity so far hidden within intercellular heterogeneity.
Conclusion. Metabolic labeling using 4sU is applicable to all cell types and major model organisms including vertebrates, insects, plants and yeast. Though we envision that scSLAM-seq will be become a corner stone technology to look at early infection events.
[1] Saliba AE, Li L, Westermann AJ, Appenzeller S, Stapels DA, Schulte LN, Helaine S, Vogel J (2016) Single-cell RNA-seq ties macrophage polarization to growth rate of intracellular Salmonella. *Nature Microbiology* 2:16206

Presentation on Tuesday, February 26, 2019 from 15:45 – 15:55 in room Hörsaal 104.

063/MPV

Rapid cell division of *Staphylococcus aureus* during colonization of the human nose

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Staphylococcus aureus is an important opportunistic pathogen and a commensal bacterium, thriving in the nasal cavities of 20% of the human population. Little is known about the dynamics of asymptomatic colonization and the occasional transition to infectious disease. In this study, we inferred that *S. aureus* cells replicate every one to three hours on average while colonizing the human nose, based on two independent lines of genomic evidence. We collected nasal swab samples from human subjects, extracted and sequenced metagenomic DNA, and analyzed the distribution of sequencing coverage along the staphylococcal chromosome. Calibration of this data by comparison to a laboratory culture enabled measuring *S. aureus* cell division rates in nasal samples. In addition, we applied mutation accumulation experiments paired with genome sequencing to measure spontaneous mutation rates at a genome scale. Relating these mutation rates to annual evolutionary rates confirmed that nasal *S. aureus* continuously pass several thousand cell divisions per year when averaged over large, globally distributed populations and over many years, corresponding to generation times of less than 90 minutes. The cell division rates we determined were higher than the fastest documented rates during fulminant disease progression (in a mouse model of systemic infection) and much higher than those previously measured in expectorated sputum from cystic fibrosis patients. This paper supplies absolute *in vivo* generation times for

an important bacterial commensal, suggesting that colonization of the human upper respiratory tract is characterized by a highly dynamic equilibrium between bacterial growth and shedding. In addition, our data suggests that the predominant lifestyle of a bacterial pathogen and the associated cell division rate may directly affect its rate of molecular evolution.

Presentation on Tuesday, February 26, 2019 from 16:00 – 16:10 in room Hörsaal 104.

064/MPV

***Chlamydia trachomatis* stabilizes the proto-oncogene c-Myc to de-route glutamine metabolism for its survival**

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Chlamydia trachomatis (Ct), one of the most frequent causes of sexually transmitted diseases, is an obligate intracellular bacterium that strictly depends on the intake of nutrients and metabolites from the host. We show here that *Chlamydia* directly take up glutamine and shuttle it into its partial TCA cycle. Intriguingly, in axenic medium significant[K1] amounts [MOU2] of glutamine are used to synthesize DAP, a component of the bacterial cell wall. The increased requirement of the infected cells for glutamine is achieved by reprogramming the glutamine metabolism in a c-Myc dependent manner. Glutamine was taken up via the glutamine transporter SLC1A5 and metabolized via glutaminase to boost the host TCA cycle. Indeed, interfering with different steps of reprograming glutamine metabolism of the infected cells limited the growth and development of *Chlamydia*. Thus, we report on the mechanism of reprograming the host cell glutamine metabolism by an obligate intracellular pathogenic bacterium, which may provide a basis for innovative anti-infective strategies.

Presentation on Tuesday, February 26, 2019 from 16:15 – 16:25 in room Hörsaal 104.

065/MPV

The olfactory epithelium as a port of entry in neonatal neurolisteriosis.

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Bacterial infections of the central nervous system (CNS) remain a major cause of morbidity and mortality in the pediatric population. Commonly used parenteral infections models, however, do not reflect the early course of the disease, leaving initial mechanisms of host susceptibility route of infection and underlying mechanisms of inflammation in the CNS largely unexplored. Therefore, our aim was to establish a model of neonatal CNS infection with *Listeria monocytogenes* following mucosal challenge. We intranasally infected one-day-old mice with *L. monocytogenes* and sacrificed them at various time points to determine bacterial dissemination. Furthermore, tissue tropism and immune responses were analyzed by immunohistochemistry, electron microscopy, flow cytometry and qRT-PCR.

Bacteria were mainly re-isolated from the brain (particularly from the olfactory bulb and the cerebrum) but only occasionally from the blood, indicating a non-hematogenous dissemination from the nasal cavity to the CNS. Notably, mucosal invasion was restricted to the olfactory epithelium and was independent of the two major listerial invasins InlA and InlB. During early infection time points, electron microscopic examination revealed that *Listeria* resided in olfactory sensory neurons. Later, wild-type bacteria were found to be associated with axon bundles projecting from the olfactory epithelium 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 CNS, *Listeria* induced a multifocal meningo-encephalitis and a strong influx of various immune cells: flow cytometric and immune-histochemical analyses showed an accumulation of bacteria with a concomitant recruitment of $CD45^{+}CD11b^{+}$ microglia/macrophages as well as a significant increase of infiltrating $Ly6C^{hi}$ monocytes/macrophages and $Ly6C^{+}$ neutrophils. As expected, mRNA of key cytokines mediating intracranial inflammation and monocyte as well as neutrophil attraction, such as *Tnfα*, *Cxcl2*, *Ccl2* and *Ccl7*, was highly upregulated.
Taken together, we propose an alternative port of entry and route of infection for neonatal cerebral listeriosis and present a novel *in vivo* infection model to mimic the clinical features of late onset disease in human neonates.

Presentation on Tuesday, February 26, 2019 from 16:30 – 16:40 in room Hörsaal 104.

Workshop 13
Infection Immunology (FG II)
26. Febr. 2019 • 15:15-16:45

066/IIV

Na+-boosted antibacterial defense is dependent on HIF1A and NFAT5

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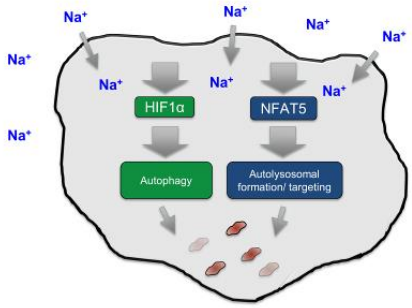
[12]*Institute of Experimental Immunology, University of Bonn, Bonn, Germany*

Infection and inflammation induce diet-independent Na+-accumulation without commensurate water retention in afflicted tissues, which favors the pro-inflammatory activation of macrophages and augments their antibacterial and antiparasitic activity. While Na+-boosted host defense against *Leishmania major* is mediated by increased expression of the leishmanicidal type 2 NO synthase (NOS2), the molecular mechanisms underpinning this enhanced antibacterial defense with high Na+ (HS) exposure are unknown. Here, we provide evidence that HS-increased antibacterial activity against *E. coli* was neither dependent on NOS2 nor on the phagocyte oxidase. In contrast, HS-augmented antibacterial defense hinged on HIF1A/ hypoxia-

inducible factor 1α-dependent increased autophagy, and NFAT5/ nuclear factor of activated T cells 5-dependent targeting of intracellular *E. coli* to acidic autophagolysosomal compartments. Overall, these findings suggest that the autophagolysosomal compartment is a novel target of Na+-modulated cell autonomous innate immunity.

Figure 1



Presentation on Tuesday, February 26, 2019 from 15:15 – 15:25 in room Hörsaal 007.

067/IIV

Francisella tularensis-Macrophage Interaction: A dual RNA Sequencing Approach"

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As a gram-negative, aerobic, non-motile, non-sporulation small coccobacillus, *Francisella tularensis* (*Ft*) causes the zoonotic disease tularemia. Being highly infectious, this bacterium can infect more than 250 hosts from amoebae to mammals and can be transmitted to humans in various ways. These can be direct contact with sick animals or contaminated water or food, as well as tick, mosquito or fly bites or even inhalation. *Ft* subspecies (subsp.) *tularensis* (*Ftt)* is highly virulent and found in North America. Note, that *Ftt* is apprehended as a potential class A agent in bioterrorism since 2001. *Ft* subsp. *holarctica (Fth)* appear in both North America and Europe and these type B strains are mainly less virulent. So, by classical attenuation even live vaccine strains have been generated from type B strains. During infection, intracellular replication of *Ft* mainly occurs in macrophages (MΦs), but also dendritic cells, neutrophils, epithelial cells, fibroblasts and hepatocytes.

Vaccine candidate development against tularemia until now is mainly ineffective in *Ftt*-challenged mice by means of protection or induction of sterile immunity. Therefore, new prevention and treatment strategies are required for this disease, but are aggravated by the limited knowledge of *Francisella"s* pathomechanisms. Developing a new vaccine against virulence factors might be a promising strategy to overcome actual limitations. Our project aims to detect such virulence factors of *Ft* facilitating intra-MΦ replication and immune evasion as targets for the vaccine and therapy development against tularemia.

We established an infection model for THP-1 MΦs with *Ft* and optimised the infection rates combined with a preferably low rate of apoptotic THP-1 MΦs for high (*Ftt*), moderate (*Fth*) and low (*Fth*, attenuated) virulent strains.

Here, first data of dual RNA sequencing (dual RNA seq) are presented. This technique empowers studies on host-pathogen interactions, since it is perfectly suited to identify host factors regulated in a detrimental way by the pathogen as well as virulence factors involved in this regulation.

Presentation on Tuesday, February 26, 2019 from 15:30 – 15:40 in room Hörsaal 007.

068/IIV

Synthetic oligosaccharide-based vaccines protect mice from Clostridium difficile infections

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Introduction

Clostridium difficile is the leading cause of antibiotic-associated diarrhea worldwide. As commonly used frontline antibiotics become less effective and frequently induce recurrent disease by disrupting the intestinal microbiota, the development of vaccines to prevent this devastating and emerging disease is highly desirable. Several bacterial surface glycans such as lipoteichoic acid (LTA) and the polysaccharides I and II (PS I and II) have been identified as promising vaccine candidates to preclude infection and colonization with *Clostridium difficile*1-2.

Aims

We conjugated PS-I, PS-II and LTA to CRM197, a carrier protein used in commercial vaccines in order to assess their potential as colonization-preventing vaccines against *Clostridium difficile* infections.

Materials & Methods

Mice were vaccinated with glycoconjugate vaccines against PS I, PS II and LTA. The composition of the intestinal microbiota, *Clostridium difficile* counts and intestinal inflammation were assessed using conventional plating assays, 16S rRNA sequencing and histopathological analyses.

Results

PS I-, PS II- and LTA- glycoconjugate vaccines induced glycan-specific antibodies in mice and substantially limited colonization with *Clostridium difficile* after infection without disrupting the intestinal microbiota. The glycoconjugates were long-term protective and even superior to a toxin-targeting vaccine candidate in preventing disease.

Summary

Glycoconjugate vaccines against *C. difficile* are a complimentary approach to toxin-targeting strategies and are advancing through preclinical work.

Literature:

Martin, C. E. *et al. J. Am. Chem. Soc.***135**, 9713-9722.
Broecker, F. *et al. Cell Chem. Biol.* **23**, 1014-1022.

Presentation on Tuesday, February 26, 2019 from 15:45 – 15:55 in room Hörsaal 007.

069/IIV

Impact of lipidation and immunization route on the protection against pneumococcal colonization following vaccination with lipoproteins DacB and PnrA

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Question. Current pneumococcal vaccines have important limitations, including restricted serotype coverage facilitating replacement by non-vaccine serotypes and high manufacturing costs. Therefore, serotype-independent and protein-based next-generation vaccines are favored to combat pneumococcal infections. We have recently investigated the potential of non-lipidated pneumococcal lipoproteins DacB and PnrA to elicit protective immune responses against pneumococcal colonization. In a follow-up study, we evaluated the impact of lipidation and

vaccination route (intranasal or subcutaneous) on the magnitude of protection and humoral as well as cellular immune responses.

Methods. The non-lipidated DacB and PnrA were shown to be immunogenic and protective after intranasal immunization using CTB as adjuvant (Voß *et al.*, Front. Immunol., 2018). Here, we compared the immunogenicity and protectivity of lipidated versus non-lipidated DacB or PnrA with and without additional adjuvant using the intranasal as well as subcutaneous route of immunization. Antigen-specific systemic IgG levels following vaccination were determined by ELISA. The cytokine profiles after intranasal pneumococcal challenge in the NALT (local) and in supernatants following spleen cell stimulation (systemic) were analyzed by flow cytometry using the LEGENDplex™ Mouse Th Cytokine Panel (13-plex).

Results. Lipidation of proteins clearly enhanced humoral immune responses even in the absence of additional adjuvant. This effect was highly pronounced for lipidated DacB when applied via the intranasal route, whereas the non-lipidated version was significantly restricted in inducing antigen-specific IgGs. Intranasal vaccination of mice with DacB as well as PnrA substantially reduced colonization three days after intranasal challenge with pneumococci. The protection correlated with increased IL-17A levels. However, reduction in the bacterial load was only observed in CTB adjuvanted groups, and subcutaneously immunized mice were not protected at all.

Conclusions. Lipoproteinsare interesting candidates for future vaccine strategies as they are highly conserved, abundant and immunogenic. We identified PnrA and DacB as potential vaccine antigens to induce protection against pneumococcal colonization. However, this study highlights the importance of selecting the appropriate vaccination route and adjuvants.

Presentation on Tuesday, February 26, 2019 from 16:00 – 16:10 in room Hörsaal 007.

070/IIV

Fine control of factor H-mediated complement evasion by the malaria parasite *Plasmodium falciparum*

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The human complement system is the first defense line against invading pathogens, including the malaria parasite *Plasmodium falciparum*. We previously demonstrated that human complement represents a particular threat for the clinically relevant blood stages of the parasite. To evade complement-mediated destruction, the parasites acquire factor H (FH) via specific receptors. FH is the major regulator of the alternative pathway and able to inhibit the complement cascade by two activities, a cofactor activity during factor I-mediated C3b inactivation and a C3 convertase-specific decay-accelerating activity.

We now report that the FH-related protein FHR-1 competes with FH for binding to the malaria parasite. FHR-1, which is composed of five complement control protein domains with variable homology to FH but which lacks C3b regulatory activity, accumulates on the surfaces of the intraerythrocytic schizonts as well as of free merozoites. Quantitative Western blotting and ELISA demonstrate that while binding of FH to schizont-infected red blood cells is increased in FHR-1-deficient human serum, the addition of recombinant FHR-1 decreases FH-binding. The presence of FHR-1 in consequence impairs C3b inactivation and parasite viability.

The combined data let us conclude that FHR-1 competes with FH for the plasmodial FH receptors, which are required to promote complement evasion by the *P. falciparum* blood stages. In consequence, lack of FHR-1 benefits parasite growth in the RBCs. Future studies of our group are aimed at identifying the precise molecular mechanisms of fine control during complement evasion by malaria parasites.

Presentation on Tuesday, February 26, 2019 from 16:15 – 16:25 in room Hörsaal 007.

071/IIV

Multi-parameter serology for rapid determination of vaccine-preventable infectious diseases using protein microarrays

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A high vaccination rate within a population is an important precondition to prohibit the spread of vaccine-preventable diseases. Due to economic crisis or armed conflict, public health systems collapsed in different parts of the world leading to outbreaks of preventable diseases such as measles, diphtheria or polio. Migrants or refugees could arrive in Germany, who are not vaccinated or whose immunization status might not be known or documented. This makes it necessary to determine the IgG status for all vaccinations recommended by the STIKO individually, rapidly and preferably in a single assay that could be used outside of centralized laboratories.

Our aim was to develop a rapid test procedure on the basis of protein microarrays that simultaneously, reliably, quickly and cost-effectively determines numerous antibody levels with minimal sample volumes of capillary blood.

Initially, antigens of *Clostridium tetani*, *Corynebacterium diphtheriae*, *Bordetella pertussis*, poliovirus, hepatitis B virus, measles, mumps, rubella and varicella zoster virus (VZV) were used. Starting from 1 µL capillary EDTA blood, the analysis including automatic image analysis and data evaluation was performed within 100 minutes. When directly comparing array results for positive and negative reference sera to established ELISAs, the following concordances rates were achieved: *C. tetani* (84/85), *C. diphtheriae* (83/85), measles (81/86), mumps (79/86), rubella (83/85), VZV (81/86), and poliovirus (20/26). Additional antigens were also immobilized including *Plasmodium falciparum* (concordance rate 18/25), *Treponema pallidum* (28/29), hepatitis C virus (HCV) (10/12), *Brucella melitensis* (77/85) and hepatitis E virus (8/9). Furthermore, synthetic peptides derived from the amino acid sequences of various antigens were examined in the same assay resulting in determination of suitable peptides for poliovirus (12/19), HCV (4/5) and *Treponema pallidum* (4/6).

The test can be expanded by adding additional antigens and could also be modified to detect IgM in order to monitor the serological response to acute infections. The assay can be produced in quantities of $>10^6$/year for a very reasonable price and the antigens/peptides investigated here can also be transferred to other platforms (lateral flow, PIMA). Multi-parameter serology at a point-of-care can be expected to be less expensive and time consuming while yielding more information than conventional serological methods.

Presentation on Tuesday, February 26, 2019 from 16:30 – 16:40 in room Hörsaal 007.

Workshop 14
Epidemiology and Antimicrobial Resistance of Zoonotic Pathogens (FG MS/FG ZO)
26. Febr. 2019 • 15:15-16:45

072/MSV

Combining multi-drug resistance with virulence: the hallmark of successful ESBL-producing *E. coli*

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Multi-drug resistant (MDR) superbugs play a key role in the new post-antibiotic era in which bacterial infections cannot be treated reliably anymore. They are exemplified by extended-spectrum beta-lactamase (ESBL)-producing *E. coli*, which are not only present in clinical settings but ecologies with lower antimicrobial resistance pressures, presenting an important One Health problem due to their emergence in humans, animals and the environment worldwide. There are two main explanations for why ESBL-producing bacteria have globally thrived: i) ESBL-encoding genes are located on plasmids, which are rapidly transmitted among bacteria, and ii) ESBL-producing bacteria disseminate through international, MDR, high-risk clonal lineages, distinguished by their sequence types (STs). Interestingly, only some STs, for example ST131, ST648 and ST410, dominate the global pool of successfully distributed ESBL-producing *E. coli* clonal lineages.
We applied cutting-edge next-generation sequencing phylogenetics including accessory genomics and functional experiments to investigate MDR *E. coli* ST410, ST648 and ST131 clonal lineages. We demonstrate that *E. coli* ST410 clonal isolates circulate in humans, animals and the environment in Germany. Similar applies to ST648, which is a nascent and generalist lineage lacking global phylogeographic and interspecies host signals. Functional genomics reveal that these clonal lineages developed highly sophisticated virulence features. Specifically, our *in silico*, *in vitro* and *in vivo* results demonstrate that ST648 was well equipped with biofilm-associated features, while ST131 showed signatures indicative of adaption to urinary tract infection, potentially conveying individual niche adaptation. ST648"s and ST131"s key to success lies in the combination of MDR with a high-level of virulence. We demonstrate that ESBL-plasmid acquisition partly provides this combination in a singular genetic.
Superbugs have evolved to combine multi-drug resistance with high-level fitness and virulence, "supported" by ESBL-plasmids, which aided in shaping ESBL-producing *E. coli* clonal lineages to successfully emerge worldwide across different ecologies. Our studies crucially contribute to the understanding what factors drive the evolution and spread of emerging international, high-risk clonal lineages and help prospectively in prioritizing intervention strategies and in detecting new therapeutic targets.

Presentation on Tuesday, February 26, 2019 from 15:15 – 15:25 in room Hörsaal 005.

073/ZOV

Identification of a distinct gut microbiota mediating colonization resistance against Campylobacteriosis in murine infection models

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Background and objectives: Colonization resistance (CR) against *C. jejuni* (Cj) established by the gut microbiota of mice allows for developing novel therapeutic or preventive strategies to complement measures directed against Cj colonization and infection in farm animals and humans, respectively. We therefore surveyed the gut microbiota composition of mice with and without CR against Cj in order to identify distinct intestinal bacteria mediating colonization resistance against *Campylobacter* within the gut.
Materials and methods: The microbiota was analysed by deep sequencing (LGC Genomics) in mice with and without CR against C. jejuni. Briefly, CR against Cj is abrogated in mice treated with antibiotics such as ampicillin, ciprofloxacin, vancomycin, metronidazole or imipenem (single and in combination), in infant mice, and in mice harbouring a complex human microbiota. Conventional adult mice served as resistant controls.
Results: Analysis of the gut microbiota in mice with and without CR against Cj indicated that bacteria of the Firmicutes might be involved in the establishment of CR against *Campylobacter*.
Conclusion: Murine infection models will provide aid to validate possible therapeutic measures for the treatment of *Campylobacter* infection in humans. Further investigations will reveal, if bacteria mediating CR against Cj in mice might be of use for prevention of *Campylobacter* colonization in poultry.

Presentation on Tuesday, February 26, 2019 from 15:30 – 15:40 in room Hörsaal 005.

074/MSV

Gain and loss of mobile genetic elements drives recent evolution of heteropathogenic *Escherichia coli* of sequence type 141

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Introduction
Heteropathogenic Shiga toxin (Stx)-producing *Escherichia coli* (STEC) of multilocus sequence type (MLST ST) 141 cause both urinary tract infection and diarrhea in humans. We could show previously that they possess virulence genes typical for STEC and uropathogenic *E. coli* (UPEC) and are phylogenetically positioned between these pathogroups. However the origin and evolution of these strains are unknown.
Objectives
Our aim is to investigate the origin and the evolution of ST141 using phylogenomics.
Materials & Methods
A total of 85 ST141 whole genome sequence datasets of *E. coli* isolated from different parts of the world were sequenced using either illumina or Pac Bio technology or retrieved from the NCBI SRA database and subsequently assembled using the SeqSphere+ software (Ridom GmbH, Münster, Germany). Moreover, 25 reference genomes comprising different extraintestinal pathogenic (ExPEC), diarrheagenic and commensal *E. coli* were added. For 42 isolates with sufficient metadata, an ancestral dating using Bayesian Evolutionary Analysis Sampling Trees (BEAST) after removal of recombination sites from the genome was performed.
Results
Extracting of characteristic genes revealed that ST141 strains have a diverse repertoire of virulence genes ranging from isolates carrying genes typical only for ExPEC to isolates having genes belonging to ExPEC, EHEC and EAEC. None possess genes typical of ETEC and EIEC. BEAST analysis demonstrated that *E. coli* ST141 is divided into two distinct group which evolved from a common ancestor 138 years ago. Approximately 100 years ago, ST141 *E. coli* acquired the *stx*-prophage and 45-50 years later (in the 1960s) heteropathogenic *E. coli* acquired the Pathogenicity Island (PAI) similar to PAI II (PAI $II_{536\text{-like}}$) of the UPEC 536. Due to the unstable nature of PAI II_{536} in the genome[1], PAI $II_{536\text{-like}}$ was subsequently lost in some phylogenetic offshoots. Very recently (20 years ago), heteropathogenic *E. coli* with both *stx*-prophage and PAI $II_{536\text{-like}}$ acquired the EHEC hly plasmid.
Conclusion
Phylogenetically, ST141 is divided into two different groups. Acquisition of *stx*-prophage, PAI $II_{536\text{-like}}$, EHEC hly plasmid by ST141 *E. coli* through horizontal gene transfer is responsible for emergence and evolution of ST141 heteropathogenic *E. coli*.

Current investigations focus on the in-depth comparative genome analysis to decipher the insertion site of *stx*-prophage and PAI $II_{536\text{-}like}$.

1. Middendorf et al. *J. Bacteriol*. 186(10):3086-3096(2004).

Presentation on Tuesday, February 26, 2019 from 15:45 – 15:55 in room Hörsaal 005.

075/ZOV

RNA-seq analysis reveals a dramatic effect of the pAA plasmid on EHEC O104:H4 flagellar expression

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Introduction

The 2011 enterohemorrhagic *Escherichia coli* (EHEC) O104:H4 outbreak strain carries both a chromosomally integrated Shiga toxin 2a encoding bacteriophage and an enteroaggregative *Escherichia coli* (EAEC)-specific virulence pAA plasmid. Our group reported that EHEC O104:H4 can sporadically lose pAA in the course of illness and that pAA loss is associated with a significantly reduced correlation of HUS development in patients, which speaks for an attenuated virulence of the pAA-negative (pAA-) strain. Here, using RNA-seq we analyzed the whole transcriptome of the pAA- strain and wild type EHEC O104:H4, in order to screen for factors and mechanisms, which contribute to virulence and fitness.

Methods

RNA-seq was performed with total RNA from pAA- and wild type EHEC O104:H4 cells grown in LB medium at 37°C to mid-log phase. Directional cDNA libraries were sequenced on Illumina platform. Raw data were processed using READemption and differences in gene expression were determined by DESeq2. FliC protein levels were analysed with semi-quantitative Western blot. Computational screening for AggR binding sites was performed with FIMO.

Results

On average 12 million reads were sequenced per library and at least 95% of them could be mapped to the reference genomes. DESeq2 analysis revealed 294 upregulated and 434 downregulated genes in pAA- strain in comparison to wild type EHEC O104:H4. Gene ontology analysis showed that the most abundant class of downregulated genes (n = 53, log2fold change < 8.8) in the pAA- strain were genes involved in motility and chemotaxis. These dramatic transcriptome differences were reflected by non-detectable FliC on the protein level. Interestingly, genes throughout the whole flagellar transcriptional hierarchy were found downregulated, suggesting the differential regulation of the FlhDC master regulator as a trigger for the effect. A computational analysis revealed two high scoring binding sites of the pAA-encoded virulence transcriptional regulator AggR in the proximity of the *flhD* coding region.

Conclusions

Our study indicated a dramatic effect of the pAA plasmid on EHEC O104:H4 host gene expression and in particular in flagellar synthesis. In addition to motility, flagella are playing an important role as virulence factors in EAEC and EHEC O104:H4. An ongoing heterologous expression of AggR in *E. coli* K-12 and AggR deletion and complementation analysis should reveal the actual contribution of this regulator to flagellar expression.

Presentation on Tuesday, February 26, 2019 from 16:00 – 16:10 in room Hörsaal 005.

076/MSV

Comparative secretome analyses of human and zoonotic *Staphylococcus aureus* isolates of CC8, CC22 and CC398

T. Busche[1,2], M. Hillion[1], V. V. Loi[1], D. Berg[1], B. Walther[3], T. Semmler[3], B. Strommenger[4], W. Witte[4], C. Cuny[4], A. Mellmann[5], M. A. Holmes[6], J. Kalinowski[2], L. Adrian[7], J. Bernhardt[8], H. Antelmann*[1]
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[8]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*

Question: The spread of methicillin-resistant *Staphylococcus aureus* (MRSA) in the community, hospitals and in livestock is mediated by highly diverse virulence factors that include secreted toxins, superantigens, enzymes and surface-associated adhesins allowing host adaptation and colonization. Methods: Here, we combined proteogenomics, secretome and phenotype analyses to compare the secreted virulence factors in selected *S. aureus* isolates of the dominant human and livestock-associated genetic lineages CC8, CC22 and CC398. Results: The proteogenomic comparison revealed 2181 core genes and 1306 accessory genes in 18 *S. aureus* isolates reflecting the high genome diversity (1). Using secretome analysis, we identified 869 secreted proteins with 538 commons in eight isolates of CC8, CC22 and CC398. These include 64 predicted extracellular and 37 cell surface proteins that account for 82.4% of total secretome abundance. Among the top 10 most abundantly secreted virulence factors are the major autolysins (Atl, IsaA, Sle1, SAUPAN006375000), lipases and lipoteichoic acid hydrolases (Lip, Geh, LtaS), cytolytic toxins (Hla, Hlb, PSMß1) and proteases (SspB). The CC398 isolates showed lower secretion of cell wall proteins, but higher secretion of a- and ß-hemolysins (Hla, Hlb) which correlated with an increased Agr activity and strong hemolysis (1). CC398 strains were further characterized by lower biofilm formation and staphyloxanthin levels due to decreased SigB activity. Conclusion: Overall, comparative secretome analyses revealed CC8 and CC22-specific enterotoxin or Spl protease secretion as well as Agr- and SigB-controlled differences in exotoxin and surface protein secretion between human-specific and zoonotic lineages of *S. aureus*.

Reference:

(1) Busche T, Hillion M, Loi VV, Berg D, Walther B, Semmler T, Strommenger B, Witte W, Cuny C, Mellmann A, Holmes MA, Kalinowski J, Adrian L, Bernhardt J, Antelmann H*. Comparative secretome analyses of human and zoonotic *Staphylococcus aureus* isolates of CC8, CC22 and CC398. *Mol Cell Proteomics*, in press. doi: 10.1074/mcp.RA118.001036. (2018).

Presentation on Tuesday, February 26, 2019 from 16:15 – 16:25 in room Hörsaal 005.

077/MSV

***Clostridioides difficile* in poultry manure**

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Clostridioides difficile (*C. difficile*) is a spore-forming obligate anaerobic gram-positive bacterium that is one of the major causes for antibiotic-associated diarrheal diseases. The ubiquitous occurring *C. difficile* can be found predominantly in the intestinal tract of humans and animals. Hence, *C. difficile* spores can be shed with animal feces and contaminate fertilizers of arable land. Some evidence suggests that here lies a possible route for the pathogen to

re-enter the human food chain by sticking to plants or through wind-blown dispersion.
During a screening of poultry fattening farms we detected *C. difficile* in chicken feces and manure. We performed whole genome sequencing of collected isolates. A phlyogenetic analysis revealed a high diversity. The isolates were distributed throughout several of the major phylogenetic clades and included multiple clinically relevant PCR ribotypes.
The interdisciplinary project "SOARiAL" (Spread of antibiotic resistance in an agrarian landscape) explores the possibility of airborne transmission of *C. difficile* from fertilized fields.

Presentation on Tuesday, February 26, 2019 from 16:30 – 16:40 in room Hörsaal 005.

Workshop 15
From Bench to Bedside and Back (FG PR, StAG HY)
27. Febr.2019 • 08:30-10:00

078/PRV

Beta-lactamase gene edition by CRISPR-CAS9 promotes resistance reduction in clinical *E.Coli* and other *enterobacteriaceae* strains

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[4]*Universidade Federal de Viçosa, Department of Biochemistry and Molecular Biology, Viçosa, Brazil*

Introduction: Considering that the emergence and spreading of antimicrobial resistant mechanisms is much faster than the discovery of new antimicrobials, alternative strategies, such as reversing resistance using CRISPR-Cas9 may have a significant impact for future clinical applications. CRISPR-Cas9 represents an opportunity to develop a specific tool to switch off the resistance due to its ability of specific DNA cleavage and edition.
Aim: To reverse resistance encoded by the *bla*TEM gene using the CRISPR-Cas9 technology.
Materials and Methods: First, a gRNA was designed using Pearl scripts to target specifically a conserved region of the *bla*TEM gene in a reference strain. The selected sequence was synthesized and inserted into the CRISPR-Cas9 vector. The functionality of the genetic device was verified by qPCR of the RNA transcripts. The phenotype reversal was assessed by growth curves in the presence and absence of ampicillin. Furthermore, the modified *bla*TEM gene was sequenced and its expression evaluated. The CRISPR-Cas9 system was also applied to clinical isolates of *E. coli*, *Klebsiella* sp. and *E. cloacae.*
Results: After gRNA designed followed by construction and cloning of the CRISPR-Cas9 system, the expression of the Cas9 enzyme could be demonstrated in all control experiments by qPCR. Growth curve experiments demonstrated resistance reversal in the *E. coli* model. Sequence analysis of the edited gene showed a frameshift mutation and reduced expression of *bla*TEM. While *E. coli* was entirely re-sensitized to five antimicrobials, a reduction of resistance was also achieved in the other clinical isolates of *Enterobacteriaceae* for three antimicrobials.
Conclusion: The CRISPR-Cas9 system has the potential to reverse antibiotic resistance with appropriate gRNA in multidrug resistant Gram-negative bacteria, in both, reference and clinical bacterial strains.

Financial Support: Thaysa Tagliaferri was supported by CNPq Scholarship – Brazil.
Presentation on Wednesday, February 27, 2019 from 8:30 – 8:40 in room Hörsaal 010.

079/PRV

Species-specific activity of antibacterial drug combinations

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[7]*EMBL Heidelberg, Structural and Computational Biology Unit, Heidelberg, Germany*

Question
The spread of antimicrobial resistance has become a serious public health concern, making once treatable diseases deadly again and undermining breakthrough achievements of modern medicine. Drug combinations can aid in fighting bacterial infections, yet the molecular mechanisms underlying drug interactions remain poorly understood, and thus combinations are largely unexplored and rarely used in clinics.
Methods
With the goal of identifying general principles driving antibacterial drug combinations, as well as their degree of conservation across Gram-negatives, we profiled ~3,000 dose-resolved combinations of antibiotics, human-targeted drugs and food additives in 6 strains from three Gram-negative pathogens, *Escherichia coli*, *Salmonella* Typhimurium and *Pseudomonas aeruginosa* (Brochado AR et al, *Nature* 2018). We followed up the most relevant results by deciphering the molecular mechanism underlying drug interactions and testing potent synergies against MDR clinical isolates.
Results
Despite their phylogenetic relatedness, more than 70% of the detected drug-drug interactions are species-specific and 20% display strain specificity, revealing a large potential for narrow-spectrum therapies. Overall, antagonisms are more common than synergies and occur almost exclusively between drugs targeting different cellular processes, whereas synergies are more conserved and enriched in drugs targeting the same process. We elucidate mechanisms underlying this dichotomy and further dissect the interactions of the food additive vanillin, which strongly potentiates the neglected antibiotic spectinomycin against *E. coli* clinical isolates. Finally, we demonstrate that several synergies are effective against MDR clinical isolates *in vitro* and during infections of *Galleria mellonella* larvae, with one reverting resistance to the last-resort antibiotic, colistin.
Conclusion
Beyond unraveling general principles, our work provides an unparalleled number of drug-drug interactions in Gram-negative species. We demonstrated the potency of several synergistic pairs against MDR clinical isolates. Many more drug pairs are still to be uncovered within our dataset. Interestingly, human-targeted drugs were among the most frequent antibiotic adjuvants in our screen. Profiling more such drugs and food additives in future combinatorial screening may also lead to efficient treatments against MDR pathogens.
Reference
Brochado AR et al. *Nature* 2018. DOI:10.1038/s41586-018-0278-9

Presentation on Wednesday, February 27, 2019 from 8:45 – 8:55 in room Hörsaal 010.

080/PRV

Tracking plasmid evolution dynamics of *bla*$_{IMP-8}$ carbapenemase harbouring Gram-negative bacteria in a hospital setting

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Question: Infection or colonization of patients with multidrug-resistant (MDR) bacteria often leave very limited treatment options. Not only clonal transmission of MDR resistant bacteria, but also the spread of multidrug resistance genes carrying plasmids by horizontal gene transfer between different bacterial species represents an important mode of expansion of antimicrobial resistance genes. In 2009 we experienced an outbreak with an extensively multidrug resistant *P. aeruginosa* harbouring a carbapenemase enzyme (*bla*IMP-8). In March 2012 we detected the first *Citrobacter freundii* harbouring the same enzyme *bla*IMP-8 carbapenemase. Since this enzyme is rarely encountered in Europe we hypothesised, that horizontal gene transfer between the different Gram-negative species had occurred within our hospital.

Methods: All *bla*IMP-8 positive strains from patients or patient-related environmental water sources (n=54) over a 6 years" period were included in the study. This included *Citrobacter species* (n=9) and *P. aeruginosa* (n=45) strains. Short-read whole genome sequences were generated from all isolates. For plasmid analysis, single molecule (long read) sequencing (Oxford Nanopore) was conducted of all *Citrobacter* species isolates (n=9) and selected *P. aeruginosa* isolates (n=5) representing different time points. In order to obtain finished genomes and circularized plasmids from the long-range read sequences, a new analysis pipeline was developed.

Results: We identified a 40 kb plasmid (plasmid A) harbouring *blaI*MP-8 in *P. aeruginosa* and *Citrobacter freundii*, indicating that plasmid transfer had occurred between the two species. Within the *Citrobacter* species the plasmid underwent further evolution resulting in the detection of a *bla*IMP-8 harbouring 164 kb megaplasmid (plasmid C) in *C. werkmanii*. The megaplasmid is most likely the result of plasmid fusion, since it contains a 40 kb region with 100% genetic homology of with plasmid A, in addition to an 88 kb region highly homologous to another plasmid (plasmid B) detected in *C. freundii*. Moreover, changes of the multidrug resistance gene cassette on the class I integron were noted, including deletions and translocations of complete antimicrobial resistance genes.

Conclusion: The results demonstrate, that the chosen approach enabled us to track plasmid evolution dynamics during a hospital outbreak, driven by plasmid transfer, plasmid fusion and evolution of the antibiotic resistance gene cassette on the plasmids.

Presentation on Wednesday, February 27, 2019 from 9:00 – 9:10 in room Hörsaal 010.

081/PRV

Comparison of conventional typing methods with whole genome sequencing to investigate suspected transmission of multidrug-resistant *Enterobacter cloacae* in a neonatal intensive care unit.

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Question

Whole genome sequencing (WGS) has become the gold standard for outbreak diagnostic over the past years as this technology become more accessible and affordable. However, there is still room for improvement, in terms of economic factors and time-to-result. In the WGS era, conventional typing methods such as pulse field gel electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD) and multi locus sequence typing (MLST) might be obsolete. In this study, we compared PFGE, RAPD, MLST and WGS to assess the reliability and concordance of conventional typing methods to WGS.

Methods

Between January and May 2018, we observed a suspicious cluster of 10 patients, who were colonized with third-generation cephalosporin-resistant *Enterobacter cloacae*in a NICU during weekly screening for multi-drug resistant organisms (MDRO). We took this opportunity to compare the different typing methods. PFGE, RAPD (using 2 primers) and WGS were performed on 11 *E. cloacae*isolates (9 with resistance to 3rdgeneration cephalosporin and 2 susceptible E. cloacae). Classical MLST were extracted from WGS data for comparison purposes.

Results

All typing methods found two major clusters A (ST664) and B (ST742). WGS and PFGE clustered 5 isolates to cluster A (SNP<13 in WGS), whereas MLST and RAPD clustered 6 isolates to cluster A. All methods designated 3 isolates to cluster B (SNP <13 in WGS) and identified 2 non-related isolates. All control isolates from a previous transmission case and a non-related control isolate were clustered correctly using all four methods. The 2 susceptible *E. cloacae*isolates from a triplet clustered to cluster A along with two resistant isolates from their sibling. There were no acquired beta-lactam resistant genes in all of the isolates. The phenotypic resistance to third-generation cephalosporin were most probably due to the induction of the chromosomal *AmpC* by perinatal antibiotic prophylaxis.

Conclusion

We found that conventional methods delivered concordant results with WGS. Although RAPD and MLST falsely designated one isolate to cluster A, both methods correctly identified non-related isolates. Validation with a larger cohort is needed but our data suggest that conventional typing methods can still be used for outbreak diagnostic, especially in settings without access to WGS yet.

Presentation on Wednesday, February 27, 2019 from 9:15 – 9:25 in room Hörsaal 010.

082/PRV

Impact of antibiotics on the gut resistome and mobilome: A prospective clinical metagenomics multicenter cohort study

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Question Antibiotics impact the gut resistome significantly, often leading to a selection and an increase of antibiotic resistance genes (ARGs). ARGs become particularly harmful when located on a mobile genetic element. However, the potential differential effect of antibiotics on the gut mobilome is still not comprehensively investigated and quantified in clinical cohorts.
Methods We collected stool samples in two cohorts of hematological patients receiving either ciprofloxacin (n=20) or cotrimoxazole (n=21) prophylaxis and determined the resistome and gut mobilome composition using shotgun metagenomics before treatment and at three timepoints during treatment. Sequence reads were assembled using SPAdes, and plasmid classification of scaffolds was performed with PlasFlow.
Results Ciprofloxacin and cotrimoxazole both reduced the gut microbiome diversity, while effects on the gut resistome differed with increasing or decreasing abundance of various ARG classes. For instance, CTX-M and glycopetide ARGs were positively selected on ciprofloxacin, while aminoglycoside ARGs decreased. In the cotrimoxazole group, sulfonamide and trimethoprim ARGs increased during drug administration, while a decrease was observed for beta-lactamase ARGs. Plasmid diversity (Shannon index) decrease on both antibiotics, while the abundance of plasmids originated from proteobacteria only decreased on ciprofloxacin ($p = 0.002$) and not on cotrimoxazole ($p = 0.86$). Proteobacteria plasmids that harbored ARGs experienced a massive mobilization on cotrimoxazole but not on ciprofloxacin, most likely due to the selection of sul resistance genes that are regularly located on plasmids with other resistance determinants.
Conclusion Our study shows a differential impact of two antibiotics on the resistome and gut mobilome, with cotrimoxazole having a much greater mobilization potential, possibly attributed with a greater potential for the spread of resistance via horizontal gene transfer.

Presentation on Wednesday, February 27, 2019 from 9:30 – 9:40 in room Hörsaal 010.

083/PRV

Understanding T6SS activation in P.aeruginosa using CRISPR interference.

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Pseudomonas aeruginosa is a life-threatening nosocomial Gram-negative pathogen that was recently identified by the World Health Organization as a priority for the development of new antibiotics to combat its multidrug resistance. In addition, P. aeruginosa is a model organism to study the type VI secretion system (T6SS). Although much is known about T6 dynamics and regulation, the signal that is sensed by P. aeruginosa resulting in a counter attack has not been elucidated so far. It has been suggested that P. aeruginosa either senses disturbances in the outer or inner membrane or that contact-independent P. aeruginosa response to antagonism (PARA) is responsible for T6 activation.
In this study, we established a CRISPR interference (CRISPRi) system in P. aeruginosa, a method that can be used to easily manipulate expression levels of (essential) genes in a high-throughput fashion. We integrated a catalytic inactive version of Cas9 (dCas9) of Streptococcus pyogenes on the genome of P. aeruginosa under the control of an inducible promoter, whereas the single guide RNA (sgRNA), mediating target specificity, is expressed from a plasmid with constitutively active promoter. We determined the optimal conditions for the targeted down-regulation of essential genes, by addressing strand specificity and dose dependency to the inducer. CRISPRi in P. aeruginosa reliably reduces the expression of essential genes which results in growth inhibition and reduced viability. With the CRISPRi technology, we studied how down-regulation of essential genes in the periplasm, inner- and outer-membrane results in modulation of T6 activity using live cell imaging and competition assays with T6+ and T6- Vibrio Cholerae. These experiments shed light on signal sensing and activation of the T6SS.

Presentation on Wednesday, February 27, 2019 from 9:45 – 9:55 in room Hörsaal 010.

Workshop 16
Microbial Interactions (FG MP, FG EK)
27. Febr. 2019 • 08:30-10:00

084/MPV

Investigating the role of blood plasma on the adhesion forces of *Staphylococcus aureus* to central venous catheters by single-cell force spectroscopy

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Background: *S. aureus* biofilms which are formed on medical devices, such as central venous catheters (CVCs), may yield in life-threatening infections. A wealth of information was gained on bacterial and host factors that are contributing to the adhesion of *S. aureus* to CVCs from flow chamber studies. The exact forces, however, with which this bacterium adheres to the implanted material remain unknown yet.
Aims: Here, we tried to fill this gap by using single-cell force spectroscopy to determine the bacterial adhesion forces. Additionally, we intended to clarify the role of blood plasma on bacterial adhesion, and to identify plasma factors that alter the adhesion strength of *S. aureus* to this type of implant material.
Materials and methods: Cantilevers were functionalized with single, viable *S. aureus* cells and probed with CVC fragments that were either left untreated or were pre-incubated with human plasma to mimic the situation seen *in vivo*. Blood plasma-coated CVC fragments were analyzed by electrophoresis for the protein composition that was deposited on the CVC surface upon contact with this type of body fluid, and contact angle measurements were

carried out to evaluate the impact of plasma coating on surface hydrophobicity

Results: Our studies revealed that *S. aureus* adheres to naive CVC fragments with forces between 1.3 and 9.7 nN. Retraction curves indicated that adhesion of *S. aureus* to this kind of surface was primarily driven by hydrophobic interactions between macromolecules on the bacterial cell surface and the hydrophobic surface of the implant material. This phenotype markedly changed when CVC fragments were pre-incubated with human blood plasma. Here, the adhesion forces dropped down to 0.5 nN and below. Electrophoresis identified serum albumin as the major plasma factor that was deposited on the CVC surface, for which *S. aureus* possesses a low binding capacity. Contact angle measurements revealed a clear decrease in surface hydrophobicity for the plasma-incubated CVC surface, an effect that has been linked with serum albumin as well.

Conclusions: Our findings indicate that the primary adhesion capacity of *S. aureus* to implant material can be significantly reduced by modifying the surface of the implant material. Pre-coating catheters with serum albumin, a procedure already suggested to reduce the formation of thrombi on CVCs, might help to reduce the colonization of the implant material with this potentially life-threatening bacterium.

Presentation on Wednesday, February 27, 2019 from 8:30 – 8:40 in room Hörsaal 104.

085/MPV

Structural determinants of *Staphylococcus epidermidis* Extracellular matrix binding protein Embp and its interaction with fibronectin

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S. epidermidis biofilm formation depends on interactions between *S. epidermidis* and fibronectin (Fn). Here, we investigate the structural basis of *S. epidermidis* – Fn interactions, focusing on the 1MDa extracellular matrix binding protein (Embp). *S. epidermidis* attachment to immobilized Fn was analysed by mapping the potential interaction sites using Far-Western Blot analysis and peptide library screening, revealing Fn type III repeat 12 (FN12) to be the major site of interaction. Further structural analysis using recombinant Embp fragments showed that Embp contains two core structural units referred to as F- and FG-repeat. Both, 170 aa F- and 126 aa FG-repeat exhibit Fn-binding activity mediated via interactions within FN12. To test the functional importance of this interaction for bacterial adherence, Embp-isoforms consisting of either F- or FG-repeats were expressed in *S. carnosus* and a *S. epidermidis embp* knockout mutant. Expression of both constructs induced bacterial adherence to immobilized Fn and FN12, showing that F- and FG-repeats are sufficient for bacterial interactions with surface-organized Fn. Intriguingly, Embp-producing staphylococci were unable to recruit soluble Fn to the surface, contrasting to *S. aureus* FnBPA that readily binds soluble Fn via interactions with Fn type I domain. In line with evidence that Fn undergoes defined structural changes within Fn type III repeats during fibrillogenesis, it thus appears that Embp interaction sites are only accessible in a surface immobilized state of Fn. The ability of *S. epidermidis* to use Embp for specific binding to defined structural Fn conformations could promote defined lifestyles during colonization and infection.

Presentation on Wednesday, February 27, 2019 from 8:45 – 8:55 in room Hörsaal 104.

086/MPV

Distinct stabilities of a polycistronic mRNA as expression level regulator:
The methionine biosynthesis operon in *Staphylococcus aureus*

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N-formyl methionine is the universal N-terminal amino acid of prokaryotic proteins making methionine indispensable for bacterial growth. The common human pathogen *Staphylococcus aureus* is capable of synthesising methionine *de novo* and therefore to sustain in niches where the amino acid is lacking. Recently, we identified a unique hierarchical control pathway regulating *de novo* methionine biosynthesis in *S. aureus* involving stringent-response control in combination with a T-box riboswitch and RNA decay [1]. Riboswitches are *cis*-acting RNA regulatory elements, located in 5'-UTRs of genes. The T-box family of riboswitches represents transcription termination control systems which bind uncharged cognate tRNAs as effector molecules. The T-box riboswitch residing in the 5'-UTR of the *S. aureus metICFE-mdh* methionine biosynthesis operon specifically interacts with uncharged methionyl-tRNAs $tRNA^{Met}$. In addition to T-box riboswitch-mediated transcription control, the *met* leader/*metICFE-mdh* mRNA was shown to undergo processing and rapid degradation involving various RNases [1]. Here we demonstrate that stability of the *metICFE-mdh* mRNA varies over the length of the transcript with a longer lifespan towards the 3'-end of the transcript. Protein detection data suggest that the enzyme levels directly reflect the respective mRNA stability.

Furthermore, we were able to determine the exact processing sites of the *met* leader and *metICFE-mdh* RNA, respectively by RACE approaches. RNases III and J1/J2 showed to be central to *met* leader RNA processing and degradation. We solved the secondary structure of this exceptionally long (440 nt) T-box riboswitch RNA using in-line probing to confirm the presence of structures required for RNase III cleavage. The uncommon, immediate physical separation of the *met* leader RNA from the *metICFE-mdh* mRNA appears to be the driving force for *met* mRNA 5' destabilisation.

From the combined data we hypothesise that targeted RNA decay represents another level in the hierarchical methionine biosynthesis control network influencing translational efficiency and adjusting the protein amounts of the distinct enzymes of the pathway to current requirements.

[1] Schoenfelder, S. M.K. et al. 2013. Methionine Biosynthesis in *Staphylococcus aureus* Is Tightly Controlled by a Hierarchical Network Involving an Initiator tRNA-Specific T-box Riboswitch. Plos Pathogens 9(9).

Presentation on Wednesday, February 27, 2019 from 9:00 – 9:10 in room Hörsaal 104.

087/EKV

Biofilm formation, growth and morphology of the black yeast-like fungus *Exophiala dermatitidis* is influenced by the presence of *Pseudomonas aeruginosa* under *in vitro* cystic fibrosis conditions.

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Exophiala dermatitidis, belonging to the black-yeast like fungi, is a frequent colonizer found in the respiratory tract of cystic fibrosis (CF) patients. It was recently reported that *E. dermatitidis* is capable to form biofilms in a strain-dependent manner. However, little is known about biofilm formation in co-culture with other CF-relevant pathogens, e.g. *Pseudomonas aeruginosa*. The growth, biofilm formation capabilities and morphology of *E. dermatitidis* in an artificial sputum medium (ASM), mimicking the CF sputum

conditions, were assessed in mono- and in co-culture with *P. aeruginosa.*

P. aeruginosa (ATCC 9027) and *E. dermatitidis* (clinical isolate) were analyzed in growth experiments over a period of 48 hours at 36°. Growth was determined by colony forming unit (CFU) counts. Biofilm, formed on polystyrene surfaces under standard protocols, was determined after 24 and 48 hours of incubation at 36°C without agitation by stain with crystal violet, by CFU counts after biofilm detachment using 0.1% dithiothreitol, for species-specific cell counts. In addition, confocal laser scan microscopy was carreid out determining the thickness of extracellular matrix (ECM). Morphology of the dimorphic *E. dermatitidis* was monitored in presence and absence of *P. aeruginosa.*

P. aeruginosa showed growth inhibiting effects on *E. dermatitidis.* Cell count of the fungus was decreasing after inoculation and was not detectable anymore after incubation in co-culture with *P. aeruginosa* for 10 h. In contrast, *E. dermatitidis* biofilm formation was not highly affected by co-cultivation with *P. aeruginosa.* In CV stain assays, a synergetic induction of biofilms of both species was investigated. This was supported by the detection of a weak increase in ECM thickness in co-culture after 24 hours of incubation. However, the species-specific cell count in biofilm was only slightly induced in comparison to pure culture. In addition, morphology of *E. dermatitidis* was altered in co-culture with *P. aeruginosa.*

Interactions between *P. aeruginosa* and *E. dermatitidis* result in altered growth, biofilm formation capabilities and morphology of the fungus in an *in vitro* CF model.

Presentation on Wednesday, February 27, 2019 from 9:15 – 9:25 in room Hörsaal 104.

088/EKV

Keeping *Candida albicans* commensal: How Lactobacilli protect intestinal cells against cytotoxicity

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Introduction: The gut is the main origin of *Candida albicans* that causes systemic infections in immunocompromised patients or patients with damaged barrier functions. Here the fungus normally exists as a harmless commensal that interacts with the host and the microbiota. However, removal or imbalance of the bacterial microbiota by antibiotic treatment, for instance, can initiate fungal overgrowth – a significant predisposing factor for disseminated *Candidiasis.*

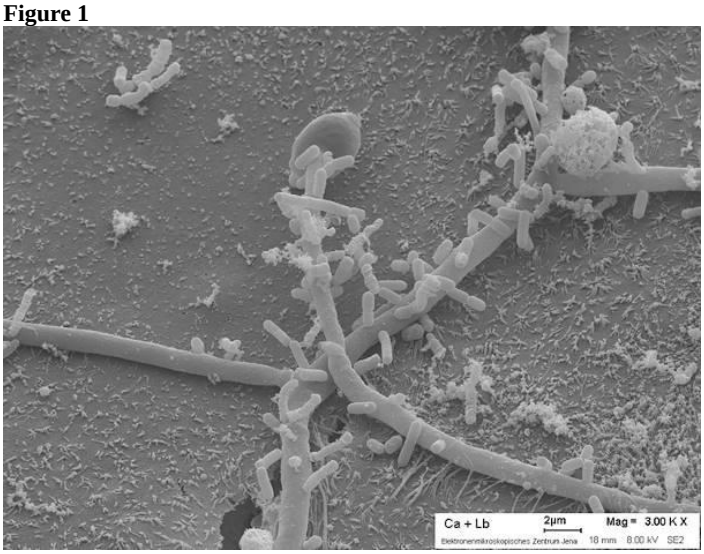
Question: Our aim is to investigate the commensal-to-pathogen-shift of *C. albicans* and how it can be prevented using Lactobacilli.

Methods: To study the complex interactions between *C. albicans*, lactobacilli (e.g. *L. rhamnosus*) and intestinal epithelial cells in a gut like environment, we established an *in vitro* commensal gut model. In this model enterocytes (C2BBe1) and mucus secreting goblet cells (HT-29MTX) represent the gut barrier. Using different techniques, including LDH measurement, assessment of adhesion, hyphal length, translocation and nutrient consumption interactions between epithelium, *C. albicans,* and lactobacilli are characterized.

Results: Using this *in vitro* gut model, we were able to show a time-, dose-, and species-dependent protective effect of different lactobacilli against *C. albicans*-induced cytotoxicity. This effect required viable bacteria, the presence of host cells, and was not related to a competition for adhesion sites. Lactobacilli affected hyphal elongation, ramification, and induced shedding of hyphae from the host cell surface at later stages. Most likely, this loss of contact leads to the damage-protection observed in the presence of bacteria. Via transcriptional profiling, metabolome and secretome analysis of all interaction partners, we aim at identifying factors involved in the commensal-to-pathogen-shift of *C. albicans* and responsible for the underlying protective mechanism of lactobacilli.

Conclusions: Collectively, using an *in vitro* commensal gut model we experimentally dissect the complex interactions of the human-associated pathogenic fungus *C. albicans*, the bacterial microbiota, and the host epithelial barrier in order to investigate their impact on disease development. Bacterial induced shedding of fungal hyphae has been discovered as a novel potential mechanism of antagonistic activities of lactobacilli towards pathogenic fungi.

Figure 1



Presentation on Wednesday, February 27, 2019 from 9:30 – 9:40 in room Hörsaal 104.

089/EKV

A live cell-based approach to study the dynamics of phagosome maturation in *Aspergillus fumigatus* infected amoebae

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The human pathogenic fungus *Aspergillus fumigatus* causes fatal infections in immunocompromised individuals. Highly specific virulence factors are absent in this fungus, but its ability to withstand alveolar macrophages through an arrest of phagolysosome maturation is regarded as one of its essential virulence attributes. The environmental reservoir of A. *fumigatus* further suggests that such general virulence traits have emerged long before the appearance of innate immune systems of vertebrates, making the professional phagocyte *Dictyostelium discoideum* an attractive model to study the defense strategies of this pathogen against innate immune cells.

Here, we have used this amoeba model to follow the antagonistic interaction of conidia of *A. fumigatus* and *D. discoideum* in real time to identify factors that drive the infection process. We found that conidia covered with the green pigment dihydroxynaphtalene DHN-melanin were internalized at far lower rates when compared to those lacking the pigment, despite high rates of initial attachment. Immediately after uptake of the fungal conidia, nascent phagosomes were formed through sequential membrane fusion and fission events. Using single-cell assays and various fluorescent reporter cell lines, we could show that acidification of the phagolysosome was transient and was followed by neutralization, and finally the expulsion of the conidium. For unpigmented conidia the cycle was completed in less than 1 h, while conidia covered with DHN-melanin delayed the process, leading to prolonged persistence in this phagocyte. The total outcome of the infection for the entire population was further supported by a computational model which integrated the results of the different dynamics in internalization and phagolysosome maturation. At later stages of infection, damage in infected phagocytes was detected through the recruitment of the repair escort machinery and autophagy markers. We are currently aiming to elucidate which fungal factors target the host cell, as this knowledge could provide a vital key to better antifungal drug design.

Presentation on Wednesday, February 27, 2019 from 9:45 – 9:55 in room Hörsaal 104.

Workshop 17
Zoonotic Diseases (FG ZO)
27. Febr. 2019 • 08:30-10:00

090/ZOV

Identification of intestinal luminal metabolites mediating colonization resistance against Campylobacteriosis in murine infection models

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Background and objectives: Detailed knowledge about intestinal luminal metabolites providing colonization resistance (CR) of mice against *C. jejuni* (Cj) is well suited to develop strategies directed against *Campylobacter* colonization and infection in farm animals and humans, respectively. In order to identify metabolites combating Cj in the gut we investigated the metabolomes of mice with and without CR.
Materials and methods: The intestinal metabolomes were analysed by Metabolomics Discoveries (Potsdam, Germany) in mice with and without CR. Briefly, colonization resistance is abrogated in mice treated with antibiotics such as ampicillin, ciprofloxacin, vancomycin, metronidazole or imipenem (single and in combination), in conventional infant mice, and in mice harbouring a human microbiota. Conventional adult mice served as controls with CR.
Results: Analysis of the intestinal metabolomes in mice with and without CR against Cj indicated that phenolic compounds might be involved in mediating CR against *Campylobacter*.
Conclusion: Gut metabolites conferring CR will be used for prevention of Cj colonization in poultry as well as for treatment capacities in murine infection models within the PAC-Campylobacter consortium. Murine infection models will aid to validate preventive or therapeutic measures for the final transfer to the pharmaceutical or product level in humans or farm animals, respectively.

Presentation on Wednesday, February 27, 2019 from 8:30 – 8:40 in room Hörsaal 007.

091/ZOV

Polyphenolic compounds alleviate *Campylobacter jejuni* induced acute enterocolitis in secondary abiotic IL-10$^{-/-}$ mice

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Background and objectives: Our recent intestinal metabolomic analyses revealed that phenolic compounds might be involved in mediating colonization resistance against *Campylobacter*. We here addressed whether peroral application of synthetic resveratrol or curcumin might be therapeutic measures for combating *C. jejuni* induced immunopathology.
Materials and methods: Secondary abiotic IL-10-/- mice were subjected to resveratrol or curcumin treatment via the drinking water starting four days prior peroral challenge with viable *C. jejuni* 81-176 strain (day 0).
Results: Six days post *C. jejuni* infection (p.i.), polyphenol-treated mice developed significantly less severe symptoms as compared to placebo controls - with most beneficial effects in the curcumin cohort. Particularly curcumin-treated mice further displayed less pronounced apoptotic cell and pro-inflammatory immune responses that were not restricted to the intestinal tract, but could also be observed in extra-intestinal compartments and, remarkably, systemically. Strikingly, intestinal *C. jejuni* loads of curcumin-treated mice were approximately 7 log orders of magnitude lower at day 6 p.i. as compared to untreated controls with median fecal burdens of 109 CFU per g.
Conclusion: Due to its potent anti-*Campylobacter* and anti-inflammatory effects in murine infection models, curcumin represents a promising option for treatment and prophylaxis of *Campylobacter* infection and colonization in humans and farm animals, respectively.

Presentation on Wednesday, February 27, 2019 from 8:45 – 8:55 in room Hörsaal 007.

092/ZOV

Differentiation of Shiga toxin (Stx) subtypes released by Stx-producing *Escherichia coli* wild-type strains using real-time interaction analysis and mass spectrometry

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Introduction: Shiga toxins (Stxs) of Stx-producing *Escherichia coli* (STEC) and enterohemorrhagic *E. coli* (EHEC), the human-pathogenic subgroup of STEC, represent the key virulence factors released by these pathogens. The Stx subtypes Stx1a and Stx2a are associated with the life-threatening hemolytic uremic syndrome (HUS), whereas Stx2e in human STEC isolates may predict a milder disease with a minimal risk of HUS. The Stx B-pentamer (AB5-toxin) of all Stx subtypes analysed so far preferentially binds to the glycosphingolipid (GSL) globotriaosylceramide (Gb3Cer) exposed on endothelial cells of human kidneys and the brain [1].
Objective: Since the initial binding process of Stx to membrane-inserted GSL receptors is poorly understood, we focused in this study on the label-free real-time interaction analysis of Stx subtypes with Gb3Cer-spiked biomimetic membranes.
Materials and Methods: Stx1a, Stx2a, and Stx2e of EHEC wild-type strains were affinity-purified using Gb3-functionalized magnetic beads. Real-time interaction analysis was performed employing the surface acoustic wave (SAW) technology. The dissociation constant KD, which describes the strength of binding between Gb3Cer and Stx, was calculated for Stx1a, Stx2a, and Stx2e. Structural identification of Stx holotoxins was performed by electrospray ionization mass spectrometry (ESI MS) and Stx-caused cytotoxic effects were determined in Vero cell culture assays.
Results: The miniaturized one-step affinity purification resulted in highly purified native Stx1a, Stx2a, and Stx2e subtypes derived from EHEC culture supernatants. SAW real-time interaction analysis revealed characteristic binding kinetics of each Stx subtype. Determined KD-values were in the nanomolar range and differences in the association and dissociation curves indicated distinct receptor binding strength of the three Stx subtypes. ESI MS investigations on proteolytic digests yielded Stx subtype-specific diagnostic peptide ions allowing for a fast and facile MS-based identification of Stx1a, Stx2a, and Stx2e. Cytotoxicity studies revealed differing toxin-mediated cell damage ranking with Stx1a > Stx2a > Stx2e.
Conclusion: Collectively, this matched procedure represents a promising clinical application for the differentiation of life-endangering Stx subtypes on protein level.
[1] Legros N, Pohlentz G, Steil D, Müthing J. (2018). *Int. J. Med. Microbiol*., pii: S1438-4221(18)30362-X.

Presentation on Wednesday, February 27, 2019 from 9:00 – 9:10 in room Hörsaal 007.

093/ZOV

Dissecting Native EHEC Outer Membrane Vesicles by Creating Synthetic Surrogates

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Introduction: Enterohaemorrhagic *Escherichia coli* (EHEC) are the major cause of the life-threatening haemolytic-uraemic syndrome (HUS). Major virulence factors include a panel of different toxins, the composition of which can differ significantly between strains, with Shiga toxin (Stx) being the most important one in molecular pathogenesis. Apart from free, soluble toxins the delivery via outer membrane vesicles (OMVs) is being increasingly recognized as a possible route. However, due to native OMVs (nOMVs) carrying a

toxin cocktail the contribution of each individual toxin to EHEC pathogenesis remains enigmatic.
Objectives: To dissect the influence of single toxins in pathogenesis, we established a method to create defined OMV-like molecular structures, synthetic OMVs (sOMVs), and compared them with nOMVs.
Materials and Methods: The first step in sOMV production was the creation of homogenous liposomes resembling the *E. coli* outer membrane lipid composition by sonication/extrusion. The quality of the liposomes was controlled via dynamic light scattering. Second, Stx-loaded liposomes were assembled by dehydration/rehydration. Then, the cytotoxicity towards epithelial cells of these sOMVs, containing only one toxin, was compared with nOMVs from EHEC strain HUSEC029, which bears no other OMV-associated toxin except Stx, and also free Stx.
Results: Using the described method we were able to reproducibly prepare Stx-loaded sOMVs with an encapsulation efficiency of ca. 52% which are also stably storable at 4°C for several months. Applying free Stx, Stx-sOMVs , and nOMVs to epithelial HCT-8 and Caco-2 and endothelial HBMEC, all administration forms had an cytotoxic effect on the cell lines with HBMEC being in general the most sensitive and Caco-2 the least sensitive cells. Moreover, application of free Stx had the most pronounced cytotoxic effect, with sOMVs and nOMVs having lower, but comparable effects.
Conclusions: The successful establishment of a preparation method for Stx-sOMVs and their actual cytotoxic effect allows the usage of sOMVs as surrogates of nOMVs and the testing of other toxins and their combinations with this method in the future.

Presentation on Wednesday, February 27, 2019 from 9:15 – 9:25 in room Hörsaal 007.

094/ZOV

Genotypic analysis of antibiotic resistance patterns in Coxiella burnetii

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Question: The obligate intracellular organism *Coxiella (C.) burnetii* is the causative agent of the Q fever disease, a worldwide Zoonosis. Human infection usually occurs after contact with infected animals, predominantly sheep and goats. Most of the infections are an acute disease but up to 2% can show a chronic form. Therapy is based on doxycycline, but gyrase inhibitors and macrolide antibiotics have been used also for therapy. Due to its intracellular lifestyle antibiotic susceptibility testing is not routinely performed. In the here presented study we examined for the first time all published 55 genomes of Coxiella burnetii for the presence of and variations in antibiotic resistance (AR) genes for six classes of antibiotics.
Methods:
A pipeline was written using the command language Bash and the high-level programming language Python. Via the stand-alone version of blast+, possible antibiotic resistance determining genes were located in whole genome data of *C. burnetii*. These genes were then aligned using the MAFFT algorithm. Additionally, ABRICATE was used to screen for known antibiotic resistance genes using the databases CARD, ARGANNOT and Resfinder. After analysis of genetic markers, a PCR-based single probe DNA assay was designed for a reliable genotypic identification of potential antibiotic resistances in various strains.
Results:
No mobile genetic elements containing AR genes have been found. The previously annotated beta lactamase ampC of *C. burnetii* str. Dugway was found with high sequence similarity in all studied genomes. Additionally, a previously unknown mutation in the Topoisomerase IV subunit b gene parE outside the Quinolone-resistance determing regions (QRDR) was identified. 26 *C. burnetii* strains from different countries and species were screened and four presented this new polymorphism.
Conclusion:
Coxiella burnetii does not harbour any acquired AR genes. The occurrence of beta lactamase ampC could serve as a hint for the ineffectivity of beta lactams in Q fever therapy. The described variation of the parE gene is associated with increased resistance against fluoroquinolones in combination with gyrA mutations in E. coli. The possible role of the parE polymorphism without this gyrA variation in the antibiotic resistance profile of C. burnetii still needs to be determined. Using the recent described axenic culture media ACCM2/-D will offer the ability to correlate pheno- and genotypic characteristics of isolates in the future.

Presentation on Wednesday, February 27, 2019 from 9:30 – 9:40 in room Hörsaal 007.

095/ZOV

New Bacterial Taxa Recovered from the Porcine Nasal Cavity

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The domestic pig population became the main host population within the evolution of the livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) clonal complex (CC) 398 lineage. Since LA-MRSA CC398 contribute to the MRSA burden in human health care, knowledge on the porcine nasal microbiota is needed to develop eradication strategies; however, there are limited data available on the composition of this habitat. Here, a culturomics approach has been applied to catalog and analyze the nasal microbiota of pigs in an MRSA CC398 endemic region.
Using a culturomics approach, the porcine nasal microflora has been analyzed with special regard to unknown bacterial species.
Nasal cavities and snout surfaces of 24 pigs were each sampled using cotton swabs and transported in Amies medium. Sodium chloride solutions were prepared and plated onto blood, chocolate, CAP, and MacConkey agar plates and incubated under aerobic conditions (chocolate agar:+ 5 % CO2). Likewise, Schaedler, Schaedler + K/V, chocolate and CAP agar plates were inoculated and incubated anaerobically. Incubation conditions were 35 °C for 48 hours. Sequencing of the 16S rRNA gene was carried out for isolates which failed to be identified with MALDI-TOF MS. The BLAST algorithm was used for comparison of sequences to the NCBI database. Similarity scores of <98.7 % and 95 % were taken to determine candidates for new species and new genera, respectively. Isolates were frozen at -80 °C.
To date, 34 candidates for new bacterial taxa were found. Of these, seven are candidates for new bacterial genera. The closest matching genera of the seven isolates are *Propioniciclava*, *Dysgonomonas*, *Globicatella*, *Tissierella*, *Gallicola*, *Sporanaerobacter*, and *Luteimicrobium*. The remaining 27 candidates represent new species within the genera of *Acinetobacter*, *Leucobacter*, *Aerosphaera*, *Globicatella*, *Bacillus*, *Caryophanon*, *Jeotgallicoccus*, *Helcococcus*, *Anaerocella*, *Propionibacterium*, *Tessaracoccus*, *Dermabacter*, *Rothia*, *Myroides*, *Wohlfahrtiimonas*, *Comamonas*, *Lampropedia*, *Luteimonas*, *Stenotrophomonas*, *Pseudomonas*, and *Moraxella*.
The porcine nasal culturome is immensely diverse and in part unexplored. The colonization with multiple hitherto undescribed bacteria indicates that it is important to include culturomics in future microbiota studies. The cataloged, characterized and biobanked isolates will form a strain collection as basis for future studies on antagonistic inter-taxa interactions.

Presentation on Wednesday, February 27, 2019 from 9:45 – 9:55 in room Hörsaal 007.

Workshop 18
Out-Patient, in-Patient, no Patient: Bacteria, Infections and Intervention Strategies (FG PR, StAG HY)
27. Febr. 2019 • 08:30-10:00

096/PRV

Molecular Typing of *Neisseria gonorrhoeae* Strains in Germany (2014-2017) by *Neisseria gonorrhoeae* Multiantigen Sequence Typing (NG-MAST)

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Question and Goals

Worldwide, antimicrobial resistance (AMR) of *Neisseria gonorrhoeae* (NG) is on the rise, posing a threat for treatment and control of gonorrhoea. As NG infections are not reportable in Germany, data on circulating sequence types and associated AMR are scarce. The Gonococcal Resistance Network (GORENET) is a laboratory network to monitor trends in the occurrence of AMR in Germany, which links data from sequence typing to epidemiological data. This allows us to describe prevalence of gonococcal sequence types in Germany and associations to AMR to improve future treatment and prevention strategies.

Methods

From the NG samples collected between April 2014 and December 2017 within the framework of the GORENET, isolates were selected for antimicrobial susceptibility testing by E-test and sequence typing by Neisseria gonorrhoeae multiantigen sequence typing (NG-MAST; Martin et al., 2004). For sequence typing, DNA was extracted and internal fragments of *porB* and *tbpB* were amplified by polymerase chain reaction (PCR). Fragments were purified and sequenced by Sanger sequencing. Sequence types were evaluated using a global database (www.ng-mast.net). Genogroups were assigned to sequence types which shared one allele and exhibited ≥99% homogeneity in the other allele.

Results and Conclusions

In total, 1220 isolates were sequence typed (106 in 2014, 96 in 2015, 525 in 2016, and 495 in 2017). Approx. 90% of these were isolated from men, 10% from women. The median age was 33 years (IQR 17 years). In general, the NG population in Germany appears highly diverse. In 2014/15, genogroup G1407 was predominant in Germany. This genogroup has been associated with cefixime resistance. In 2016, prevalence of G1407 decreased and fewer AMR isolates were detected in all isolates monitored. Interestingly, prevalence of several new genogroups increased in 2016 and 2017. To verify these results, continuous testing with an increased number of isolates should be performed.

References

Martin IM, Ison CA, Aanensen DM, Fenton KA, Spratt BG. 2004. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J. Infect. Dis. 189:1497–1505.

Presentation on Wednesday, February 27, 2019 from 8:30 – 8:40 in room Hörsaal 005.

097/HYV

Infectious disease prevention – the "diagnostics-as-prevention" (DasP) strategy against sexually transmitted infections (STI)

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Introduction: Diversification has recently been observed in the field of prevention of transmission of sexually transmitted infections (STI). For all those for whom monogamy or sexual abstinence are no desirable options, condom use largely remained without alternative as a preventive strategy against STI for a long time. Recently, however, alternative options have emerged. While HIV pre-exposure prophylaxis (PrEP) is now widely known, this is much less true for the "diagnostics-as-prevention" (DasP) strategy developed in the demimonde of the red light milieu. The potential sexual partners are examined before intended unprotected sexual risk contacts in a concerted manner with point-of-care rapid diagnostic tests (RDT) in order to minimize the individual transmission risk by abstaining from unprotected sex in case of positive test results. Fundamental preventive effectiveness of this strategy is demonstrated by modeling-based approaches.

Methods: Based on performance characteristics data from available immunochromatographic and molecular RDT for STI as well as on incidence and prevalence data of the assessed STI, reduction of exposure risk to causative agents of STI by applying DasP was calculated. Condom-based prevention was considered as reference standard.

Results: For HIV, DasP even allowed a clearer reduction of exposure than condom use in the most situations in the modeling. The weakness of rapid immunochromatographic tests in the early infective stages of HIV infection can be partially compensated by the addition of PCR-based RDT systems, which are increasingly available as point-of-care options. As DasP is not necessarily limited to HIV prevention, multi-test prevention approaches can be extended to other STI depending on the availability and affordability of reliable rapid test systems, further reducing the overall exposure risk for methodological reasons.

Conclusions: With a technically broad "diagnostics-as-prevention" approach, sexual risk-taking populations could considerably reduce their STI exposure risk. Most recently, RDT for HIV have become available for home testing. With condom, HIV-PrEP and DasP, a number of strategies for the individual prevention of the transmission of STI are available. Each of them has different directions of impact and their practical feasibility and acceptance in society beyond mathematical modeling ultimately remain to be seen.

References on pubmed: PMID:29655384, PMID:29997911, PMID:30074254.

Presentation on Wednesday, February 27, 2019 from 8:45 – 8:55 in room Hörsaal 005.

098/PRV

Detection of risk factors for long-term vancomycin-resistant enterococci colonization

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Introduction

Vancomycin-resistant enterococci (VRE) have raised concern worldwide due to their antibiotic resistance and capability to spread in healthcare settings, with rates of nosocomial infections increasing continuously in the last years in Germany and several

European countries. Actively detecting colonization is a key to avoiding further transmission. Official guidelines recommend hygiene measures such as isolation of colonized patients and environmental disinfection. These may only be discontinued once a spontaneous clearance occurs, as specific eradication strategies are lacking. However, VRE can persist over long periods of time. Although several risk factors for acquisition of VRE have been identified, little is known about those determining a prolonged colonization.

Objectives

The aim of this study is to identify the rate of VRE persistence and risk factors favoring long-term VRE colonization status.

Materials and Methods

Patients at risk of acquiring VRE were screened upon admission over a period of 24 months. The persistence and clearance of colonization was assessed by performing subsequent screening of positive patients on readmission. Risk factors were identified in order to determine their association with long-term colonization.

Results

In total, 1157 patients (48.5% male, median age 59 years) with evidence of VRE upon first hospital admission were included in the study. Results of the preliminary data analysis indicate that VRE persisted on readmission in approximately 30% of the cases. After analyzing the presence of different factors, several of them were more frequently observed in subjects continuously colonized. Conditions showing a significant association with this long-term colonization were long hospitalization duration, liver dysfunction, dialysis and antibiotic therapy. Of the patients studied, ca. 70% experienced a spontaneous clearance of the colonization.

Summary

First results of our study suggest that VRE colonization persistence is potentially overestimated. Long-term colonization is favored by patient-associated risk factors such as prolonged hospitalization, liver dysfunction, antibiotic therapy and hemodialysis, also known to promote initial VRE colonization as well.

Presentation on Wednesday, February 27, 2019 from 9:00 – 9:10 in room Hörsaal 005.

099/PRV

Asymptomatic carriage rate of toxigenic *Clostridioides difficile* in a geriatric hospital department in Germany

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Background:

Clostridioides (*C.*) *difficile* strains harbouring the toxin gene *tcdB* can cause severe enteritis once the physiological microbiota has been disrupted in patients, in particular by antibiotics. The aim of this study was to determine the asymptomatic carriage rate with toxigenic *C. difficile* strains in patients treated in a geriatric hospital department.

Methods:

This study was a partial evaluation (April - September 2017) of stool samples taken weekly on 4 geriatric wards in a 95-bed geriatric department in a standard care hospital (project ReToCdiff, funded by Innovationsausschuss, project number: 01VSF16059). Samples were plated onto chromogenic agar media and incubated at 37°C for at least 48 h in an anaerobic atmosphere. Colonies displaying morphological features suggesting *C. difficile* were stored at -80°C and later underwent PCR for the detection of *gluD* for species confirmation as well as *tcdB.*

Results:

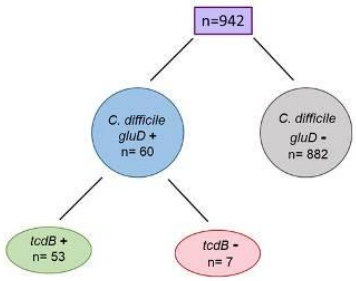
Stool samples were available from 942 patients with a median age of 82.4 years. *C. difficile* was found in samples of 60 patients (6.4%). Of these, a toxigenic strain was found in 53 patients, i.e., in 5.6% of all patients from which stool samples were available, and in 88.3% of all patients carrying *C. difficile*.

Discussion:

Tests on asymptomatic carriage of toxigenic *C. difficile* are not performed routinely, and therefore data are scarce. Our data of 5.6% carriage rate fall between those reported for nursing home residents in Hesse (4.6%) and in German rehabilitation centres (11.1%). Knowing the asymptomatic carriage rate for toxigenic *C. difficile* is important to estimate the proportion of patients at risk for an endogenous symptomatic *C. difficile* infection. Moreover, asymptomatic carriage might serve as an underestimated reservoir for transmissions.

Figure 1: Stool samples from 942 patients without diarrhea were screened by culture for *C. difficile*. Colonies displaying morphological features suggesting *C. difficile* were stored at -80°C and later underwent PCR for the detection of *gluD* for species confirmation as well as *tcdB.*

Figure 1



Presentation on Wednesday, February 27, 2019 from 9:15 – 9:25 in room Hörsaal 005.

100/PRV

Dissemination of antibiotic resistant bacteria into German wastewater and surface waters

N. Zacharias*[1], C. Timm[1], C. Schreiber[1], R. Schmithausen[2], T. Kistemann[1], M. Exner[2]

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The intense use of antibiotics in human and veterinary medicine, in animal farms and in agriculture has led to a wide spread of antibiotics and antimicrobial resistance in the environment. Antibiotic resistance is defined by the WHO as a threat to the world population. The WHO highlighted the gaps in the importance of the water path for the dissemination of antibiotic resistance. In a note from 2014 they point out: "There is growing public health concern that antimicrobial-resistant bacteria and AMR genes in human waste could potentially enter water sources via untreated or treated wastewater effluent and result in the exposure of human populations […] Overall, there is a lack of reliable, well documented and validated human health risk assessments for antimicrobial-resistant bacteria and AMR genes from such environmental exposures [...]" (WHO, 2014).

Within a sub-study of the multidisciplinary joint research project HyReKA the pathways by sewage from a clinical/urban and rural/municipal wastewater were investigated. Clinical/urban water samples were taken from wastewater of patient rooms´ sanitary facilities, at different sampling points within the connected sewer

system and the sewage treatment plant (STP) as well as from the receiving surface waterbody. The rural/municipal catchment area was sampled at eight different surface water sampling sites of a river system influenced and not influenced by municipal wastewater, including influents and effluents of four discharging municipal STPs (not influenced by hospitals or industry).
Antibiotic resistant bacteria were grown on selective agar plates, differentiated by morphological and physiological characteristics and confirmed using MALDI-TOF MS. The resistant strains were epidemiologically classified and the antibiotic resistance was further tested using the minimal inhibition concentrations.
Urban/Clinical wastewaters were charged with a higher load of multidrug resistant bacteria in comparison to the rural/municipal wastewater. Although most of these bacteria were eliminated during wastewater treatment, dissemination into surface waters is possible as single resistant bacteria were still present in the effluents of the wastewater treatment plants.Human wastewater is the main source of fecal contamination - and consequently most likely the origin of ARB and ARGs.
WHO (2014): Antimicrobial resistance: global report on surveillance.

URL:
http://www.who.int/drugresistance/documents/surveillancereport/en/

Presentation on Wednesday, February 27, 2019 from 9:30 – 9:40 in room Hörsaal 005.

101/PRV

Full recording of nosocomial postoperative infections during a pre-intervention phase of a planned intervention study in a German Level A Traumacenter over a period of seven months

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Introduction
As part of the BMG-funded project "HygArzt" (ZMVI1-2516FSB111) nosocomial infection rates are to be minimized in the long term prevention through an evidence based intervention bundle by an infection control practicioner. To assess the initial situation, a surveillance system consisting of 97 outcome parameters which included all nosocomial infections was developed and introduced in three orthopedic/trauma surgery normal wards.
Methods
In the pre-intervention phase, 1503 patients who had undergone orthopedic/trauma surgery (1972 surgeries - partly consisting of several surgeries on the same patient) and were admitted to hospital were included. To identify and record nosocomial infections, clinical signs of infection according to KISS and CDC definitions were recorded in a checklist. For this, data from pre-existing conditions as well as current and previous infections found out from the hospital administration system, admission forms, discharge letters and nursing documentation, were aggregated with current patient data as well as with laboratory findings. In addition, three times a week the morning visit was attended to record not documented signs of infection.
Results
A total of 52 nosocomial infections according to CDC definitions (infection rate by 2.6%, CI 95% 1.92%; 3.28%) 85 brought infections in 1972 surgeries were documented. Among the most common procedures were 233 lower leg procedures including metal removal, VAC changes and wound revisions, 167 knee arthroscopy, 115 cruciate ligament replacements. The nosocomial postoperative wound infections formed the majority with 39 cases (2.0% crude infection rate, CI 95% 1.40%; 2.60%). The number of surgeries in patients with postoperative wound infections was considered only up to the time the infection occurred. The pathogens found were predominantly *S.aureus* (MSSA) with 20 infections, followed by coagulase-negative Cocci mainly with *S.epidermides* (11 infections) and Enterobacteriaceae with *P. aeroginosa, E. cloacae complex* and *C.koseri* (8 infections). In addition to the wound infections, 4 catheter-associated urinary tract infections, 3 pneumonia, 3 *C. difficile* infections, 1 bronchitis and 1 thrombophlebitis could be detected nosocomially. So far, no infection rates have been calculated for these, as data on patient days are not yet available. Currently, a follow up of 30 days can take place in wound infections.
Summary
In the pre-intervention phase of the HygArzt study an overall nosocomial infection rate of 2,6% was determined. As main infection prevention target postoperative wound infections were identified, which should mainly be addressed by planned infection prevention measures bundle during the upcoming intervention period.

Presentation on Wednesday, February 27, 2019 from 9:45 – 9:55 in room Hörsaal 005.

Workshop 19
Intestinal Microbiota - a Track Record of DFG Priority Programme 1656 (FG PW)
27. Febr. 2019 • 08:30-10:00

102/PWV

Modulation of intestinal homeostasis and inflammation by *Prevotella intestinalis* (nov. sp.)

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Prevotella is a complex genus of anaerobic Gram-negative bacteria of the Bacteroidetes phylum. Several studies have suggested *Prevotella copri* may be a beneficial member of the gut microbiota in humans since it has been found to improve glucose metabolism and it is predominantly prevalent in people who consume a plant-rich diet. In contrast, other studies have associated *Prevotella copri* with autoimmune diseases and gut inflammation. In mouse models, *Prevotella*-dominated microbiome was associated with higher susceptibility to chemically-induced colitis suggesting that *Prevotella* may have the ability to promote intestinal inflammation. Detailed investigation of the cause for divergent modulation of host physiology by *Prevotella* is however limited by the poor characterization of *Prevotella* species and the lack of diverse intestinal *Prevotella* isolates. Here we isolated a novel intestinal *Prevotella* specie (*Prevotella intestinalis)* and investigated the impact of its colonization on the interplay between host and the microbiota during intestinal homeostasis and inflammation. We found that *P. intestinalis* colonization of WT specific pathogen free (SPF) mice, devoid of any *Prevotella* spp. in the intestine, reshapes the resident intestinal microbial community and it significantly alters the metabolic profile in the intestine. *Prevotella*-induced changes in the levels of short-chain fatty acids (SCFA) modulated colonic interleukin (IL)-18 expression and production during homeostasis which significantly increased the susceptibility of *Prevotella*-colonized mice to intestinal inflammation in DSS-colitis model. We are further investigating whether *P. intestinalis* is directly effecting the concentration of SCFA in the intestine, or indirectly by altering the abundance of SCFA-producing bacteria.

Presentation on Wednesday, February 27, 2019 from 8:30 – 8:40 in room Hörsaal 004.

103/PWV

Mucispirillum schaedleri protects mice against non-typhoidal _Salmonella_ colitis by interfering with virulence factor expression

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The microbiota and host genotype determine susceptibility to enteric *Salmonella enterica* serovar Typhimurium (*S.* Tm) infection. In particular, the intestinal mucus layer plays an important role in innate immune defense. Here, we analyzed the course of *S.* Tm colitis in *Agr2*-/- mice lacking a functional mucus layer in the gut. Unexpectedly and in contrast to *Agr*+/- littermates, *Agr2*-/- mice were protected against *S.* Tm induced gut inflammation in the streptomycin colitis model. This correlated with microbiota alterations and enrichment of bacteria of the phylum Deferribacteres. Using gnotobiotic mouse models, we identified *Mucispirillum schaedleri*, currently the sole known representative of the phylum Deferribacteres present in the mammalian microbiota, to be causally involved in protection against *S.* Tm colitis. Inhibition of *S.* Tm by *M. schaedleri* involves interference with invasion gene expression, possibly by competing for anaerobic electron acceptors. In conclusion, this study establishes *M. schaedleri*, a core member of the murine gut microbiota, as key antagonist of *S.* Tm virulence in the gut.

Presentation on Wednesday, February 27, 2019 from 8:45 – 8:55 in room Hörsaal 004.

104/PWV

Functional Characterization of Microbial Signatures in Inflammatory Bowel Disease Using Gnotobiotic Humanized Mice

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Substantial effort has been dedicated to evaluate the use of microbial signatures as a diagnostic tool for IBD. While cross-sectional studies showed an association between disease activity and an altered microbial composition, a causative role is not yet clear. We characterized gut microbiota from 3 longitudinal IBD cohorts to identify microbial signatures linked to change of disease state, or response to therapy. Dysbiosis, as measured by community diversity and stability was pronounced in active patients compared to patients in remission. Patients' gut microbiota composition fluctuated dramatically through the course of disease, however stratification of patients by disease phenotype or response to therapy led to significant separation between subgroups. To address the functional impact of microbial profiles in IBD, we established a humanized IBD model by colonizing germfree IL-10-/- mice with fecal samples from CD patients. We selected 3-paired samples from CD patients representing different disease activities and community clusters. Humanized mice recapitulated the disease phenotype and microbial dysbiotic features of their respective human donors after 4 weeks of colonization. 16S rRNA gene sequencing showed that different microbial profiles could drive inflammation in IL10-/- mice humanized with CD-associated microbiota. Consistent with individually diverse microbiota profiles in CD patients, inflammation in gnotobiotic mice was driven by various community profiles. Using a machine-learning approach, we identified a panel of 10 OTUs that discriminates humanized mice by inflammatory status. A microbial signature characterized by an overabundance of *Bacteroides fragillis* and *Desulfovibrio* classified humanized mice by inflammation with high accuracy. To define changes in the gut metabolome, we used a targeted metabolomics approach to measure bile acids in fecal samples from human donors and humanized mice. Metabolic profiles varied between disease-associated and remission-associated humanized mice, suggesting a microbiota-dependent alteration of metabolic functions in driving disease in the host. The amount of certain metabolites, including Taurocholic acid and Lithocholic acid varied significantly between inflamed and non-inflamed mice. Our data suggests that IBD pathogenesis involves disruption of the functional diversity and structural complexity of gut microbial ecosystems, and support the translational validity of the gnotobiotic mouse models.

Presentation on Wednesday, February 27, 2019 from 9:00 – 9:10 in room Hörsaal 004.

105/PWV

Enrich the cultured diversity of mouse gut microbiota towards computation-based design of minimal bacterial consortia

T. Streidl[1], N. Kumar[1], I. Lagkouvardos[2], A. Afrizal[1], T. Hitch[1], T. R. L. Lesker[3], N. Smit[3], H. Huang[2], T. Riedel[4], B. Abt[4,3], D. Haller[2], T. Strowig[5], B. Stecher[3,2], J. Overmann[4,3], T. Clavel*[1]

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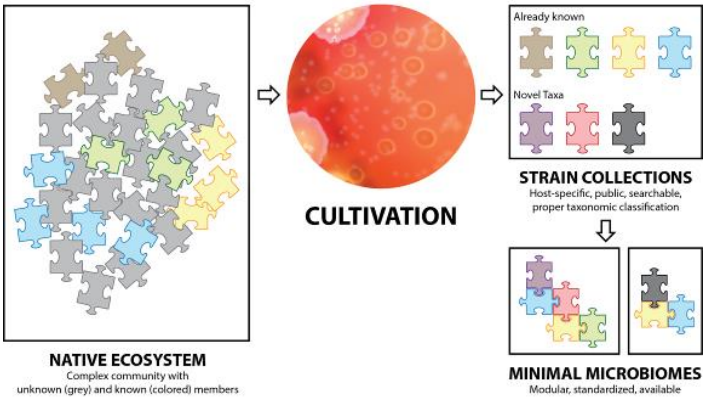
Background: Many bacteria within mammalian gut microbiota are still unknown, which prevents proper investigation of microbe-host interactions. There is also an urgent need to guarantee public access to isolates and simplified communities for the purpose of standardized and individualized gnotobiotic studies.

Aims: Extend diversity of the mouse intestinal bacterial collection (www.dsmz.de/miBC) and develop a new computational tool, MIMIC, for genome-guided design of personalized minimal bacterial consortia.

Results. We reached 100 species available in miBC via the inclusion of approx. 20 taxa not represented in the original collection, including eight new bacteria (four genera and four species). Specific analyses included sequence-based and cultivation study of the mouse-specific family S24-7, for which we propose the name *Muribaculaceae* to resolve its still ambiguous taxonomy. Interesting functions were identified within miBC members, such as the ability to produce lipases or to dehydroxylate bile acids. As it is challenging to study microbial community dynamics in the gut because of the high ecosystem diversity, it is important to create model systems of reduced complexity. However, comprehensive strategies for construction of such simplified communities are lacking. Using all bacterial genomes available in NCBI (n = 105,507) and our own collections, we built a binary matrix (presence/absence) of protein families (Pfam = 16,295) and linked it to taxonomic lineage and BacDive entries (e.g. cultivability). We showed that Pfam vectorization retains enough resolution to distinguish metagenomic profiles from various environments and from mouse gut microbiota associated with healthy or inflamed conditions. The current version of MIMIC predicts minimal bacterial compositions using an iterative scoring system based on maximal match-to-mismatch ratios between single genomes and the given Pfam-vector of an input metagenome. First results on mock communities (mixtures of known microbes) indicated that MIMIC-selected species provided a better coverage of metagenomic functions compared to 500 randomly picked sets. Current work includes refinement of the tool by reducing

redundancy of the Pfam-annotated genome database, weighing functions during scoring, and development of modelling approaches for testing inferred communities.
Conclusions. miBC and MIMIC are helpful resources that will facilitate the implementation of novel functional studies of the gut microbiome.

Figure 1



Presentation on Wednesday, February 27, 2019 from 9:15 – 9:25 in room Hörsaal 004.

106/PWV
Role of host glycans in mediating infection and susceptibility upon *Salmonella* infection
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The gastrointestinal tract expresses a substantial amount of glycoconjugates which constitute an important factor contributing to the composition and physiology of the intestinal microbiota. Variation in host glycosylation can mediate susceptibility to intestinal inflammation caused by infection with enteric pathogens. Indeed, changes in the glycosylation profile of the GI tract is often mediated by variation in histo-blood group glycosyltransferases. In this study, we investigated the role of two histo-blood group glycosyltransferase genes, B4galnt2 and Fut2. The glycosyltransferase gene *B4galnt2* encodes a beta-1,4-N-acetylgalactosaminyltransferase known to catalyze the last step in the biosynthesis of the Sd(a) and Cad blood group antigens and is expressed in the GI tract of most mammals, including humans. The *Fut2* gene encodes a α-1,2-fucosyltransferase responsible for the expression of ABO histo-blood group antigens on the gastrointestinal mucosa and bodily secretions.
To investigate the role *B4galnt2* and *Fut2* genes in the susceptibility to bacterial-induced inflammation and their influence on microbiota diversity.
Using a *Salmonella* Typhimurium infection model, *B4galnt2* and *Fut2*-deficient mice were infected. Bacterial counts, histopathological scoring and immune parameter were analysed in the intestines of infected wild-type and knock-out mice.
In our study,we found *B4galnt2* intestinal expression was strongly associated with increased susceptibility to *Salmonella* infection as evidenced by increased histopathological changes, intestinal inflammatory cytokines and infiltrating immune cells. Fecal transfer experiments demonstrated a crucial role of the *B4galnt2* dependent microbiota in conferring susceptibility to *Salmonella* infection. Similarly, *Fut2* intestinal expression was also associated with increased susceptibility to *Salmonella* infection at day 7 after infection. In contrast, the deletion of *std* fimbriae known to be important for adherence of *S.* Typhimurium to fucosylated intestinal epithelial cell lines, displayed similar levels of colonization and immune parameter in both *Fut2*+/+ and *Fut2*-/- mice.
Overall,the expression of *B4galnt2* and *Fut2* genes mediate *Salmonella* colonization and intestinal inflammation. Our B4galnt2 study showed that the difference in microbiota composition is responsible for the different susceptibility to Salmonella infection. Whereas our Fut2 study demonstrated the importance of Std-fucose interaction during infection.

Presentation on Wednesday, February 27, 2019 from 9:30 – 9:40 in room Hörsaal 004.

107/PWV
Gut microbiota-derived short chain fatty acids are precursors for hepatic lipid synthesis
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Interactions between the gut microbial ecosystem and host lipid homeostasis are highly relevant to host physiology and metabolic diseases. We present a comprehensive multi-omics view of the effect of intestinal microbial colonization on hepatic lipid metabolism, integrating transcriptomic, proteomic, phosphoproteomic, and lipidomic analyses of liver and plasma samples from germfree and specific pathogen-free mice. Microbes induce monounsaturated fatty acid generation by stearoyl-CoA desaturase 1 and polyunsaturated fatty acid elongation by fatty acid elongase 5, leading to significant alterations in glycerophospholipid acyl-chain profiles. A composite classification score calculated from the observed alterations in fatty acid profiles in germfree mice clearly differentiates antibiotic-treated mice from untreated controls with high sensitivity. Mechanistic investigations reveal that acetate originating from gut microbial degradation of dietary fiber serves as precursor for hepatic synthesis of C16 and C18 fatty acids and their related glycerophospholipid species that are also released into the circulation.

Presentation on Wednesday, February 27, 2019 from 9:45 – 9:55 in room Hörsaal 004.

Workshop 20
Basishygiene: Wissen alleine reicht nicht - auf die Umsetzung kommt es an (FG PR, StAG HY)
27. Febr. 2019 • 13:00-14:30

108/PRV
Measurement of adherence to infection prevention measures in operating rooms as part of the HYGARZT Project
J. Hoffmann*[1], S. Herbrandt[2], M. Strybos[1], S. Akca[3], U. Ligges[2], C. Kugler[3], F. Mattner[1]
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[2]*Technische Universität, Fakultät Statistik, Dortmund, Germany*
[3]*Albert-Ludwigs-Universität Freiburg, Institut für Pflegewissenschaft, Freiburg i. Br., Germany*

Question In the *HYGARZT* Project the implementation and effectiveness of infection prevention and control (IPC) measures in operating rooms (OR) by infection prevention and control link practitioners (IPC-LP) will be investigated. The adherence with existing IPC measures (KRINKO requirements, 5 moments of WHO) was evaluated. Here, the results of the pilot phase are presented.
Method Adherence with existing IPC-measures (hand hygiene (HH), donning of personal protection equipment (PPE), private conversation, opening of the doors to the OR, entering and exiting

of persons) was measured, according to staff group and staff function. Items on the checklist were based on findings of a systematic literature review. IPC measures were observed from the beginning of the preparation phase until the end of wound dressing. The findings were descriptively analyzed using "R" as software for statistics.

Results 95 surgical procedures were observed in three operation rooms. The duration between incision and suture was 57 min (± 42 min), the observation time was 82 min (± 38 min). Overall 1945 HH indications were identified. Adherence to HH during preparation before incision (without surgical hand disinfection) was 5,5% concerning surgeons, 13,1% concerning nursing staff respectively, 14,6% concerning surgeons, 19,8% concerning nursing staff after surgery respectively. Among all staff 69 HH was performed without indication. The correct donning of PPE was: surgical caps surgeons 78,9%, nursing staff 38,1% or surgical masks surgeons 92,2%, nurses 86%, respectively. In 31,6% of the procedures, private conversation was held, most of them (23,2%) were held by the surgical nurse, followed by the surgeon (22,1%). The doors to the OR were opened in 57 cases (60%) and in 30 cases (31,6%) persons entranced or leaved the OR.

Conclusion Maybe the OR suggests the staff, to be in a particularly clean area, where IPC measures might not be necessary, as in other parts of the hospital. A change in the awareness of the role of IPC measures in the staff is of vital importance to reduce nosocomial infections. Overall, the low adherence to single IPC measures, show targets for possible interventions. The increase of HH in OR will be an important aim. The correct donning of PPE and discipline to refrain private talking, as well as organizational changes in providing material or staff might constitute a potential for the prevention of nosocomial infections.

Presentation on Wednesday, February 27, 2019 from 13:00 – 13:04 in room Hörsaal 010.

109/PRV

Measuring hand disinfectant consumption in operating theatres with a focus on anaesthesiology and the impact of different dispenser types

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Introduction

Hand hygiene (HH) compliance is frequently monitored by quantifying and benchmarking hand rub consumption. While this approach provides a rough estimate of quality, it can barely be applied to operating theatres (OTs) due to problems in differentiating hygienic from surgical HH. HH compliance is specifically crucial during anaesthesiological procedures, accompanied with a high microbiological contamination.

Modern disinfectant dispensers using electronic monitoring systems (EMS) allow HH-monitoring on individual positions by automated and real-time data collection. These dispensers are available as manual or touchless versions.

Aims

The aims of this study are 1. to evaluate the impact of touchless and manual dispensers on HH in the OT, and 2. to assess the dispenser positions in place. Study preparation was also used to optimize dispenser position according to user demands and work environment.

Methods

Ten OTs were equipped with EMS-dispensers (Ophardt, Germany) including new positions for the anaesthesiology work area (Fig. 1). Five OTs were equipped with manual and five with touchless dispensers. The prospective study is ongoing at the time of writing. Two periods of two months each, in which touchless and manual dispensers are exchanged between the OTs, are assessed.

Data collection includes type and location of dispensers, date and time of HH-events, volume of disinfectant used (total and per activation) and count of HH-events. Data will be referred to the number of surgical procedures per day and room.

Results

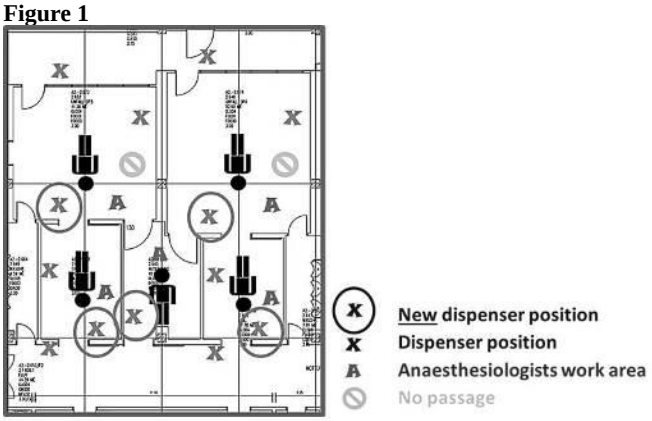
Preliminary analyses suggest that the newly introduced dispensers for anaesthesiologists are among the top used dispensers. The total volume of disinfectant used per day is higher for manual dispensers, whereas the total number of HH-events is higher for touchless dispensers. The daily average amount of disinfectant used per HH-event is about 3ml for manual and 2ml for touchless dispensers. Oral feedback from anaesthesiologists is positive, particularly related to the facilitated access to dispensers and the touchless version.

Conclusion

Dispenser availability needs to be adapted according to the needs of the users, as also shown by human factor engineering approaches (1). Comparative data analysis will be provided. However, performance differences between manual and touchless devices became already evident.

1. Jacob JT, Herwaldt LA, Durso FT, Program CDCPE. Preventing healthcare-associated infections through human factors engineering. Current opinion in infectious diseases. 2018;31(4):353-8.

Figure 1



Presentation on Wednesday, February 27, 2019 from 13:06 – 13:10 in room Hörsaal 010.

110/HYV

There are numerous opportunities for hand hygiene in the operating theatre: *Does anyone actually observe that?*

B. Schock*[1], D. Beck[1], P. Schipper[1], I. F. Chaberny[1]
[1]*Leipzig University Hospital, Institute of Hygiene, Hospital Hygiene and Environmental Medicine, Leipzig, Germany*

Basically known that hand hygiene is a key measure to prevent healthcare-associated infection. To promote hand hygiene nationally the German campaign "Aktion Saubere Hände" (ASH) was launched in 2008, based on the World Health Organization (WHO)'s "Clean Care is Safer Care" initiative. Therefore, hand hygiene compliance has been collected in many hospitals at various wards for years. The WHO recommends direct observation as the gold standard. Since 2015, at the Leipzig University Hospital, hand hygiene compliance has been directly observed in the ward context and improved through continuous efforts. But there are also numerous opportunities for hand hygiene in the operating theatre (OT). For this reason, hand hygiene compliance was also directly observed in the OT. The results will be used in feedback sessions to improve infection control.

A modified form of the ASH hand hygiene collection sheet is used for observation in the OT. Compliance has been monitored at "central operating theatre" since 2015, with the years followed more observations in other areas of operating theatre. Surgeons and surgical care (together OT-team) as well as anesthetists and anesthesia care (anesthesia-team) are stratified. The results are reported to the employees in feedback sessions and analyzed together.

It can be affirmed that numerous opportunities for hand hygiene are in the OT. Over 700 hand hygiene opportunities were collected. Total compliance in the OT is lower than the total ward compliance

(compliance$_{OT\ 2017}$:34% vs. compliance$_{ward\ 2017}$: 74%; p<0,001). It becomes clear that the compliance of the surgeons improved over the years (compliance$_{2015}$: 27% vs. compliance$_{2017}$: 39%; p=0,019). The compliance level of the anesthesia-team is generally higher than the total compliance of the OT-team (compliance$_{OT\text{-}team}$ 2017: 37% vs. compliance$_{anesthesia\text{-}team}$ 2017: 48%; p<0,001)
While acknowledging that the surgical hand disinfection is very well implemented, there was the hypothesis that total compliance in the OT is much better than the ward compliance. By means of the feedback discussions, it became apparent that the employees did not know that they also had to behave according to the 5 moments of hand hygiene (WHO) in the OT and they indicate that they do not know the associated indications. Tailored training and feedback will hopefully bring a positive change over the next few years.

Presentation on Wednesday, February 27, 2019 from 13:12 – 13:16 in room Hörsaal 010.

111/HYV

Infection control in medical education – hygiene should be practice from the beginning

B. Schock*[1], A. Richter[1], A. Surikow[1], I. F. Chaberny[1]
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Insufficient infection control knowledge increases the risk of hospital-acquired infections through insufficient compliance and therefore poses a potential risk to patient safety. Therefore, in 2015 the teaching project "OT Training" was introduced at the Leipzig Faculty of Medicine and a restructuring of the series of lectures and practical training on the topic of "Hygiene" was developed and integrated in the medical study curriculum.
The "OT Training" in the pre-clinical component and the didactic restructuring of the hygiene workshops in the hospital semester were comprehensively developed by means of the currently applicable learning objective catalogues and have already been tested in existing teaching (per year N=320 students; 2015-17: N= 960). The "OT Training" and the series of lectures and practical training are evaluated externally by the Faculty of Medicine. In addition, a self-developed questionnaire (for "OT Training") and an internal evaluation (for practical stations as part of the practical training series) were used.
Overall the "OT Training" was evaluated as "very good" (N=492; RR=51%). Alongside the high importance of hygiene in the hospital and operating theatre (Overall$_{hospital}$=97% and Overall$_{OT}$=98%) the salient feature of hygiene for self-protection and in particular for patient safety was also recognised at an early stage. Through the series of lectures and practical training which were also evaluated positively, the self-reported level of knowledge and the importance of hygiene for the students improved significantly (level of knowledge M_{before}=2.8 vs. M_{after}=3.9; p>0.000; importance M_{before}=3.3 vs. M_{after}=4.2; p>0.000; 5 point Likert scale; t-Test).
Lack of hygiene constitutes a potential risk to patients. Consequently the early and continuous focus on hygiene in student education makes a contribution to increasing patient safety in the healthcare sector. It is clearly obvious that the healthcare system requires hygiene-friendly offspring - the Leipzig Faculty of Medicine has already started.

Presentation on Wednesday, February 27, 2019 from 13:18 – 13:28 in room Hörsaal 010.

112/PRV

Glove disinfection and compliance: not as easy.

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[1]*University Medical Center, Georg August University, Department of Infection Control and Infectious Diseases, Goettingen, Germany*
[2]*University Medical Center, Georg August University, Department of Anaesthesiology, Goettingen, Germany*
[3]*University Medical Center, Georg August University, Department of Medical Statistics, Goettingen, Germany*

Compliance with hand hygiene (HHC) is upper most important to prevent healthcare-associated infections. However, implementation remains a challenge. The goal is to find effective, easy to implement, sustainable, resource saving and scalable methods. This study investigates glove disinfection as one new approach aiming at HHC improvement.
A three phase study was conducted in a surgical intensive care unit (40 beds, 200 healthcare workers) at a University Hospital. Observation units were patients isolated in single rooms with a high number of hand hygiene indications occurring consecutively. HHC was determined by direct observation using a modified WHO protocol.
After baseline observation (phase I) glove disinfection was allowed (phase II) and thereafter withdrawn (phase III) to evaluate time and training effects (figure 1). Primary endpoint was HHC; as secondary endpoints healthcare-associated infections (HAI) and hospital-acquired multi-drug resistant bacteria were addressed. Statistical computation of confidence intervals, chi2-statistics or p-values was performed by R 3.5.1.
After allowing for glove disinfection HHC increased moderate, but significantly (phase II 30%, phase II 42%, p<.001). For indication-specific HHC see figure 2. Phase III as well as data analysis of HAI and hospital-acquired multi-drug resistant bacteria is currently under investigation, also the question if this effect is caused by the intervention itself or by time or training. In contrary to another setting (stem cell transplant unit) glove disinfection seems not to be of great impact for this surgical ICU. Thus, glove disinfection seems to be one piece in the mosaic, but not the one fits all solution.

Figure 1

figure 1 study design and workflow

Figure 2

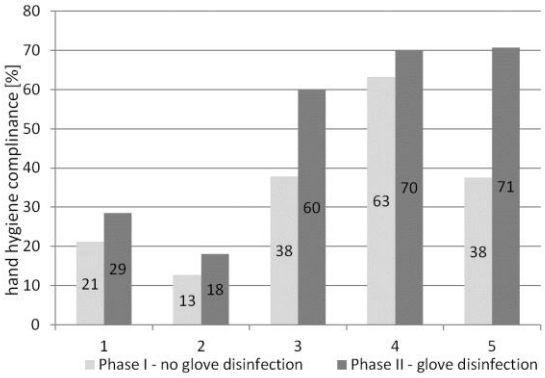

figure 2 hand hygiene compliance at a combined surgical intensive care unit (stratified by indications 1-5 according WHOs my-five-moments)

Presentation on Wednesday, February 27, 2019 from 13:33 – 13:43 in room Hörsaal 010.

113/HYV

How important are attitudes and assessments by the infection control team for the success of tailored intervention?

B. Schock*[1]
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The improvement of hygiene measures is a constant challenge. For years, numerous intervention programs have been tested in various study designs. The PSYGIENE study has shown that tailored behavioral psychological intervention on medical staff lead to a lasting improvement in hand hygiene compliance [1]. Innovative concepts enable promising results during the study period, but it often remains unclear how the research impulses can be integrated into the infection control routine. Therefore, a reality check is

carried out after three years behavioral psychological infection prevention at the Leipzig University Hospital.
Standardized interview of the infection control team from the Institute of Hygiene, Hospital Hygiene and Environmental Medicine with a short questionnaire on the benefits and acceptance of behavioral psychology-oriented infection prevention (5-Likert scale, 1 = fully up to 5 = does not apply at all).
The survey was carried out with all staff members of the institute (N = 15, physicians = 5). Nearly all employees say that they like receiving feedback. The benefit of tailored intervention is well-known to all, 80% of the participants say that they were not convinced from the beginning. As a reason for their own resistance, the employees indicate fear and no capacity to understand and lack of understanding of tailored intervention. The routine application of the interventions is explained with positive experiences, change of attitude and communication with the team.
Up to now research has been carried out about the resistance to infection control measures by the medical staff. In order to achieve long-term success in infection control, the infection control team should be supported with regard to their attitudes and motivation for tailored intervention. The establishment of tailored intervention takes time: everyday hospital practice as well as the stage of adaptation in the infection control team.
[1] von Lengerke T, Lutze B, Krauth C, Lange K, Stahmeyer JT, Chaberny IF. Förderung der hygienischen Händedesinfektion. Clusterrandomisierte kontrollierte Studie PSYGIENE zur Evaluation maßgeschneiderter Interventionen. Dtsch Arztebl Int 2017; 114(3): 29-36.

Presentation on Wednesday, February 27, 2019 from 13:48 – 13:58 in room Hörsaal 010.

114/PRV

Identification of adherence predictors for effective implementation of infection prevention measures in trauma surgery and orthopedics

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Background
Effective infection prevention (IP) succeeds with health care professionals" adherence to infection prevention guidelines, which currently showed to be not sufficient. The BMG-funded intervention study HygArzt (ZMVI1-2516FSB111) aims at postoperative, nosocomial infection reduction in trauma surgery and orthopedics through implementing infection prevention measures (IPM). Effective implementation requires promotion of desired behavior by considering psychological predictors of adherence, this present study aimed to identify.
Methods
Based on Ajzen's theory of planned behavior, a Likert-scaled questionnaire was developed, piloted, and distributed to physicians and nurses in three trauma surgical and orthopedic wards (N = 83). Results were generated using factor- (VARIMAX rotation), reliability- (Cronbach's alpha), correlation- (Pearson), and descriptive analyses.
Results
The response rate was 52% (N=43). Relevant questionnaire items were bundled to content categories (scales) with scale reliability coefficients ranging between $\alpha = .67$ and $\alpha = .89$. Preliminary physician-specific results reflect the initial situation: 70% show positive attitude to IP, 78% very good to good knowledge about IPM, 53% value the priority of IP, 56% score commitment of supervisors as high, 56% rate satisfaction with implementation of IPM as moderate. 94% intend to adhere to IPM, which succeeds, at 34% always, at 42% almost always. In 72%, work environment is experienced as implementation-favorable. Adherence-relevant correlations were identified between: Adherence and internal control (r=.67, p<.002), negative emotions regarding IPM (r=-47, p<.04), behavioral intention (r=.56, p<.002), knowledge about IPM (r=.63, P<.004), and priority of IP (r=.49, p<.05), between satisfaction with implementation of IPM and priority of IP (r=.75, p<.000), supervisors" commitment (r=.74, p<.000), supervisors" commitment and priority of IP (r=.65, p<.003).
Conclusions
Most physicians reported positive attitude, good knowledge, high implementation intention, and implementation-favorable work environment. Potential for optimization exists with satisfaction with implementation of IPM, the priority of IP, supervisors" commitment, and adherence. The promotion of these, also of adherence-relevant personal characteristics, control expectancy, emotions, behavioral intention, knowledge, should be targeted in intervention phase for effective implementation of IPM.

Presentation on Wednesday, February 27, 2019 from 14:03 – 14:13 in room Hörsaal 010.

115/PRV

The practice of obtaining blood samples from peripheral venous catheters decreases the likelihood of timely catheter removal

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Introduction: With an estimated prevalence of approximately 4.6% in German acute care hospitals, healthcare-associated infections (HAI) pose a challenge in medicine. Bloodstream infections (BSI) are among the most frequently documented HAI. Central and peripheral venous catheters (CVC and PVC) are relevant risk factors for BSI. Although the risk for BSI is higher for CVC, PVC are utilised more frequently and are thus relevant in the context of HAI prevention. Robust data on the prevalence of PVC and associated infections in German hospitals are scarce to this date. Question: The objectives of this study were to estimate the prevalence of PVC and PVC-associated infections on peripheral wards of a large tertiary care hospital in Germany. The collected data may be utilised for a tailored infection prevention intervention in the future. Methods: A point prevalence study was conducted in peripheral wards of a tertiary care hospital with more than 3000 beds. Data were collected between August 2017 and February 2018. Standardised data collection forms were used for collecting ward, patient and PVC-related data. As endpoints, PVC-prevalence, PVC-associated infections and PVC without usage in the 24 hours prior to the survey and without documentation of intended usage in the 24 hours after the survey ("unused PVC") were chosen. For data analysis, Kruskal-Wallis test was employed for continuous variables and Chi-squared test or Fisher"s exact test for categorical variables. Multivariate analysis and logistic regression were performed for the endpoint "unused PVC". Results: Data from 2092 patients in 110 wards were collected. The overall prevalence of patients with PVC was found to be 33%. Infections were recorded in 16 patients. Except one case of BSI, these were all local infections at the site of insertion. Of 725 documented PVC, 77 (11%) were unused PVC. Multivariate analysis and logistic regression revealed wards with the practice of regularly obtaining blood from PVC, PVC with dirty or loose insertion dressing, paediatric ward specialty and last inspection of the PVC more than one day ago as significant risk factors for unused PVC. Conclusions: A substantial proportion of patients presented with a PVC on the day of survey. Too few infections were recorded to allow for detailed analyses. Various risk factors for unused PVC were identified. We hereby present a solid method to obtain an overview about PVC use and to increase awareness for PVC-associated risks.

Presentation on Wednesday, February 27, 2019 from 14:18 – 14:30 in room Hörsaal 010.

Workshop 21
Microbiota and Gastrointestinal Pathogens (FG GI, FG PW)
27. Febr. 2019 • 13:00-14:30

116/GIV

Phage resistance/inactivation mechanisms adopted by the probiotic strain *E. coli* Nissle 1917 to battle the T4 phage infection

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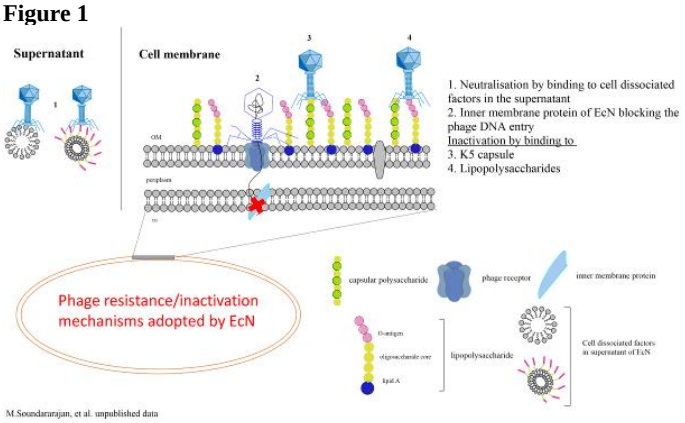
Introduction: *E. coli* Nissle 1917 (EcN) is one of the few successful probiotics that is licensed as a drug. Bacteriophages are reported to be a dominant player in maintaining the gut microbial community. Hence, phage resistance is an important aspect of a probiotic to survive in the gut and to not acquire any genetic material (antibiotic resistance, toxin-encoding genes) by phage infection. Recently we have shown that EcN was not infected by tested lambdoid phages [1]. In this study, we aimed to investigate EcN"s resistance towards an entirely different class of phage: lytic T4 phages.
Methods: T4 phage resistance of EcN was tested by phage plaque assays (ppa) and to understand the resistance mechanism, EcN cells incubated with phages were examined with confocal and electron microscopy. In addition, the transcriptome of EcN was analyzed in the presence of T4 phages. To understand the phage inactivation by EcN, phage titer (pfus/ml) was determined by ppa after static coincubation (37°C) of phages and EcN/ EcN supernatant (EcN_S). To characterize the factor(s) responsible for inactivation, heat-killed EcN (HK) and 1 mg/ml proteinase K (PK) and/or 40 mM sodium metaperiodate (SMP) treated EcN were used.
Results: EcN was not infected by the tested T4 phages and microscopic examination of EcN after infection with phages showed the phages were found to be attached to the intact EcN cells. Transcriptome analysis of EcN in presence of T4 phages indicated the possible involvement of an inner membrane protein (EcN_1772) in EcN"s phage resistance.
EcN cells and EcN_S reduced the T4 titer by ~100-fold after 24 h of incubation and with microscopic examination, we could show that T4 phages were bound to spherical structures in the supernatant. Interestingly, when the EcN cells were treated with a combination of HK, PK, and SMP the phage titer reduction ability was reversed by ~50 to 80-fold, indicating the possible co-involvement of proteins and polysaccharides. Noteworthy, EcN K5 capsule negative mutant was not able to inactivate the phages and was sensitive to T4 phage infection.
Conclusion: We conclude that EcN is resistant against T4 phage infection and inactivates the T4 phage in coincubation. Regarding the mechanisms, it can be deduced from the presented results that more than one mechanism is adopted by EcN to combat the T4 phage infection and a hypothetical model is summarized in Fig 1.

1. Bury, S., et al. Front Microbiol, 2018. **9**: p. 929.

Figure 1



Presentation on Wednesday, February 27, 2019 from 13:00 – 13:10 in room Hörsaal 104.

117/GIV

Naturally occurring variation in microbiota composition influence colonization with the intestinal pathogen *Citrobacter rodentium*: the role of SCFAs and facultative anaerobes

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Question:
The composition of the intestinal microbiota has an enormous impact on the outcome of enteric infections in human and experimental animals. Specifically, the microbiota prevents pathogen colonization and host entry via direct and indirect microbiota-pathogen interactions, collectively termed colonization resistance. The roles of specific individual members or community signatures contributing to disease severity are still underexplored.
Hence, our aim was to study how the microbiota influences susceptibility to enteric infections with *Citrobacter rodentium*, which is commonly studied as model of enteropathogenic *E. coli* (EPEC) due to the many shared pathogenicity traits.
Methods:
We studied initially isogenic mouse lines with distinct microbiota profiles as demonstrated by 16S rRNA gene sequencing. In addition we utilized germfree mice and co-housing experiments to identify specific bacteria and microbiota signatures associated with altered susceptibility to *C. rodentium*. Aerobic and anaerobic cultivation of intestinal bacteria was utilized to isolate candidates responsible for protection. Targeted metabolomics for short-chain fatty acids (SCFA) was performed to quantify levels of acetate, butyrate, and propionate.
Results:
Isogenic mice demonstrated highly varying disease kinetics after infection with C *rodentium* with more than 1000-fold difference in colonization. Transfer of communities from susceptible (SPF-1) and resistant (SPF-2) mice into germ-free mice verified that the varying susceptibilities are determined by microbiota composition. The strongest differences in colonization were observed in the cecum and could be maintained *in vitro* by coculturing cecal bacteria with *C. rodentium*. Analysis of the SCFA concentrations revealed significantly elevated level of butyrate in resistant mice. Butyrate supplementation strongly impaired growth of *C. rodentium in vitro* and *in* vivo indicating that butyrate producing bacteria are responsible for inhibition of *C. rodentium* early on. Interestingly the transfer of a mixture of cultivable, facultative anaerobic bacteria from resistant to susceptible mice also altered disease susceptibility. Whether these two observations are linked is not known yet.
Conclusions:
Both, the SCFA butyrate produced by diverse anaerobic bacteria and facultative anaerobic bacteria contribute to the initial colonization resistance against the pathogen *Citrobacter rodentium*.

Presentation on Wednesday, February 27, 2019 from 13:15 – 13:25 in room Hörsaal 104.

118/GIV

Germination Capacity of Commercial Bacillus thuringiensis strains ABTS-351 and ABTS-1857 Exposed to Caco-2 cells

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Due to the common usage of commercial *Bacillus thuringiensis* (Bt) strains as a sustainable pest control tool in agriculture, they have occasionally been isolated in conjuncture with diarrheal food poisoning incidents. The purpose of the present work was to determine the potential of commercial Bt strains ABTS-351 and ABTS-1857 to germinate under the current theoretical model for *B. cereus* (Bc) infection of adhering to the host epithelium, multiplying in the intestine and producing enterotoxins. Previously, it was shown that these commercial Bt strains do not germinate in the nutrient-rich Simulator of the Human Microbial Ecosystem

(SHIME®); however, this system does not include host factors that might act as germinants.
The impact of the host gut wall on spore germination and outgrowth was evaluated for strains ABTS-1857 and ABTS-351 as compared to the Bc strain ATCC 14579 and a probiotic *B. clausii* (Enterogermina, Sanofi, Italy). To assess the impact of germinants released from host cells, spores were either exposed to the supernatant of a 4 or 16 hr incubation of Caco-2 cells in a minimal medium (indirect exposure) or to a Caco-2 cell monolayer (direct exposure).
First, a minimal medium (HBSS) was identified which did not induce germination of any of the test strains in the absence of Caco-2 cells. Indirect exposure to human cell-derived molecules caused an increase in the total viable count (TVC) of the Bc strain by more than 1.7-log after 4 hrs in both supernatants derived from Caco-2 cell incubation. In contrast, ABTS-1857 TVC only increased 0.7-log and 0.9-log when exposed to the Caco-2 supernatant collected after 4 and 16 hrs, respectively. No meaningful germination of ABTS-351 or *B. clausii* was observed. After a 4 hr direct exposure to Caco-2 cells, 50% of ABTS-1857 spores remained in the cell fraction while only 5-15% of *B. clausii* or ABTS-351 adhered. Neither commercial Bt strain nor the probiotic B. clausii germinated. This was in contrast to a strong 1.6-log increase in Bc TVC after 4 hrs.
Overall, spores from the commercial Bt strains behaved similar to the probiotic control *B. clausii* as opposed to the Bc strain, which germinated strongly upon both indirect and direct exposure to Caco-2 cells. Similarly, direct exposure to human intestinal cells, where adhesion of Bt spores was shown, did not induce germination of either Bt strain. These data suggest that ABTS-351 and ABTS-1857 do not conform to the current model for Bc infection.

Figure 1

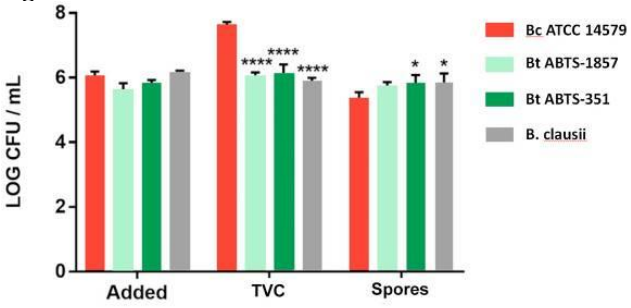

Figure 1. During a 4 hr direct exposure of spores to a Caco-2 monolayer, *Bacillus cereus* (Bc) germinated while neither *B. thuringiensis* (Bt) nor the *B. clausii* probiotic control exhibited any outgrowth.

Presentation on Wednesday, February 27, 2019 from 13:30 – 13:40 in room Hörsaal 104.

119/PWV
Modulation of the Gut Microbiota by Antisense Oligonucleotides as Novel Antibiotics
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The emergence of resistance, and the detrimental effects of antibiotics on resident microbes, require novel types of antimicrobials. Antisense oligonucleotides (ASO), such as peptide nucleic acids (PNAs), are promising candidates for such novel antimicrobials. In bacteria, ASOs bind in a sequence-specific manner to bacterial mRNA and inhibit ribosome initiation or elongation by steric hindrance. Essential or virulence specific genes are targeted to compromise or deplete pathogen populations. However, delivery of uncharged ASOs into bacteria can be challenging. To overcome this, the ASOs are coupled to cell-penetrating peptides (CPPs), which have been shown to enhance delivery across the cell membrane. As a platform technology, CPP-ASO conjugates can be easily and rapidly adapted to new target genes, which can be utilised to counteract emerging resistances.
The gut microbiota is a complex community of bacteria and other microbes, which is of immense importance for human health. The microbiota contributes to protection from disease and in recent years, a variety of human diseases, such as colorectal cancer, have been linked to dysbiosis in the gut. Therefore, it is critical to consider the gut microbiota in the design of novel antibiotics.
The ideal next-generation antibiotic is able to target a specific pathogenic species in the complex microbial communities and has minimal off-target effects on the host and commensal bacteria.
By carefully selecting the bacterial target genes, antibacterial ASOs have the potential to achieve this high level of species-selectivity.
Herein, we study the uptake, mechanism of action, target specificity and efficacy of CPP-ASO conjugates in gut pathogens, including *Salmonella enterica*, *Escherichia coli* and *Fusobacterium nucleatum* with the goal of developing highly species-selective antibiotics.

Presentation on Wednesday, February 27, 2019 from 13:45 – 13:55 in room Hörsaal 104.

120/PWV
The neonatal window of opportunity: long-lasting consequences of microbial colonization and early-life infections on the tolerogenic properties of stromal cell subsets in gut-draining lymph nodes
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[5]*Helmholtz Centre for Infection Research, Department Microbial Immune Regulation, Braunschweig, Germany*
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Gut-draining mesenteric and celiac lymph nodes (mLNs and cLNs, respectively) are important for inducing peripheral tolerance towards food and commensal antigens by providing an optimal microenvironment for efficient *de novo* generation of Foxp3+ regulatory T cells (Tregs), and LN stromal cells were identified as critical cellular players in this process. Transplantations of mLN from either specific pathogen-free (SPF) or germ-free (GF) mice further proved that the high Treg-inducing capacity of mLN stromal cells is stably imprinted by intestinal microbiota. This process is taking place already in the neonatal phase and renders the mLN stromal cell compartment resistant to inflammatory perturbations later in life. Transient neonatal infections either with enteropathogenic *Escherichia coli* (EPEC) or *Yersinia pseudotuberculosis* (*cnfY* knock-out strain YP147) resulted in a severly impaired *de novo* Treg induction in cLNs as late as twelve weeks post infection, demonstrating the importance of this early phase in life for the establishment of long-lasting intestinal tolerance. LN transplantation and single-cell RNA-seq under steady-state conditions allowed identification of expression signatures stably imprinted by microbiota in mLN fibroblastic stromal cells. Subsetting common stromal cells across gut-draining mLNs and skin-draining LNs further refines their location-specific immunomodulatory functions, and mLN stromal cells were demonstrated to shape resident dendritic cells to attain high Treg-inducing capacity in a Bmp2-dependent manner. Thus, crosstalk between mLN stromal and resident dendritic cells provides a robust feedback mechanism for the maintenance of intestinal tolerance, a process that can be substantially disturbed upon early-life infections.

Presentation on Wednesday, February 27, 2019 from 14:00 – 14:10 in room Hörsaal 104.

121/PWV

Epithelial STAT1-signaling maintains intestinal tissue homeostasis during *Salmonella* infection by controlling host cell death

I. Stolzer*[1], M. T. Chiriac[1], M. F. Neurath[1], C. Günther[1]
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The invasive bacterium *Salmonella enterica* is a common gastrointestinal pathogen and causes severe foodborne illness in humans worldwide. Intestinal immune homeostasis and the maintenance of the intestinal epithelial barrier are essential components of host defense during gastrointestinal infection. Maintaining a functional barrier requires a strict regulation of cell death. However, molecular mechanisms of cell death regulation as well as novel forms of cell death are not fully understood. Thus, we elucidated the contribution of different forms of cell death and upstream regulatory components during gastrointestinal infection. *In vitro* and *in vivo*, we revealed that MLKL-mediated necroptosis is impaired as a result of deletion of STAT1 in the context of gastrointestinal infection. Mice lacking Caspase8 (*Casp8*$^{\Delta IEC}$ mice), a negative regulator of necroptosis, are highly sensitive towards gastrointestinal infection and inflammation associated with lethality of these mice. Our results demonstrate that epithelial STAT1 signaling controls *Mlkl* gene expression in intestinal epithelial cells during *Salmonella* Typhimurium infection and that lethality of *Casp8*$^{\Delta IEC}$ mice is mediated by epithelial STAT1 signaling. A deletion of STAT1 ensures the survival of *Casp8*$^{\Delta IEC}$ mice during the early phase of a *Salmonella* infection with a milder course of disease based on decreased epithelial cell death and increased epithelial integrity. Further our data uncovers that STAT1 is particularly important during disease initiation in the early phase of infection but not during disease progression. Tissue destruction and elevated Mlkl levels in Casp8$^{\Delta IEC}$xStat1$^{-/-}$ mice during the late phase of infection suggest a switch of upstream regulators of cell death. This pathway seems to be pathogen specific since STAT1 has only a minor contribution to coordinate cell death during *Citrobacter rodentium* infection. Collectively, our study reveals that STAT1-signaling maintains the homeostasis of intestinal epithelial barrier by altering *Mlkl* expression. Our data suggest that interferons and STAT1 signaling is essential to coordinate the early host response by regulating epithelial cell death during *Salmonella* typhimurium infection.

Presentation on Wednesday, February 27, 2019 from 14:15 – 14:25 in room Hörsaal 104.

Workshop 22
Molecular Microbial Pathogenesis applied (FG MP)
Mittwoch, 27. Februar 2019 • 13:00-14:30

122/MPV

DEAD-box helicase helP identified as prognostic biomarker in patients with *Pseudomonas aeruginosa* bloodstream infection

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[3]*Genomic and Epigenomic Variation in Disease Group, Barcelona, Spain*
[4]*Proteome Center Tübingen, Tuebingen, Germany*
[5]*Medical Center, Department of Hematology, Oncology, Immunology, Rheumatology & Pulmonology , Tuebingen, Germany*
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Introduction: Pathogen virulence factors could be valuable prognostic biomarkers and potential therapeutic targets, but their utility for clinical application is not comprehensively investigated.
Methods and Findings: To identify clinically relevant pathogen-derived prognostic biomarkers, a genomic multicenter cohort study of patients with Pseudomonas aeruginosa bloodstream infection (n = 166) was conducted. We performed whole genome sequencing and quantitative proteomics on the entire P. aeruginosa strain collection (first blood culture isolate of a patient). In a second step, a multi-level Cox regression analysis was performed to determine the relation between patient characteristics and pathogen features with 30-day mortality. We analyzed 2298 accessory genes, 1078 core protein levels, and 107 parsimony-informative variations in reported virulence factors. Additionally, antibiotic susceptibility profiles were explored as potential pathogen-related risk factors. Phylogenetic analysis of the core and accessory genome revealed several distinct clusters. One accessory genome cluster was associated with a poor outcome (hazard ratio 1.95, p = 0.048) and was functionally enriched for GO-terms of peptidyl-histidine phosphorylation (false discovery rate < 0.05). Deep genomic exploration uncovered that the presence of one gene from P. aeruginosa - a putative DEAD-box helicase that we named helP - was independently linked to fatal outcome (HR 2.01, p = 0.05). Sequencing of five helP positive strains on a PacBio RS II instrument confidently localized helP on a pathogenicity island 1 related region in close proximity to a conjugative type IV pili system. This suggested that helP is potentially transferable to other strains or even species. This suspicion was supported by the fact that helP appeared in strains from phylogenetically distinct clusters.
Conclusions: We identified a novel and clinically relevant prognostic biomarker marker candidate in P. aeruginosa. The helP genotype could be useful in clinical routine due to its adequate predictive power and ease of measurement.

Presentation on Wednesday, February 27, 2019 from 13:00 – 13:10 in room Hörsaal 007.

123/MPV

C26 - a novel compound inhibiting the expression oftype III secretion system 1 of *Salmonella* Typhimurium

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[2]*German Center for Infection Research (DZIF), Helmholtz Centre for Infection Research, Braunschweig, Germany*

Resistance of Gram-negative bacteria is a serious emerging threat and hence the search for new antibiotics is of high priority. A novel class of antibiotics that block infection instead of killing bacteria is hought to exhibit a lower potential for the development of resistance because of a reduced selection pressure compared to conventional antibiotics. These antiinfectives target virulence mechanisms of pathogenic bacteria such as adhesion determinants or toxin delivery systems. Type III secretion systems (T3SS) of gram negative bacteria, often enteropathogens, enable the injection of bacterial effector proteins into eukaryotic target cells to promote infection and colonization. Type III secretion systems are not only excellent targets for antiinfectives because the virulence of many pathogens depends on these systems but also because they are highly conserved and hence a single drug has the potential to act against a broad spectrum of enteropathogens. We have identified C26, a novel compound exhibiting a strong inhibition of the almonella
T3SS-1. Preliminary results suggest a high potential for hit-to-lead development

Presentation on Wednesday, February 27, 2019 from 13:15 – 13:25 in room Hörsaal 007.

124/MPV

A Phagemid-based expression of synthetic sRNA to silence shiga toxins in the enterohemorrhagic *E. coli* (EHEC) O157; a strategy towards RNA-based therapeutics

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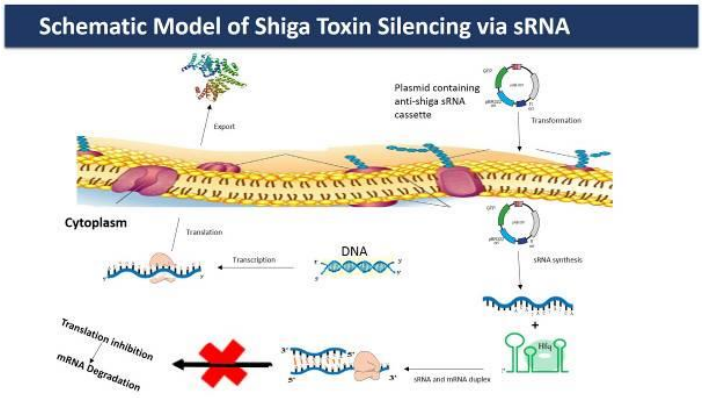
The emergence of resistance in bacteria against antibiotics and the dwindling number of new antibiotics pose a double threat to our continued protection from bacterial pathogens. In bacteria, the small non-coding RNAs recruit RNA chaperone proteins, majorly the Hfq, and affects mRNA stability and expression level. These natural sRNAs have inspired the design of synthetic trans-acting RNA molecules to efficiently control gene expression in microorganisms. Reversion of antibiotic resistance using synthetic RNAs can be a potential tool in combating antibiotic resistance in pathogenic bacteria. The enterohemorrhagic *E. coli* (EHEC), a cause of worldwide outbreaks is a food borne pathogen. Its strong pathogenicity is due to virulence factors such as shiga toxin 1 (Stx-1) and shiga toxin 2 (Stx-2), commonly known as Vero toxins owing to their cytotoxic effect on vero cell lines. Our study involves designing a specific synthetic sRNA against the stx-1 and stx-2 genes of *E. coli* followed by assessing the repression capability of anti-shiga sRNA through computational prediction tools. After successfully expressing the anti-shiga sRNA via the M13 phagemid pAB001 which harbors the *hfq* downstream to the sRNA transcript. The effect of anti-shiga sRNA on the mRNA level of the target gene was determined by quantitative real time RT-PCR, while reduction in the toxin level was verified via MTT cell viability assay. Toxin filtrates from wild type O157 strain were compared to the filtrate of O157 strains with anti-shiga sRNA expression cassette. Higher cell viability indicates reduced levels of shiga toxin in the supernatant of *E. coli* O157 due to repression efficiency of the the sRNA expressed. For perfect delivery of the sRNA to the bacterial cells, we are using a helper plasmid for expression of the M13 bacteriophages, which can easily be used as phage therapy strategy for turning the pathogenic bacteria into a normal flora. Synthetic sRNA could be a revolutionary approach for targeting multidrug resistant bacteria. Interrupting expression of resistance genes by using synthetic sRNA, we can not only restore the antibiotic susceptibility of these bacteria extending the lifespan of existing antibiotics but can also efficiently eradicate their infections sustainably.

Figure 1



Presentation on Wednesday, February 27, 2019 from 13:30 – 13:40 in room Hörsaal 007.

125/MPV

Towards a causative therapy for EHEC infections: transcriptional and translational inhibitors block ciprofloxacin induced shiga toxin production

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Introduction

Shiga toxins (Stx) are the main virulence factors of enterohemorrhagic *Escherichia coli* (EHEC) and induce the symptoms of life-threatening hemolytic uremic syndrome (HUS). The expression of Stx 1/2 is strictly dependent on the bacterial gene expression apparatus. The bacterial SOS response is the essential signal for Stx2 production and in addition to iron limitation the strongest signal for Stx1 induction. SOS inducing antibiotics are still one of the most widely prescribed and effective anti-bacterial therapies. However, their use in the therapy of EHEC infections is contraindicated as their application resulted in increased frequencies of HUS development. Several studies have shown that most transcriptional and translational inhibitors are effective at blocking Stx 1/2 production *in vitro*. Thus, causative antibiotic therapies for EHEC infections that block toxin synthesis and eliminate the toxin producers are theoretically available. However they are not often applied since the results of clinical studies that employ these agents are confounded by the inclusion of antibiotics inducing the bacterial SOS response within the trials. It is also not clear whether these antibiotics can also be used to inhibit Stx 1/2 production after the SOS response has been induced.

Results and discussion

We constructed a reporter gene based test system for the time-resolved, simultaneous read-out of the SOS response (*rec*AP-*cfp*) and Stx1 production (*stx1::yfp*), both in a single EHEC O157:H7 cell, and on the population level. We did not observe an induction of the SOS response when cells were exposed to inhibitory or subinhibitory concentrations of rifaximine, azithromycin, tetracyclin, gentamycin or ampicillin. In contrast, ciprofloxacin induced the SOS response at concentrations that did not affect growth or led to cell lysis. Cell lysis and the peak in Stx1 production were substantially delayed with respect to the peak of the SOS response. This feature allowed us to block Stx1 production in a concentration depended manner by adding rifaximine, azithromycin, tetracyclin and gentamycin even after the SOS response was fully induced. As expected the cell wall synthesis inhibitor ampicillin did not have a similar effect. QPCR based tests with other clinically relevant EHEC isolates showed similar results for Stx1 and Stx2. These observations suggest that an early and aggressive antibiotic combination therapy may be of value in treating EHEC infections.

Presentation on Wednesday, February 27, 2019 from 13:45 – 13:55 in room Hörsaal 007.

126/MPV

Deprivation of the periplasmic chaperone SurA reduces virulence and restores antibiotic susceptibility of multidrug-resistant *Pseudomonas aeruginosa*

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[3]*University of Oslo, Department of Biosciences, Section for Evolution and Genetics, Oslo, Norway*

Pseudomonas aeruginosa (Pa) is one of the main causative agents of nosocomial infections, rising. Therefore, novel strategies for therapy are urgently required. The outer membrane composition of Gram-negative pathogens and especially of *Pa* restricts the efficacy of antibiotic entry into the cell and determines virulence. For efficient outer membrane protein biogenesis, the BAM complex in the outer membrane and chaperones within the periplasm are crucial. We hypothesized that by global disturbance of the outer membrane composition, it might be possible to achieve two effects

simultaneously: increased entry of antibiotics and reduced virulence. To this end, deletion mutants of the non-essential BAM complex components BamB and BamC, of the periplasmic chaperone HlpA as well as a conditional deletion mutant of the periplasmic chaperone SurA were created and tested for antibiotic sensitivity and virulence. The most profound effects for both traits were associated with a lack of SurA, characterised by increased membrane permeability, enhanced sensitivity to antibiotic treatment and attenuation of virulence in a *Galleria mellonella* infection model. Strikingly, the conditional deletion of *surA* in a multidrug-resistant clinical bloodstream isolate re-sensitized the strain to treatment with antibiotics to which the strain was resistant before. From our data we conclude that SurA serves as a promising target to develop antiinfective drugs or drugs that could be used as sensitizers in combination with available antibiotics.

Presentation on Wednesday, February 27, 2019 from 14:00 – 14:10 in room Hörsaal 007.

127/MPV

A periplasmic chaperone as novel pathoblocker and resistance breaking target

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SurA is a periplasmic chaperone expressed by Gram negative bacteria. It is essential for shuttling outer membrane (OM) proteins across the periplasm of Gram negative bacteria and thus decisively shapes the composition and essential barrier function of the OM. Lack of SurA induces a profound rearrangement of the OM protein composition (including significant reduction of virulence factors and strong reduction of proteins involved in LPS biosynthesis) and sensitizes the bacteria to antibiotic treatment with antibiotics normally excluded by the Gram OM (e.g. Vancomycin, Erythromycin; Weirich et al., 2017). We have generated evidence that in the absence of SurA an infection with the model enteropathogen *Yersinia enterocolitica* can be cleared utmost efficiently by the host *in vivo*, presumably because SurAdeficient bacteria do not only exhibit reduced levels of important virulence factors but are also highly sensitive to serum killing and detergents such as SDS or bile salts due to global rearrangements of the OM. Consequently, the inhibition of SurA function could support the therapy of infections caused by Gram enteropathogens at different levels: (I) increase sensitivity to serum complement mediated killing (especially relevant in bloodstream infections) (II) increase sensitivity to detergents (important during passage of the gastrointestinal tract), and (III) increase bacterial sensitivity towards antibiotics normally excluded by the OM and thus broaden the repertoire of drugs that could be used for therapy. Consequently, we have established a screening assay to search for inhibitors of *E. coli* SurA and screened ~480.000 compounds in cooperation with the European Lead Factory. We obtained a qualified hit list recently, comprising ~ 50 compounds that were identified as inhibitors of SurA during the screen. We currently re-validate the hits by the primary assay, biophysical interaction analyses and secondary assays to select the most promising ones. Additionally, all the hit compounds were docked into the crystal structure of *E. coli* SurA bioinformatically (Coop. Antti Poso, Thales Kronenberger). Potential interactions are currently validated by molecular dynamics simulations. Thereby we aim to identify compound clusters, preferred binding pockets and will get more information about how compounds interact with our target. Based on this and on our wetlab data we will select compounds for re-synthesis, derivatisation and further development.

Presentation on Wednesday, February 27, 2019 from 14:15 – 14:25 in room Hörsaal 007.

Postersession 01
25 Febr. 2018 • 19.00-21.00

Diagnostic Microbiology and Clinical Microbiology (FG DKM)

128/DKMP

Improved sample preparation for the MALDI-TOF mass spectrometry direct-on-target microdroplet growth assay (DOT-MGA) to determine antibiotic susceptibility in *Enterobacterales*

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Introduction. The increasing number of multi-drug resistant clinical microorganisms is a global threat requiring accurate and rapid detection of their resistance patterns. The recently described MALDI-TOF MS-based direct-on-target microdroplet growth assay (DOT-MGA) is a novel approach to determine antibiotic susceptibility of bacteria.
Objective. Here, we investigate the influence of culture medium removal on the reliability of the DOT-MGA technique to determine the antibiotic susceptibility status of bacteria within a few hours.
Methods. A set of 20 *Enterobacterales* comprising different resistance patterns were employed to analyze the susceptibility to ceftazidime at its breakpoint concentration according to EUCAST. MICs were determined by broth microdilution method. DOT-MGA was performed in triplicates at two different sites applying incubation times of 4, 4.5, and 5 hours. One site employed Whatman paper as previously described (1) to remove the culture supernatant. The other site used novel filter plates that were directly placed on the spots to absorb the culture supernatant in a single step. Subsequent procedures were identically performed at both sites as published (1).
Results. The MALDI-TOF MS DOT-MGA prototype software results from each site after 4 h, 4.5 h, and 5 h incubation were compared to the results of the microdilution method after 20 h incubation. For the already described approach applying Whatman paper, a categorical agreement of 93%, 93%, and 92% was achieved after 4 h, 4.5 h, and 5 h incubation, respectively. Application of the novel filter plates for removal of culture supernatants resulted in 95%, 95%, and 100% agreement after 4 h, 4.5 h, and 5 h incubation, respectively. The use of the filter plates allowed a nearly complete removal of the culture supernatant compared to the Whatman paper. Residual culture medium as detected in the case of applying the Whatman paper, resulted in an increased concentration of MALDI-TOF MS incompatible components leading to poor spectra quality and thereby to an increased false susceptible rate.
Conclusion. The study demonstrated that an increased performance of the DOT-MGA is achieved by an enhanced method for removal of the culture supernatant. Further studies are warranted to optimize the assembly and standardization of the MALDI-TOF MS DOT-MGA.

- (1) Idelevich EA, Sparbier K, Kostrzewa M, Becker K., Clin Microbiol Infect. 2018 (7):738-743. doi: 10.1016/j.cmi.2017

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

130/DKMP

Evaluation of the eazyplex®MRSAplus system for the detection of the Panton-Valentine leucocidin (PVL) genes

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Introduction. Panton-Valentine leucocidin (PVL)-possessing *Staphylococcus aureus* strains are responsible for severe skin and soft-tissue infections and feared as causative agent of necrotising pneumonia. PVL-positive isolates have been associated with community-acquired (CA) *S. aureus* lineages comprising both methicillin-susceptible (MSSA) and methicillin-resistant *S. aureus* (MRSA) strains; hence, the need for rapid detection and confirmation of PVL-harbouring isolates.
Objectives. In this study, the PVL-extended, multiple loci-targeting and ready-to-use LAMP-based amplification system, eazyplex®MRSAplus (AmplexDiagnostics, Gars Bahnhof, Germany) was challenged with a well-characterized collection of PVL-positive and PVL-negative MRSA as well as MSSA strains. The assay combines species confirmation of *S. aureus* with the identification of the *mecA*/*mecC* genes and, in addition, allows the detection of the *lukS/F-PV* encoding genes.
Methods. Overall, 70 clinical *S. aureus* (*nuc* gene-positive) strains including 35 PVL-positive and 35 PVL-negative isolates comprising 48 different *spa* types were tested. Of these, 61 isolates harboured the *mecA* gene. Five of nine of the MSSA strains included were PVL-positive.
Results. All 61 MRSA and nine MSSA challenge strains were identified as belonging to the species *S. aureus* and the presence or absence of the *mecA* gene was correctly identified. *lukS/F-PV* encoding genes were correctly detected for all PVL-positive MRSA (n = 30) and MSSA (n = 5) strains. The 35 PVL-negative *S. aureus* strains were correctly tested negative.
Conclusions. Applying to an epidemiologically diverse collection of different PVL-positive/negative challenge strains, the eazyplex®MRSAplus system was proven as a convenient extension tool for the detection of the possession of the PVL-encoding genes simultaneously to the molecular verification of the methicillin resistance and the *S. aureus* species affiliation.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

132/DKMP

Comparative Analyses of Different Methods for the DNA Extraction of *Cryptosporidium parvum* for Molecular Detection

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Background: Cryptosporidiosis is a widespread diarrheal disease of animals and humans caused by the uptake of the protozoan parasite *Cryptosporidium (C.)*. Every year, more than eight million human infections are registered worldwide. Even though the majority of cases come from poor countries, the number of reported diseases in industrial nations should not be neglected. Moreover large scale underreporting is suspected.
Objectives: Currently, there is a lack of harmonized molecular methods for sensitive and specific pathogen detection. Therefore, the aim of this study was to compare different methods for the pulping of oocysts to optimize molecular detection of *Cryptosporidium*.
Materials and methods: Water and faeces were spiked with different numbers of *C. parvum*. Various sample preparation methods (e.g. heat treatment, thawing/freezing, ultrasound, six different DNA extraction kits, homogenization with different matrices via FastPrep®24 and proteinase K treatment) were compared to provide oocysts for nested-PCR detection of the specific gene sequences 18SrDNA, COWP & GP60.
Results: The most efficient molecular detection in water was achieved with prefixed thawing/freezing cycles and mechanical lysis with FastPrep®24 using the lysing matrix E (detection of one oocyst/µl). Preliminary results of compared methods in faeces indicate that the use of certain DNA extraction kits is the most promising approach for the molecular detection of *Cryptosporidium* in faeces (detection of one oocyst/µl).
Conclusion: There are many different methods that were employed to disrupt oocysts of *Cryptosporidium* for molecular detection. The results of this study show that the choice of the method should depend on the sample matrix. Besides, not all methods are similarly suitable for an effective disruption of the oocysts.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

133/DKMP

A novel digital image platform for the detection and quantification of FISH stained bacteria in adhesion assay

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Introduction. The adhesion of bacteria is a widespread phenomenon and a crucial step in infection and colonization of hosts. Bacterial adhesion to organic material like heart valves or non-organic material like prostheses causes high risk for chronic infections. In order to determine the presence of bacteria adhered to different materials, the determination of attached bacteria species is of high interest.
Objectives. The aim was to develop a tool to recognize and count bacterial cells stained with fluorescence *in situ* hybridization (FISH) using automated digital image VideoScan technology. Thereby, infection-adhesion assays should be carried out to infect human tumour cell lines and further porcine tissue sections. We intended to apply different algorithms enabling the determination of cells within diverse sizes of bacterial colonies.
Material & Methods. Using specific mathematical tools, we developed a novel algorithm for the detailed analysis of bacterial cells adherent to mammalian cells (HEp-2) and porcine tissue sections. Infections of hosts were carried out using cell line infection-adhesion assay and novel tissue infection-adhesion assay technique. Specific eubacterial FISH probe (EUB338 Atto647N) and species specific *E.coli* probe (ECO453 Atto647N) were used to stain bacteria. DAPI staining was done for host analysis.
Results. Performing infection-adhesion assays for cell lines and tissue sections we were able to infect hosts with EPEC and EAggEC. Thereby, a novel software algorithm was used to count FISH stained bacteria even in colonies. Compared to standardized technologies like lysis assay or propidium iodide staining, the FISH analysis showed higher sensitivity for the determination of bacterial cells.
Conclusion. Automated digital image analysis of FISH stained bacteria is a promising alternative for time-consuming manual methods.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

134/DKMP

Culture and single cell analysis of microorganisms associated with eukaryotic hosts

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Introduction

99 % of the microorganisms present in many natural environments are not cultivable with conventional approaches due to difficulties and limitations associated with cultivation techniques (1, 2). Thus, cultured microorganisms do not reflect the functional and phylogenetic diversity present within a natural habitat. However, continuous efforts have been invested to improve efficiency of microbial cultivation techniques (3, 4). Significant progress has been achieved, e.g. by simulation of the natural conditions, refinement of the culture media and culturing microbial consortia.
Furthermore, several DNA-based molecular methods, the so called "metagenomics", have been developed in order to explore the diversity of microbial communities by analyzing the complex genomes. Primarily employed to study non-cultivable microbiota

for a better understanding of global microbial ecology (5), metagenomics data also provide information on the functional role of the different microbes. A recent addition to the omics repertoire is single-cell genomics (6), which has advanced the field of microbiology from the analysis of microbial metagenomes by recognizing each microbial cell as a separate and unique entity.

Objectives

The main purpose of our service project is the development of a platform that allows the isolation and identification of microorganisms associated with animal and plant metaorganisms that are studied within the Collaborative research center (CRC) 1182 using standardized tools throughout all subprojects. Established methods and media for microbial cultivation as well as cutting edge tools for separating and analyzing single cells are employed towards this goal. Overall we aim to achieve a high standardization of isolation and identification of host-associated microorganisms, which are naturally difficult to enrich and to grow in pure cultures.

Materials and Methods

We use classical cultivation techniques and further analyze hosts" microbial community structure by using 16S rDNA amplicon sequencing approaches and coordinated protocols for DNA extraction, in order to guarantee comparable data sets of host associated consortia throughout all CRC projects. Furthermore, fluorescence activated cell sorting (FACS) is applied as a tool to singularize individual microbial or eukaryotic cells from complex consortia and allow single cell analysis (e.g. single cell genomics) as well as cultivation of slow growing bacteria.

Results

Overall, we enriched, isolated and identified 84 bacteria from the jellyfish *Aurelia aurita* and 78 associated bacteria of the comb jelly *Mnemiopsis leidyi*. Furthermore, we were able to isolate 149 microbes from 3 different life stages of the sea anemone *Nematostella vectensis.* 54 of those isolates were used for genome sequencing and construction of metabolic networks. Moreover, we isolated 183 wheat-associated bacteria from the leaves and roots. Overall, all obtained isolates are crucial for recolonization experiments of the respective host to get a deeper insight into function of the respective microbiota and the interplay between host and bacteria.

Still ongoing collaborations are focused on a targeted isolation approach of methanogenic archaea from *Drosophila melanogaster* gut and the isolation of methanogenic archaea from wild mouse gut. A further collaboration is focused on the isolation of *Subdoligranulum sp*. from the gut of great apes, as there is increasing evidence that it represents a key species in the core microbiome of great apes. Until today, we were able to enrich *Ruminococcaceae*, which points to an enrichment of *Subdoligranulum sp.*. For validation, these samples were now used for a metagenome analysis. All obtained results will be presented and discussed with regard to their importance.

Conclusion

Taken together, an increasing percentage of the microbiome of different hosts can now be cultured and thus is available for functional analyses, which will greatly advance our understanding of the host-microbiome interplay in health and disease.

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Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

135/DKMP

Establishment and optimization of new FISH probes for the detection of *Escherichia* and *Staphylococcus*

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Introduction. Fluorescence *in situ* hybridization (FISH) is a rapid method for the *in situ* detection and differentiation of microorganisms. *Escherichia* and *Staphylococcus* representing two highly prevalent pathogens which can cause severe human infections like urinary tract infections, meningitis, bacteremia and endocarditis.

Objectives. In consequence of unavailable probes (genus *Escherichia*) and unspecific binding (genus *Staphylococcus*) the aim was to establish genus-specific FISH probes for *Escherichia* and *Staphylococcus*. Furthermore, we intended to optimize the FISH protocol to achieve stable and high fluorescence intensities.

Material & Methods. Novel genus-specific probes targeting 16S rRNA of *Escherichia* (ESCH448) and *Staphylococcus* (STA465) were designed using DECIPHER software. The specificity of the probes was evaluated using probeCheck. We applied our novel probes on 50 different bacterial isolates, including various genera and species. Additionally, we tested probes labeled with different fluorophores. FISH assays were analyzed using our automated VideoScan technology (Rödiger et al. 2013).

Results. Previous results showed that we could identify the specific genera, respectively. Regarding all 50 tested bacterial isolates we achieved a probe specificity of 66% for ESCH448 and 86% for STA465. FISH protocols were optimized by exchanging ethanol with methanol resulting in more stable and higher fluorescence intensities presumably due to a better decomposition of the bacterial cell envelope. Regarding the verified fluorophores, Atto647N proved to be the most suitable fluorescent dye compared to Atto488, Atto465 and Cy3 and was therefore used as a probe labelling for further FISH assays.

Conclusion. FISH staining of bacteria is an easy, robust, cheap and rapid method. This culture-independent method can be used for the *in situ* identification of important bacterial pathogens. In further studies we will optimize the probe specificities. Additionally, a simultaneous detection of different pathogens within one sample using mixed colored oligonucleotide probes shall be achieved.

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Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

Clinical Microbiology (StAG KM)

136/KMP

Rare, but present – About dermatophytes that do not occur daily in routine diagnostics

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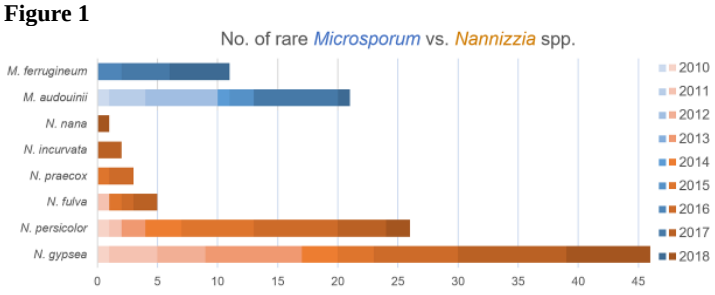
Introduction: Dermatophyte infections affect a high number of people at least once in their lifetime. Especially inflammatory tinea corporis and tinea capitis often caused by the zoophilic *Microsporum (M.) canis* are extremely unpleasant. However, severe tineas can be also caused by less frequently occurring anthropophilic *M. audouinii* and *M. ferrugineum* or by geophilic *Nannizzia* spp. that were previously described in a new clade delimited from *Microsporum* (de Hoog *et al.*, 2017). An appropriate diagnosis is therefore essential to ensure a successful treatment, determine the origin of infection and eventually eliminate the risk of transmission. In this study, we aimed to access the number of rare *Microsporum* and *Nannizzia* spp. identified during routine diagnostics.
Methods: Since 2010, *Microsporum* and *Nannizzia* spp. strains have been prospectively searched in the routine diagnostic laboratory in Mölbis. The dermatophytes were identified by conventional methods based on their colony appearance and microscopic morphology. In most of the cases, a uniplex PCR-ELISA assay confirmed the suspected dermatophyte species or genus. Sanger sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA and/or the translation elongation factor alpha (*TEF1α*) gene was used to finally identify dermatophytes if routine diagnostics could not give a clear result.
Results: Out of 501 positive *Microsporum* cases, 32 (6.4%) were identified as rare *M. ferrugineum/audouinii* and 469 (93.6%) as *M. canis*. One third of all rare *Microsporum* spp. is represented by *M. ferrugineum*. Moreover, 83 *Nannizzia* spp. strains have been identified since 2010, showing 25 (31.3%) positive *N. persicolor* strains. The number of rare *Microsporum* and *Nannizzia spp.* identifications per year increased during the last years, e.g. 100% of all *M. ferrugineum* and 73.1% of all *N. persicolor* strains were isolated between 2015 and September 2018 (see Fig. 1).
Conclusions: This study indicates especially *M. ferrugineum* and *N. persicolor* not to be as rare as suspected. As standard methods including culture detection and microscopy are only based on morphological characteristics a reliable species differentiation stays difficult in many cases. In order to increase the number of correctly identified rare dermatophytes, a suspected infection can be only confirmed by sequencing after considering morphological features and patient´s anamnesis (e.g. contact to animals, travel return, sports, gardening).

Figure 1



Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

137/KMP

Ethambutol and pyrazinamide drug susceptibility testing of rifampicin resistant Mycobacterium tuberculosis isolates - a diagnositic algorithm for a low incidence setting

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Introduction: Isoniazid (INH), rifampicin (RMP), ethambutol (EMB), and pyrazinamide (PZA) are first-line drugs used to treat tuberculosis (TB) caused by susceptible *Mycobacterium tuberculosis* complex (MTBC). While phenotypic drug susceptibility testing (pDST) for INH and RMP is reliable, it is less accurate for EMB and PZA. In the context of multidrug-resistant (MDR) TB, EMB and PZA are recommended as "add-on agents" regardless of pDST results.
Aim: To overcome ambiguous pDST results for EMB and PZA we investigate an algorithm combining phenotypic and molecular DST to determine PZA and EMB susceptibility in rifampicin resistant *M. tuberculosis* isolates in Germany.
Materials/Methods: We included all Rifampicin resistant isolates referred to the German National Reference Laboratory for Mycobacteria, Borstel between January 2016 and March 2017. *pnc*A and *emb*B306 Sanger sequencing was routinely performed in parallel to pDST for all first and second-line drugs using the proportion method in the MGIT960 system (Becton Dickinson, Sparks, Md.). Minimal inhibitory concentration (MIC) and next generation sequencing (NGS) were applied to investigate discordances between phenotypic and molecular results.
Results: In total 85 isolates (7 RMP mono-resistant and 78 INH and RMP resistant isolates) were included. There was 100% concordance between pDST and *pnc*A sequencing results for 49 PZA resistant and 33 PZA susceptible strains. For three strains no PCR result could be obtained. 24 of 42 isolates (57.14%) with phenotypic EMB resistance had an *emb*B306 mutation. For 16 of the remaining 18 strains, NGS analysis revealed non-synonymous, non-phylogenetic mutations in the *emb*CAB operon. The mutation *emb*B Q497R was present in 7 isolates and *emb*B G406A was found in 5 isolates. Eleven of 12 strains with *emb*B M306, *emb*B D354A, *emb*B G406A, or *emb*B Q497K mutations that tested EMB susceptible had an elevated MIC below the critical concentration or were tested resistant on repeated pDST.
Conclusions: Although reliability has been questioned pDST results for PZA were consistent with *pnc*A sequencing for all isolates included in this analysis. In contrast, discordance was high between pDST Sanger sequencing and NGS for EMB. Sanger sequencing or NGS together with pDST should be employed in low-incidence countries to ensure reliable EMB DST.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

138/KMP

Detection of bacterial pathogens from heart valve tissue by 16-PCR/sequencing

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Question: Detection of nucleic acids is increasingly used to supplement conventional culture for the diagnosis of bacterial infections. It has been suggested to improve diagnostic yield especially in cases of antibiotic pretreatment or infection with fastidious organisms. We have evaluated the applicability of 16S-PCR/sequencing for the detection and identification of bacterial pathogens in heart valve tissue samples.
Methods: Over a two year period, 172 tissue samples from 137 surgical interventions have been analyzed using a commercial 16S-PCR/sequencing-assay and conventional culture. Tissue samples were cut in pieces, homogenized with pestle and mortar, plated to

Columbia blood agar, Chocolate agar and Schaedler agar and incubated for up to 14 days at 37°C in CO2-enriched and anaerobic atmosphere. DNA extraction and PCR testing were performed as recommended by the assay manufacturer. Purified PCR products were sequenced by a commercial sequencing service provider. Species identification from 16S-sequences was done with the online tool provided by the assay manufacturer.
Results: Samples from 77 of 137 episodes (56 %) tested negative by culture and PCR. In 22 (16 %) cases, bacterial pathogens were concordantly detected by both methods. Samples from 38 (28 %) patients tested positive by PCR only. In 15 of these 38 cases, the corresponding pathogen had also been recovered from preoperative blood cultures sent to our laboratory. Microorganisms from samples of the remaining 23 episodes included Viridans streptococci (8), Cutibacterium spp. (3), Aggregatibacter actinomycetemcomitans (2), Bartonella spp. (2) and Tropheryma whipplei (1).
Conclusions: The application of 16S-PCR/sequencing largely increased the detection rate for bacteria in heart valve tissue. In the majority of cases, microorganisms detected by PCR only were also recovered from blood culture samples or represented typical agents of infective endocarditis. A notable proportion of culture-negative samples yielded pathogens not amenable to conventional culture.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

Eukaryotic Pathogens (FG EK)

139/EKP

Fungal determinants in the interaction of eosinophils with *Aspergillus fumigatus* in the context of allergic bronchopulmonary aspergillosis (ABPA).

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Introduction: The saprobic mold *Aspergillus fumigatus* is the causative agent of allergic bronchopulmonary aspergillosis (ABPA). This inflammatory lung disease is prevalent in patients suffering from cystic fibrosis or severe asthma due to impaired fungal clearance of inhaled conidia.
Objective: Eosinophils play a crucial role in allergic inflammations such as ABPA. Therefore, we aim at investigating the *in vitro A. fumigatus*-eosinophil interaction. Insights are gained by analyzing fungal determinants in the eosinophil activation and fungal killing in co-culture experiments by employing defined *A. fumigatus* mutant strains.
Methods: To investigate fungal survival of an *A. fumigatus* wild type isolate compared to mutant strains in co-culture with eosinophils, an XTT-based viability assay was established. The ability of strains to activate eosinophils is quantified by measuring the release of IL-4 via ELISA. These assays are then used in a screening approach of a transcription factor deletion library. For fungal transcriptome profiling upon confrontation with eosinophils, we aim at RNA-seq with the results to be validated by qRT-PCR.
Results: Reduced fungal viability in the *in vitro* co-cultures could be observed for *A. fumigatus* wild type conidia as well as pathogen-induced release of IL-4 by eosinophils. In a next step, it is investigated to which extent these effects are influenced by deletions of different transcription factor genes in *A. fumigatus*. Furthermore, RNA-seq studies reveal significantly regulated transcripts as potential determinants of the interaction of eosinophils with *A. fumigatus*.
Conclusion: The results confirm an anti-fungal effect of eosinophils. To characterize fungal determinants in the interaction of *A. fumigatus* with eosinophil, screening of a transcription factor deletion library will assist in the identification of relevant regulatory factors. Furthermore, the comprehensive RNA-seq approach will provide an overview on the fungal transcriptome as it is shaped upon confrontation with eosinophils to elucidate relevant determinants of ABPA.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

140/EKP

***Aspergillus fumigatus* F-Box protein Fbx15 is a substrate adaptor of SCF E3 ubiquitin RING ligases which is essential for stress tolerance, virulence and nuclear entry of transcriptional co-repressor SsnF**

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Aspergillus fumigatus is a saprotrophic, opportunistic pathogen, which is often insensitive to azoles and can cause invasive Aspergillosis in immunocompromised individuals resulting in high mortality rates. Aspergilli express approximately 70 F-box proteins (Fbx) as substrate receptors for E3 ubiquitin SkpA-Cullin-F-Box (SCF) ubiquitin RING ligases to target interacting substrates for ubiquitin mediated degradation in the 26S proteasome. The Fbx15 encoding gene is required for virulence, oxidative stress adaptation, gliotoxin production and the nuclear entry of the conserved transcriptional co-repressor SsnF [1]. The corresponding Fbx15 of *Aspergillus nidulans* is required for fungal developmental programs as the formation of asexual spores (conidia) released into the air or the building of resting overwintering fruiting bodies (cleistothecia) in the soil resulting in sexual spores released into water [2]. Additional interacting proteins of Fbx15 were identified and are currently compared in both fungi. The goal is to evaluate the exact molecular function of this F-box protein in substrate protein stability and/or nuclear import control and to analyze whether Fbx15 could be a target protein to control fungal growth.

[1] Jöhnk *et al.* (2016) SCF ubiquitin ligase F-box protein Fbx15 controls nuclear co-repressor localization, stress response and virulence of the human pathogen *Aspergillus fumigatus*. *PLoS Pathog.* 12, e1005899.
[2] von Zeska Kress *et al.* (2012) The COP9 signalosome counteracts the accumulation of cullin SCF ubiquitin E3 RING ligases during fungal development. *Mol. Microb.* 83, 1162-1177.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

141/EKP

Functional polymorphism of arginine deiminase, a putative *Giardia duodenalis* virulence factor

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Arginine depleting enzymes are considered virulence factors and the depletion of arginine is a well-known strategy of pathogens to evade immune effector mechanisms. The gut dwelling protozoan parasite *Giardia duodenalis* cause relevant morbidity in humans and animals. An arginine deiminase (ADI) secreted by the parasite has been implicated in virulence. Here we tested hypothesis that sequence variation detected between *G. duodenalis adi*-alleles of different genotypes affects functional parameters of the enzyme**.** Therefore, ADI sequences of different *G. duodenalis* genotypes

were cloned and purified in recombinant form and the Km value of the enzymes were determined in vitro. Furthermore, Km values of *G. duodenalis* ADI activities were determined in lysates of recent clinical parasite isolates. In addition, a sequence analysis of various *adi*-genes was performed. The results show that sequence variation cause changes in Km values of the respective enzyme variants and thus provide a further molecular argument to the concept that *G. duodenalis* ADI is a molecularly defined virulence factor of *G. duodenalis*.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

142/EKP

Yeast versus hyphae: How does the morphotype affect the adhesion of *Candida albicans* to Central-venous-catheters?

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Background: Central-venous-catheter-related bloodstream infections (CRBSI) are a prominent cause of sepsis and associated with high morbidity and mortality. CRBSI are usually triggered by the formation of biofilms on central-venous-catheters (CVC), rendering the pathogen less susceptible to anti-infective therapy and causing a constant release of the pathogen into the blood stream. Attachment of the pathogen to the catheter surface is a basic condition for biofilm formation, thus a pathogens ability to adhere to such a surface should be considered a fundamental virulence feature. Besides Staphylococci, the yeast *Candida albicans* is a major source of CRBSI. This fungus can form two morphotypes, the yeast state thought to contribute primarily to adhesion and dissemination, and the hyphal state thought to promote cell invasion and biofilm maturation. One physiological trigger that induces a transition from yeast to hyphal state is contact with plasma, which induces the rapid outgrowth of an initial hyphae within minutes.
Aims and Methods: By using single cell force spectroscopy and flow chamber experiments, we aimed at determining the primary adhesion capacities of the yeast and early hyphal states of *C. albicans* to CVCs. Additionally, qRT-PCR was used to determine the expression pattern of important, adhesion-relevant genes in the yeast and early hyphal state *C. albicans* cells.
Results: Our single cell force spectroscopy results indicate that *C. albicans* cells in the early hyphal state, induced by incubation of yeast cells in human blood plasma, attach with a significantly higher adhesion force to naive CVCs than yeast cells. Similarly, significantly increased adhesion rates were observed for early hyphal state cells to CVCs under high shear flow (6 dyne/cm2 and higher), if compared to the yeast state, which hardly bound to the CVC surface under this physiologically relevant flow condition. In addition yeast and hyphae cells demonstrated distinct expression patterns of important, adhesion-relevant genes under the conditions applied in our spectroscopy and flow experiments.
Conclusion: The blood contact driven transition of *C. albicans* cells from the yeast to early hyphal state enhances the adhesion capacity of this pathogen to CVCs and is likely to contribute to the pathogens success to colonize and to form biofilms on this type of medical device, especially under high shear flow rates encountered in the superior vena cava.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

143/EKP

Prevalence and relevance of the presumed *pyrethroid-knock down* resistant genotype amongst head lice in Germany

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Question: Globally, infestation with *P. humanus capitis* is one of the most frequent ectoparasitoses of man and a cause of significant distress. Insecticide resistance, in particular to pyrethroid-based pediculocides, is a suspected driver of increasing prevalence. Mutations linked to knock down resistance (*kdr*) sites in the target of pyrethroids, the α-subunit of a voltage sensitive sodium channel (VSSC) of insect nerve cells, have been proposed as a molecular reason. However, the significance of the *kdr* genotype described for head lice for clinical resistance against pyrethriod-based treatment is debated and is investigated here.
Methods: We report on the molecular analysis of the kdr genotype by sequencing respective VSSC PCR-amplified fragments of lice/nits from 221 individual cases of head louse infestations. Samples were obtained during a nation-wide, voluntary survey of human head louse infestations in 2016. The survey included collection of additional data by means of questionnaires.
Results: The main outcomes of the study confirmed that head lice in Germany carry almost uniformely the alledged *kdr* mutations in VSSCα. The head louse population sampled was further characterized using a highly polymorphic marker sequence, PM2, to assess segregation of the *kdr* or VSSC wild type genotypes in the population which was not the case. Moreover, the observational data derived from survey questionnaires indicated that a kdr genotype does not preclude successful treatment with pyrethroid-based regimens.
Conclusions A kdr-genotype is not equivalent to a clinically resistant phenotype. Thus, while *kdr* genotype-linked changes to pyrethroid susceptibility require further studies, they are unlikely to be a major driver of the high prevalence of pediculosis.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

144/EKP

Competitive fitness experiments in sequential *P. aeruginosa* isolates

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The colonization of CF airways with the common gram-negative bacterium *P. aeruginosa* is one of the few opportunities to observe the microevolution of a pathogen during chronic infection in real life. We wanted to explore if and to what extent the microevolution in the CF lungs had influenced the fitness of *P. aeruginosa* to grow in an aquatic habitat.
Longitudinal clonal *P. aeruginosa* isolates that had been collected from 12 CF patients (six patients with a mild and six with a severe course of infection), since the onset of colonisation for up to 30 years, were subjected to within-clone competition experiments. Separate precultures of serial isolates of a patient course were grown in Luria Broth (LB; nutrient rich) and minimal medium (nutrient poor, sole carbon source succinate) until exponentially phase and were then mixed in equal amounts; samples were taken at 0 h, 48 h (with continuous culturing every 12 h) and 120 h (without continuous culturing). The composition of the bacterial communities at time points 48 h and 120 h was determined by sequencing of bacterial DNA-derived amplicons spanning strain-specific SNPs. By optimizing this method as a novel approach for microbial fitness studies, intraclonal comparisons within bacterial communities become possible.
Resulting from this competitive fitness experiments there are significantly less winner strains than expected. *P. aeruginosa* isolates retrieved during the first years of colonization won the competition in the presence of progeny for ten of the twelve

examined patient courses suggesting that *P. aeruginosa* adaptation to the CF lung results in gaining fitness in this special habitat but otherwise with a loss of fitness in other environments. Three different modes of fitness could be verified: (I) gradual loss of fitness during the first years of colonization, (II) a gradual loss of fitness over the whole observation time and (III) no loss of fitness or even an increase of fitness over the years. Irrespective of clone, number of acquired mutations and horizontal transfer events, the early isolates were characterized by the highest fitness. These winner strains gained more biomass when growing in the community than in single culture. They did not suppress the growth of their clonal competitors completely. Instead, they grew faster in a community of genetically highly related strains than growing alone thereby benefitting from public goods.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

145/EKP

The fungal peroxiredoxin Asp f3 of *Aspergillus fumigatus* is essential for redox homeostasis during exposure to external reactive oxygen species

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The ubiquitous filamentous ascomycete *Aspergillus fumigatus* is generally known for its saprophytic lifestyle but very often also occurs as an opportunistic pathogen. Immunocompromised patients are at the highest risk to develop *A. fumigatus* derived infections, ranging from allergic reactions to often fatal invasive aspergillosis (IA). Especially patients suffering chronic granulomatous disease (CGD) are highly susceptible, due to a defect in their NADPH-oxidase, leading to a reduced capability to produce reactive oxygen species (ROS).
We have recently characterized the two-cysteine type peroxiredoxin Asp f3 which is also known as a major allergen and was shown to be of crucial relevance for the fungus when challenged with ROS. The deletion of *asp f3* resulted not only in a high susceptibility to ROS but moreover led to avirulence in a mouse model of pulmonary aspergillosis. The ROS sensitive phenotype was further found to depend on both conserved cysteine residues whose exchange yielded a phenotype comparable to the deletion mutant. To elucidate the biochemical targets of Asp f3 we took a redox proteomic approach comparing the oxidation status of the total protein content of the wild type and the Δaspf3 strain following hydrogen peroxide treatment to gain insight into the main targets of ROS-associated damage. We could identify central metabolic enzymes as well as proteins with proposed extracellular function in protein folding. Additionally, we established an *in vivo* assay, which allows specific exposure to external pulses of superoxide (O_2^-), the primary product of the NADPH oxidase in cells of the innate immunity, to monitor the effect of ROS and the transcriptional changes in *A. fumigatus* confronted with ROS. Here, we will present first results on how the absence of a functional *asp f3* affects gene expression in this fungal pathogen.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

146/EKP

Identification of targets of the essential protein kinase Snf1 in *Candida albicans*

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The yeast *Candida albicans* is a member of the human microbiota, wherein it typically behaves as a commensal. However, particularly among immunocompromised people, it can become a life-threatening pathogen. We have shown that the SNF1 kinase complex, which plays roles in diverse cellular processes including carbon metabolism and maintenance of cell wall integrity, is essential to the *in vivo* fitness of *C. albicans*. While the function of SNF1 is well conserved, its target proteins in *C. albicans* are not known. Interestingly, unlike in other organisms, the catalytic α-subunit Snf1 of the SNF1 complex is essential for the viability of *C. albicans*. To gain insight into the SNF1 signaling pathway and understand why Snf1 is essential in *C. albicans*, we have exploited several strategies. We introduced a library of artificially activated zinc cluster transcription factors into mutants with a defective SNF1 complex, and discovered that an activated form of the transcription factor Czf1 rescued their defects in cell wall integrity. Concurrently, we have isolated spontaneous suppressor mutants, including some in which Snf1 is no longer essential. Ongoing analyses of these suppressors seeks to clarify why Snf1 is essential as well as identify potential targets of Snf1.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

147/EKP

Abstract for SCF Ubiquitin Ligase F-box Protein Fbx15 Controls Nuclear Co-repressor Localization, Stress Response and Virulence of the Human Pathogen *Aspergillus fumigatus*

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E3 ubiquitin ligases ubiquitinate specific target proteins, eventually triggering their proteasomal degradation. F-box proteins share the F-box domain to connect substrates of E3 SCF ubiquitin RING ligases through the adaptor Skp1/A to Cul1/A scaffolds. Fbx15 is a fungal specific F-box protein, which is required for the general stress response in the human pathogen *Aspergillus fumigatus*. Oxidative stress induces *fbx15* gene expression leading to 3x elevated Fbx15 protein levels. In addition it triggers the dephosphorylation of Fbx15, which is phosphorylated during non-stress conditions. Fbx15 binds to SsnF(Ssn6), a part of the RcoA(Tup1)-SsnF(Ssn6) transcriptional co-repressor complex and is essential for its nuclear localization. Dephosphorylation of Fbx15 prevents the nuclear localization of SsnF and results in derepressed gliotoxin gene expression. Fbx15 exhibits a second function dependent on its F-box domain, which acts as SCF interaction site and is required for complete stress tolerance and gliotoxin biosynthesis repression. The absence of Fbx15 in *A. fumigatus* leads to an avirulent phenotype *in vivo* in immunosuppressed CD-1 mice. Fbx15 has a novel dual function by controlling transcriptional repression and being part of of SCF E3 ubiquitin ligases, which is essential for stress response, gliotoxin production and virulence in the opportunistic human pathogen *A. fumigatus*.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

Gastrointestinal Infections (FG GI)

148/GIP

Using the model nematode *Caenorhabditis elegans* to investigate the mode of action of plant products with anthelmintic activity: an extract of *Ailanthus altissima* (Mill.) Swingle causes infertility

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The nematode *Caenorhabditis elegans* is commonly used as model organism to find new natural products with anthelmintic activity. An ethnopharmacological study in the central-southern Apennines (Italy) reported the use of *Ailanthus altissima* leaves and bark against gastroenteritis and anthelmintic infections [1]. The family Simaroubaceae is known for producing quassinoids, degraded triterpenes, which have antimalarial, anticancer and herbicidal activity [2]. A bioassay showed a sterilizing effect on *Caenorhabditis elegans* by the stem bark extract (IC50 value 0.15 mg/mL). During the assay, L4 larvae/young adult worms were treated with different extract concentrations (0.01 to 5 mg/mL) for two days at 20 °C and hatched larvae were counted after 48 h. M9 medium, supplemented with 5 µg/mL cholesterol to guarantee the functionality of the reproductive system, served as positive control. To elucidate the mode of action we investigated the subcellular localization of different gfp-tagged stress response proteins. Only mutants with a daf-16::gfp reporter construct (strain TJ356) showed a cytoplasm-to-nucleus translocation. DAF-16, a FOXO-family transcription factor, is known to influence e. g. ageing, growth and reproduction by the insulin/insulin-like growth factor 1 (IGF-1) signaling pathway [3]. The DAF-16 target genes, which are responsible for reduced reproduction after *A. altissima* extract treatment, still need to be elucidated. To identify the bioactive compounds of the crude extract a bioassay-guided fractionation was performed. Fractions which reduced reproduction in the bioassays are activating cytoplasm-to-nucleus translocation in daf-16::gfp mutants as well. The active substance still needs to be isolated and identified by NMR and mass spectrometry.

[1] Fortini, P., Di Marzio, P., Guarrera, P. M., & Iorizzi, M. (2016). Ethnobotanical study on the medicinal plants in the Mainarde Mountains (central-southern Apennine, Italy). *Journal of ethnopharmacology*, *184*, 208-218.

[2] Alves, I. A., Miranda, H. M., Soares, L. A., & Randau, K. P. (2014). Simaroubaceae family: botany, chemical composition and biological activities. *Revista Brasileira de Farmacognosia*, *24*(4), 481-501.

[3] Henderson, S. T., & Johnson, T. E. (2001). daf-16 integrates developmental and environmental inputs to mediate aging in the nematode Caenorhabditis elegans. *Current Biology*, *11*(24), 1975-1980.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

149/GIP

***In vivo* adaptation of *E. coli* strains Nissle 1917 and 83972 obtained from deliberately colonized individuals**

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Escherichia coli strain Nissle 1917 (EcN) is one of the most well-studied non-pathogenic, probiotic bacterial strains, that has been successfully used for the prevention and treatment of a range of gastrointestinal disorders. In addition to being used for the treatment of ulcerative colitis (remission phase), constipation and diarrheal diseases, and colonization prophylaxis, EcN is used for enhancing postnatal immune competence in infants. Even though the effects of short-term colonization by EcN have been reported it has not been analyzed so far, how EcN adapts in individual hosts during long-term intestinal colonization. In order to assess this, we performed a whole genome sequence analysis of *EcN* re-isolates from stool samples of deliberately colonized individuals. To further understand *in vivo* genome plasticity of EcN re-isolates, we compared the genomic variations observed in the EcN re-isolates" genomes to those of a very closely related strain, asymptomatic bacteriuria (ABU) *E. coli* isolate 83972, and its re-isolates from deliberate bladder colonization of individual patients. *E. coli* 83972 is successfully used for the treatment of urinary tract infections and asymptomatically colonizes the urinary bladder for extended periods of time. Our comparative genomic study identified well-dispersed genomic variations within the genomes of both, EcN and *E. coli* 83972 re-isolates. Compared to *E. coli* 83972 re-isolates, EcN re-isolates were observed to have relatively stable genomes irrespective of different hosts and different colonization time points. Differences in mutational hotspots and the overall degree of genome plasticity observed between EcN and *E. coli* 83972 will help to understand differential adaptation strategies of *E. coli* in response to *in vivo* growth within the intestinal tract or the urinary bladder in different individuals.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

150/GIP

Use of Whole Genome Sequencing and FT-IR analysis for Detection of Virulence factors in the Zoonotic Pathogen *Arcobacter butzleri*

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Background:

The genus Arcobacter was previously known as aero-tolerant Campylobacter. Today more than 20 species are known, but only a few cause infections in humans. *A. butzleri* is described as the most pathogenic one, but even within this species not all isolates seems to be pathogenic. We collected more than 120 isolates from stool or rectal swabs of patients and performed antibiotic susceptibility tests. We investigated the clinical reports of these patients, but only 30% of them showed clinical symptoms like diarrhea or abdominal pain.

Materials/Methods:

To investigate the correlation between putative virulence genes and clinical symptoms, whole genome sequencing of 50 Arcobacter strains was performed using HISeq Illumina sequencing. Briefly, after sequencing reads were trimmed for good quality and assemble with SPAdes. The assembled contigs were annotated using RAST. Rapid large scale pan genome analysis was performed using ROARY pipeline. The presence of antibiotic resistance genes was verified by phenotypic susceptibility testing. We used metabolic fingerprinting by Fourier transform infrared (FTIR) spectroscopy to phenotypically analyze changes in metabolic profiles and surface patterns of the bacteria.

Results:

Whole genome sequencing analysis revealed various patterns of different metabolic genes (amino acids, respiration). Environmental genes also showed some variability. We focused on virulence factors, e.g. *cad*F, *cia*B or *hec* to correlate these to the clinical findings of the patients. Analysis of resistance genes resulted in a more frequent detection of macrolide resistance while fluoroquinolones resembled are higher susceptibility. The FT-IR analysis grouped the A. isolates in several distinct subgroups. Yet, analysis of the complete FT-IR spectrum did not parallel the clinical patterns of the hosts completely. Some clusters contain a high frequency of virulent strains, while apathogenic ones belonged to others.

Conclusions:

Arcobacter butzleri isolates depict not a homogenous genetic profile. Significant differences were found in metabolic gene clusters as well as in antibiotic resistance markers. In an ongoing research approach we did not yet found a single marker defining pathogenicity within the Arcobacter group. FT-IR is a promising tool for the clustering of Arcobacter and analysis of metabolic

profiles, but further studies are necessary to develop more standardized protocols.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

151/GIP

Risk factors for primary antimicrobial resistance of *Helicobacter pylori* in Germany, 2014-2018

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Question

First-line treatment of *Helicobacter* (*H.*) *pylori* infections is in most cases empiric and often includes clarithromycin in combination with amoxicillin or metronidazole. These therapies should not be given when the proportion of patients carrying a clarithromycin resistant strain is expected to be more than 15%. Guideline-recommended empiric second-line treatments include, among others, levofloxacin-based therapies that are less effective when the strain is fluoroquinolone resistant.
The aim of this prospective study was to provide current data on primary resistance of *H. pylori* to clarithromycin and levofloxacin; and to identify risk factors for its development in Germany.
Methods
Between 2014 and 2018 gastric tissue samples from 2024 *H. pylori* positive patients without any known prior eradication treatment were included. Samples were molecular genetically tested for mutations conferring resistance to clarithromycin (N=2024) and to levofloxacin (N=1591). Epidemiological data were analysed using multivariable logistic regression.
Results
Primary resistances were 11% (95% confidence interval (CI): 9.7–12.5) for clarithromycin and 13.3% (95% CI: 11.7–15.1) for levofloxacin. Female sex and antibiotic therapy for unrelated infections were associated with carriage of clarithromycin- (adjusted OR (aOR): 2.4, 95% CI: 1.6–3.4; aOR: 2.7, 95% CI 1.5–4.6, respectively), or levofloxacin-resistant (aOR: 1.4, 95% CI: 1.004–2.1; aOR: 1.9, 95% CI: 1.01–3.6, respectively) strains. Originating from Turkey or countries in Central/South Asia was associated with carrying *H. pylori* resistant to both antibiotics (aORs not shown). Compared with the youngest patient group (aged 18 to 24), all other age groups were associated with higher odds of levofloxacin resistance. Patients aged 65 years and older had the highest aOR (8.3, 95%CI: 3.5–20.1).
Conclusions
Clarithromycin may still be recommended as first line drug in eradication treatments in Germany. However, risk factors for resistance should be taken into consideration when choosing an empiric regimen for an individual patient. Gastroenterologists may be encouraged to have antimicrobial susceptibility tested ahead of first-line therapies as this may help identify patients that are not suitable for clarithromycin-containing regimens.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

152/GIP

CEACAM-HopQ interactions modulates murine and human neutrophil CagA phosphorylation

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Helicobacter pylori is a human-associated pathogen that exclusively colonizes in the human gastric mucosa of approximately 50% of the world population. The infection with this Gram-negative, microaerophilic, spiral-shaped bacterium causes a chronic bacterial gastritis that may lead to peptic ulcer disease, duodenal ulcer, MALT (mucosa-associated lymphoid tissue) lymphoma or gastric cancer. Despite a strong immune response upon infection, *H. pylori* is able to persistently colonize the human gastric mucosa thereby employing various strategies that either help to avoid a proper recognition by the human immune system (e.g. via pattern recognition receptors) or that lead to a direct modulation of the immune cells themselves. Recently, human carcinoembryonic antigen related cell-adhesion molecules (CEACAMs) 1, 3, 5 and 6 were identified as receptors for the *H. pylori* outer membrane protein HopQ. The HopQ-CEACAM interactions contribute to the adherence of *H. pylori* to host epithelial cells and the translocation of the bacterial oncogene CagA (cytotoxin associated gene A) via the cag type IV secretion system into host cells. After translocation CagA is phosphorylated by host kinases (e.g. c-Src) and interacts with a variety of intracellular signaling pathways, interfering with cell motility, cell elongation, proliferation and inflammatory responses. In the current study the impact of HopQ-CEACAM binding on murine and human neutrophils on translocation and phosphorylation of the bacterial oncogene CagA in neutrophils was investigated and novel results will be presented.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

Infection Immunology (FG II)

153/IIP

Gene expression profiling meta-analysis reveals novel pathways and mechanisms shared between tuberculosis and rheumatoid arthritis

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Study goals:
Tuberculosis (TB) is a severe, complex disease and among the leading causes of death by infectious diseases. An association between *Mycobacterium tuberculosis* infection and autoimmune diseases like rheumatoid arthritis (RA) has been reported but it remains unclear if it is a causal factor and if so which molecular pathways and regulatory mechanisms contribute to it. Here we tested the hypothesis that a meta-analysis of published gene expression data will increase sensitivity and permit identification of altered gene expression and associated signaling pathways shared between TB and RA.
Methods:
Gene expression data were retrieved from public databases such as NCBI GEO. 141 samples were included from studies where gene expression in whole blood or blood cell populations of patients with either active TB or RA had been compared to healthy controls. Datasets were normalized and common co-expressed genes between the two diseases were identified using the Random Effect Model in NetworkAnalyst tool, and pathway enrichment analysis was conducted by DAVID, STRING-db, and ClueGO plugin in Cytoscape program.

Results:
This analysis revealed 341 differentially expressed genes (DEGs) that were shared between the two conditions, with TLR5, TNFSF10/TRAIL and IL17RA (adjusted p-values of 2.47E-09, 4.86E-09, and 2.43E-07 respectively) among the most significantly upregulated genes and PPP1R16B/TIMAP, SIAH1, and PIK3IP1 (adjusted p-value of 3.34E-07, 1.65E-05, and 0.00042583) among the most significantly downregulated genes. Pathway enrichment analysis revealed "T cell receptor signaling pathway", "Toll-like receptor signaling pathway" and "Virus defense related pathways" among the most strongly associated common pathways.
Conclusions:
Our study shows that the approach of a meta-analysis increases sensitivity and permits the identification of candidate genes and mechanisms that may play a role in the pathogenesis of infectious and of autoimmune disease. The identification of genes previously implicated by experimental studies in either disease in the past provides validation of the approach. Novel candidates were identified that may provide a basis for future functional and epidemiological studies. The identification of a common set of deregulated genes and pathways substantiates the observation of an epidemiological association of TB and RA and provides clues on the mechanistic basis of this association.

Figure 1

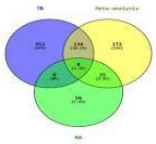

Figure 1: Comparison of the meta-analysis and the individual conditions

Comparing meta-analysis and the analysis of the data sets of the individual conditions in Tuberculosis (TB) and rheumatoid arthritis (RA). 172 DEGs were found to be only significantly deregulated by applying the meta-analysis, while we see a loss of 408 genes that were only significant in the individual disease analysis. Data sets were analyzed with the Random Effect model in NetworkAnalyst tool, and genes with P-value < 0.01 were considered as significant.

Figure 2

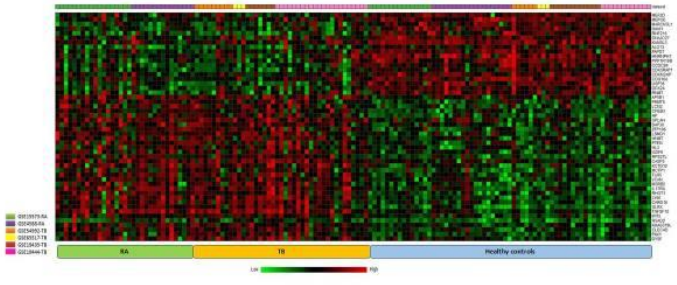

Figure 2: Heatmap of highest significantly differentially expressed genes

The heatmap of the highest significantly differentially expressed genes (DEGs) from the 341 significant DEGs identified through the meta-analysis, where 205 genes were up-regulated, and 136 genes were down-regulated (TB and RA versus control). The heatmap indicates the normalized expression value of each DEG in the individual samples, and genes were clustered based on their condition (cases vs controls) and their original datasets. The heatmap was created by the visualization module in NetworkAnalyst, and genes with P-value < 0.01 in the Random Effect Model analysis were considered as significant.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

154/IIP
High-throughput assays for bacterial uptake and ribosome binding of proline-rich antimicrobial peptides
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The antibacterial activity of proline-rich antimicrobial peptides (PrAMPs) was traced back to the interactions with an intracellular protein, i.e., chaperone DnaK, and the bacterial ribosome complex, i.e., a ribosome intermediate (43S) and mature 70S ribosomes, responsible for protein folding and translation, respectively. The uptake into bacterial cells consists of several steps: (i) electrostatic interaction with components of the bacterial surface, (ii) self-promoted translocation into the periplasmic space, and (iii) active transport into the cytosol via SbmA and YjiL/MdtM as limiting factors. Therefore, the binding constants determined for PrAMPs and isolated 70S ribosomes and the amount of internalized peptide into bacterial cells may resemble PrAMP activity and might be utilized for optimizing PrAMP lead structures stepwise.
Thus, assays determining the binding affinities to 70S ribosomes via fluorescence polarization (λexc=485 nm, λem=535 nm) [1] and the mass spectrometry methods to quantify the uptake into bacterial cells [2] were optimized and simplified to provide higher sample throughputs. Instead of using a full serial dilution, new derivatives were incubated at three peptide concentrations with ribosomes using 5,6-carboxyfluorescein-labelled Onc112 as competitor.
The uptake of Onc112 and the new derivatives were analyzed by quantifying them in the cells and medium. These quantities correlated well to the peptide quantities originally added to the cell cultures [2]. Accordingly, quantification of the peptides remaining in the medium by liquid chromatography and mass spectrometry relative to a calibration curve seemed reasonable and less challenging than efforts of establishing various multi-reaction-monitoring methods. Taken together, we present two high-throughput assays to identify peptide motifs influencing i) the uptake and ii) ribosome binding allowing a further optimization of PrAMPs as peptide-based antibiotics.
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Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

155/IIP
New Approaches to Antimicrobial Vaccine Development *"In silico* Prediction of Immunogenic T Cell Epitopes of *Leishmania donovani* GP63 Protein" as an example.
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Introduction & Objectives: Classical methods of developing antimicrobial vaccines are no longer satisfactory because they are time consuming, laborious and vaccines developed can not be standardized. Visceral leishmaniasis (VL) is a major parasitic childhood disease in sub-Saharan Africa. Expensive and toxic anti-leishmanial drugs are current control methods. Safe, effective and cheap vaccines are potentially powerful strategies to control VL. Traditional vaccine development techniques have failed to deliver an effective vaccine. Leishmania vaccine development may benefit from immunoinformatics tools. This paper describes an improved *in silico* prediction method for immunogenic *Leishmania donovani*-GP63 T cell epitopes as VL candidate vaccines.
Methodology: Using the EpiMatrix algorithm, the amino acid sequence of *L. Donovani donovani* GP63 protein (GenBank accession: ACT31401) was screened for putative T cell cluster epitopes that would bind to the most common HLA class II alleles among at-risk populations. Nine epitopes were initially identified using EpiMatrix. Based on cluster score, number of EpiMatrix hits, hydrophobicity, and number of EpiBars (an EpiBar is a 9 amino acid frame predicted to bind at least 4 different HLA molecules), four peptides (P1-P4) were selected for synthesis. In a proof of concept study, blood samples from consenting healthy, leishmanin skin test (LST) reactive and non-reactive volunteers were stimulated and IFN-γ, IL-4, and IL-10 were measured. IFN-γ and IL-4 levels were similar in both groups.
Results: However, mean IL-10 levels were significantly reduced in LST reactive individuals. To evaluate whether cross-reactivity with the human genome (HG), the human gut microbiome (HM) and common human pathogens (HP) was responsible for these

differences, the sequences of the evaluated peptides were screened using JanusMatrix. One of the peptides (P1), which increased IL-10 in the LST reactive volunteers, showed high cross-reactivity with HG, suggesting that P1 might induce a regulatory immune response in humans.
Conclusion: In conclusion, immunoinformatics tools provide a promising alternative approach for anti-parasite vaccine development. Data obtained can be used in the development of epitope-based *Leishmania* vaccine.
Keywords: T cell epitopes, HLA class II alleles, regulatory T cell epitope, visceral leishmaniasis, EpiMatrix.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

Food Microbiology and Food Hygiene (FG LM)

156/LMP
Antibacterial activity and structure-activity relationship of biflavonoids from fruits of the Brazilian peppertree
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Flavonoids, synthesized by plants across all families and found in a huge chemical variety, possess a diverse range of pharmacological properties (1). In addition to their direct antibacterial activities (2), flavonoids inhibit several bacterial virulence factors (3). Before flavonoids can be applied as natural food preservatives, it is important to understand their modes of action and structure-activity relationships.
The objective of this study was to determine the antibacterial properties of biflavonoids from *Schinus terebinthifolius* Raddi fruits. Besides the inhibitory effects of biflavonoids, the relationship between their structure and inhibitory activity was also investigated.
Biflavonoids including agathisflavone, amentoflavone, dihydroamentoflavone and tetrahydroamentoflavone were isolated from crude *Schinus terebinthifolius* Raddi extracts (4) by liquid-liquid extraction followed by semipreparative HPLC. The antimicrobial properties, including the minimal inhibitory and minimal bactericidal concentration, were measured in bacterial growth assays using *B. subtilis*, *E. coli*, *L. innocua*, *L. rhamnosus*, *P. putida*, *S. carnosus*, *S. ficaria*, *Y. rohdei* with a modified broth dilution method (DIN 58940-7). A crystal violet assay was used to investigate the biofilm inhibition.
The flavonoid monomers apigenin and naringenin showed a lower antibacterial activity than their corresponding dimers. Amentoflavone merely reduced bacterial growth at a concentration of 1 mg/ml. In contrast, tetrahydroamentoflavone showed the highest antibacterial activity with a minimal bactericidal concentration of up to 0.063 mg/mL for the gram-positive bacteria. The examined biflavonoids differ in their oxidative state of the C-ring, which influences the conformation of the molecule. These results suggest that a reduced C-ring has a positive influence on antibacterial activity. Furthermore, tetrahydroamentoflavone also significantly reduced the growth of gram-negative bacteria at a concentration of 0.25 mg/mL. Biflavonoids were also able to inhibit the formation of biofilms at an even lower effective concentration of 0.016 mg/mL.
The results indicate that the biflavonoids investigated in this study differ in their inhibitory effect depending on the degree of oxidation, with tetrahydroamentoflavone showing the highest activity.

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Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

157/LMP
Toxinogenic strains of *Clostridium difficile* in fresh retail chicken meat representing a potential source of toxin-mediated diarrhea in humans
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Clostridium (C.) difficile is a well-known opportunistic pathogen of elder humans which causes healthcare-associated infections such as antibiotic-associated diarrhea, pseudomembranous colitis, and toxic megacolon. Nowadays, *C. difficile* infections (CDI) were also registered independently of hospitalization and the age of the patients in an ambulant setting. One potential cause of the so-called community-acquired infection is a zoonotic transmission to humans based on direct contact with animals or the consumption of food, indicated by closely related isolates from humans and animals [1] and the isolation of *C. difficile* from a wide range of food products including vegetables and different kinds of meat [2]. To estimate the exposition of humans with *C. difficile* via food, we optimized and validated a detection method for *C. difficile* spore contamination of fresh chicken meat. In brief, it consists of an enrichment step followed by a species-specific Real-time PCR for screening purposes and the final isolation of *C. difficile* on selective agar plates. The method validation showed reliable results with a specificity of 100%, a sensitivity of 91.1% and a detection limit (LOD95%) in the range of 6-14 spores/ 25 g chicken meat. Furthermore, we analyzed the occurrence of *C. difficile* in 311 different retail products of fresh chicken meat with and without skin. We detected *C. difficile* in 13.3% (n= 270, with skin, mainly chicken wings, whole chicken and chicken thighs) and 0% (n= 41, without skin) of the tested chicken meat samples, respectively. Most isolates (82.9%) exhibit toxin genes *tcdA* and *tcdB*, but the binary toxin *cdtA/B* was absent. The isolates were mainly represented by PCR-ribotypes 001, 002, and 014, which are also frequently detected in CDI cases in Germany [3] and were partially detected in poultry (e.g., chicken meat or faeces) elsewhere [4]. The results of this study reveal that fresh retail chicken meat with skin in Germany is often contaminated with toxin-producing *C. difficile*. The presence of PCR-ribotypes already detected from CDI patients indicates that contaminated chicken meat may be a potential source of human CDI. Subsequent investigations also include the prevalence of *C. difficile* in poultry live stocks to provide more accurate information on the origin of *C. difficile* contamination along the food chain.
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Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

158/LMP
Identification of cereulide producing strains of *Bacillus cereus* group by MALDI-TOF MS
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Question:
The *Bacillus* (*B.*) *cereus* group is genetically highly homogenous and consists of nine recognized species which are present worldwide. Their spores are very resistant to external influences and play an important role for food intoxications by producing different toxins. The heat stable peptide cereulide causes severe

emetic syndromes immediately after ingestion. However not all strains are able to produce cereulide.
Currently, only time-consuming cell bioassays, molecular methods and tandem mass spectrometry are applied for the differentiation between emetic/non-emetic *B. cereus* strains. Thus, the aim of the present study was to set up a fast and reliable method for the differentiation between emetic/non-emetic strains by MALDI-TOF MS.

Methods:
Selected isolates of *B. cereus* group strains (n = 119) were cultured on sheep blood agar for 48h at 37 °C. The cultures were directly analyzed by MALDI-TOF MS without prior extraction steps (direct smear method). The samples were measured in linear positive ionization mode in the mass range of *m/z* 800 – 1,800 Da.

Results:
Using ClinProTools 3.0 statistical software and Flex Analyst, a differentiation between emetic/non-emetic isolates was possible with a rate of correct identification of 99.1 % by means of the evaluation of two specific biomarkers (*m/z* 1171 and 1185 Da).

Conclusion:
In the presented approach, *B. cereus* cultures were directly measured from the agar plate and the detection of cereulide positive/negative samples was performed within 5 minutes. Thus, it is possible to differentiate fast and reliably between emetic and non-emetic strains by MALDI-TOF MS.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

Microbial Pathogenesis (FG MP)

159/MPP

Investigation of the metabolism of *Legionella pneumophila* and appropriate mutants

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Introduction and Aim
Legionella pneumophila (Lp) is an opportunistic intracellular pathogen in humans causing Legionnaires" disease, a pulmonary infection. In the environment it is found in many protozoa, where it replicates within a so called *Legionella* containing vacuole (LCV) and avoids fusion with lysosomes. Restricted in the availability of the substrates within the protozoa, *Lp* has to adapt its metabolism to the host. It could be shown that *Lp* uses amino acids as a main carbon source through the Entner-Doudoroff pathway, but recent studies also indicated an *in vitro* usage of glucose in a growth-phase dependant manner and conversion into the carbon storage molecule polyhydroxybutyrate (PHB). Moreover, the degradation of glycogen and starch has been shown to be dependant on a glucoamylase A (GamA) in *Lp*. In addition, there are further genes in the *Lp* genome coding for putative enzymes of carbohydrate degradation. Addition of ^{13}C-labeled substrates generates isotopologue profiles to investigate the role of carbohydrates in the metabolism of *Lp*.

Material and Methods
In this study we used *Legionella pneumohila* Paris and different metabolic mutant strains generated by homologous recombination. Studies to characterise the mutants were performed in *Lp* YEB medium and minimal medium MDM. In vivo studies were performed in the amoeba *Acanthamoeba castellanii* (ATCC30010) and cell line U937 (ATCC RL-1593.2). Enzyme assays were performed using commercially available Kits. Relative amount of PHB was measured using Fourier-transform infrared spectroscopy (FT-IR). Isotopologue analysis was performed using GC/MS.

Results and conclusion
Here, we generated several *Lp* knockout mutant strains of putative enzymes of glycolysis and degradation of carbohydrates. *In vitro* and *in vivo* studies in growth medium and in *Acanthamoeba castellanii* and U937 respectively, could show their influence in carbohydrate metabolism of *Lp*. Differences in PHB level reflect the importance of this compound in metabolism and survival in *Lp*.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

160/MPP

***Yersinia enterocolitica* engages the macroautophagy pathway for intracellular replication and release from epithelial cells**

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Pathogenic *Yersinia spp*. employ cell-invasive as well as phagocytosis-preventing strategies for efficient colonization of the host organism. In early infection stages, enteropathogenic yersiniae display an invasive phenotype which facilitates penetration of the intestinal mucosa. Our work shows that invasion of epithelial cells by *Y. enterocolitica* is followed by intracellular survival and multiplication of a subpopulation of internalized bacteria. The replicating bacteria were enclosed in vacuoles with typical autophagy-related characteristics, including phagophore formation, recruitment of cytoplasmic autophagosomes to bacteria-containing compartments, and xenophagy. Importantly, the ability of these *Yersinia*-containing vacuoles to fuse with lysosomes and concomitant vesicle acidification were subsequently inhibited by viable bacteria. This resulted in increased intracellular replication and enhanced release of yersiniae from infected cells. Interestingly, deficiency of the core autophagy machinery component FIP-200 impaired development of autophagic features at *Yersinia*-containing vacuoles, as well as intracellular proliferation and egression of the bacteria to the extracellular milieu. Thus, our data suggest that *Y. enterocolitica* takes advantage the macroautophagy pathway to create an autophagosomal niche in epithelial cells that supports intracellular bacterial survival, replication and, finally, spread of the bacteria from infected cells.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

161/MPP

A non-death function of the mitochondrial apoptosis apparatus in infection

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Although some cell death has been observed in numerous infections, the levels of mitochondrial apoptosis are generally low, raising the question of its physiological relevance. Besides releasing molecules triggering apoptosis, mitochondria have recently been found to harbor cell-stimulatory components. We found that all six tested microbial pathogens in epithelial cells triggered a low activity of the mitochondrial apoptosis apparatus that was insufficient to kill the cell but that was able to induce DNA-damage and a DNA-damage response. We further report that low-level activation of the mitochondrial apoptosis apparatus is sufficient to induce cytokine secretion from epithelial cells, which is controlled by the Bcl-2-family of mitochondrial proteins. Blockade of mitochondrial apoptosis reduced cytokine secretion from infected cells and enhanced growth of intracellular bacteria. In addition to the control of apoptosis, mitochondria thus have a function in microbial detection and in the initiation of the innate immune response to infection.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

162/MPP

Identification of target recognition domains in EspL from enteropathogenic *E. coli*

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Enteropathogenic *Escherichia coli* (EPEC) are non-invasive gastrointestinal pathogens that translocate bacterial effector proteins into the host cell cytosol via a type-three secretion system. These effector proteins are able to subvert host cell signaling in favour of bacterial survival and replication and commonly target innate immune and cell death pathways, modulating the immune response to infection. Over the last decade, we have identified and characterized the role of a number of EPEC effector proteins that target the TNFα- and FAS receptor pathways, effectively blocking the production and release of pro-inflammatory cytokines as well as apoptotic cell death. More recently, we identified the effector protein EspL as a novel type of cysteine protease that specifically cleaves the receptor-interacting protein kinases (RIPKs) 1 and 3 in their RHIM domain, resulting in the abrogation of the caspase-independent form of cell death, necroptosis.

Here, we aimed to identify the area(s) of the EspL protein responsible for target recognition and cleavage. We found that the C-terminal 150 amino acids (EspL399-459) are completely dispensable for EspL-mediated cleavage of RIP kinases. Using random transposon-based insertion mutagenesis, we identified 5 insertion sites, which affected cleavage of RIPK3. Interestingly, site-directed mutagenesis of the amino acids surrounding the insertion sites in the context of the full-length protein had no effect on target cleavage. However, closer analysis predicted that the transposon insertions disrupted the ankyrin repeat structure of the C-terminal domain of EspL, hence interfering with the number of or distance between ankyrin repeats. This suggests that the sequence of the ankyrin repeats interacting with the target may be negligible. Future studies are required to test this hypothesis.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

163/MPP

Meningeal pathogens alter defining phenotypes of human brain endothelial cells

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Question: Bacterial meningitis is a serious infection of the central nervous system (CNS) and occurs when bacteria interact with, and penetrate brain endothelial cells (BECs). The blood-brain barrier, and other CNS barriers such as the meningeal blood-CSF barrier are comprised of BECs that exhibit phenotypes that contribute to their unique tightness and promote proper brain homeostasis. Specifically, BECs, when compared to their peripheral endothelial counterparts, exhibit complex tight junctions, low endocytosis rates, and specialized transporters. We explore how bacterial meningeal pathogens interacting with BECs alter these defining phenotypes uncovering novel mechanisms of blood-brain barrier destruction during bacterial meningitis.

Methods: To explore this host-pathogen interaction, we have employed a state-of-the-art model of BECs derived from human stem cells (hSCs). This model retains BEC phenotypes better than any other *in vitro* model to date making it an ideal *in vitro* system to study bacterial-BEC interaction. To examine tight junction integrity, we have employed trans-endothelial electrical resistance (TEER) in addition to fluorescence microscopy. To estimate global rates of endocytosis altered during infection, we assessed the uptake of fluorescent dextrans into BECs using fluorescence plate readers and flow cytometry. Finally, to determine efflux transporter function we employed an assay monitoring the flux of a fluorescent substrate using a fluorescence plate reader.

Results: During infection, tight junction integrity significantly reduced TEER and continuity of tight junction staining. In addition, the transcriptional repressor of tight junctions, Snail1, was upregulated supporting previous work suggesting a tight junction destruction mechanism. Global rates of endocytosis were increased by observing the increase of fluorescent dextrans inside BECs following infection. Efflux transporter function was lost in BECs that had been infected. Inhibitory levels were similar to that of pharmacological drug based inhibition.

Conclusions: Our findings show that the defining phenotypes of BECs are disrupted during infection. These results suggest that a global redefining of BECs may occur during infection. Understanding mechanisms of destruction may provide insight into novel therapeutic interventions. Finally, efflux transporters present a challenge for CNS drug delivery, and our results suggest a novel pool of potential transporter inhibitors.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

164/MPP

Molecular analysis of the anti-apoptotic effector protein AnkG of *Coxiella burnetii*

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Introduction

The obligate intracellular bacterium *Coxiella burnetii* is the causing agent of the zoonosis Q fever. The disease is usually acquired by inhalation of contaminated animal products. The infection can be either acute or chronic. Q fever is mainly a flu-like illness. However, the development of an atypical pneumonia, hepatitis or endocarditis is also possible.

C. burnetii inhibits apoptosis of host cells to ensure bacterial survival. The anti-apoptotic activity of C. burnetii is mediated by a type IV secretion system which secretes more than 150 different effector proteins1. The effector protein AnkG is known to exhibit anti-apoptotic properties which depend on nuclear localization. Hitherto, it has been shown that AnkG binding to host cell proteins p32 (migration)2 and Importin-alpha1 (import)3 is critical for migration into host cell nucleus.

Aims

The nuclear activity of AnkG shall be unraveled. The work focuses especially on protein-protein interactions of AnkG with host cell proteins to deduce influences of AnkG on cell survival.

Material & Methods

Different molecular techniques were used. Potential AnkG interacting proteins were determined by ectopic expression of GFP-AnkG in HEK293T cells followed by a GFP-trap and mass spectrometry analysis. Immunofluorescence was used to verify co-localization of AnkG and its possible interacting proteins.

AnkG-RNA-interactions were examined using RIP-RNA-Seq and was verified via RT-qPCR

Results

Interaction of AnkG with several host cell proteins involved in RNA metabolism was identified. Here, we focus on the binding of AnkG to DDX21. Both proteins co-localize in the host cell nucleoli. For the interaction of AnkG with DDX21 the amino acids 1-28 are necessary and sufficient. Importantly, this region in AnkG was shown to be necessary and for its anti-apoptotic activity.

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Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

165/MPP

Characterization of the *Legionella pneumophila* GDSL hydrolase PlaD

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Introduction

The facultative intracellular pathogen *Legionella pneumophila* is the causative agent of Legionnaire"s disease, a potentially fatal pneumonia. *L. pneumophila* is ubiquitous in aqueous habitats with amoebae as the natural host. However, the pathogen can also colonize lung macrophages and epithelial cells after inhalation of contaminated aerosols. After internalization *L. pneumophila* blocks maturation of the phagosome and thus evades degradation in the lysosome. Instead, the phagosome is remodeled into the *Legionella* containing vacuole which allows replication of *Legionella.* During infection *L. pneumophila* secretes more than 300 effector proteins into the host cell via the Dot/Icm type IVB secretion system. Among others, these influence host vesicle trafficking pathways and host cell death.

Aims

L. pneumophila expresses a multitude of phospholipases. We here focus on the characterization of the phospholipases PlaA, PlaC and PlaD which belong to the family of GDSL hydrolases. In the presented project we aim to understand the importance of PlaD in infection.

Methods

We investigate the lipolytic activities of the GDSL-enzymes via lipid hydrolysis assay and thin layer chromatography. Moreover, we analyze the replication of *L. pneumophila* wild type and mutants in standard and coinfections. Further, we investigate the mode of secretion of PlaD and possible interactions with other proteins by means of proximity labelling and pulldown experiments.

Results

PlaA and PlaC show strong lysophospholipase A (LPLA) and phospholipase A activity, respectively. Moreover, both enzymes exhibit glycerophospholipid:acyltransferase activity. In contrast, only weak LPLA activity has been detected for PlaD so far. In infections of *Acanthamoeba castellanii* and RAW264.7 macrophages an *L. pneumophila plaD*- mutant replicates like wild type. Strikingly, the *plaD*- mutant prevails in competitive infections with the wild type.

Summary

No strong activity has been assigned to PlaD but lack of PlaD seems to benefit replication in competitive infections. Current experiments focus on the mode of secretion of PlaD, possible interactions with host proteins and on its impact on host signaling pathways.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

166/MPP

The genetic transformation of *Chlamydia pneumoniae*

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Chlamydia pneumoniae infect humans and animals causing a wide range of different diseases. The absence of tools for the genetic manipulation of *C. pneumoniae* has severely hampered research into all aspects of its biology. Here, we demonstrate the genetic transformation of *C. pneumoniae* using a plasmid shuttle vector system which generates stable transformants.

The equine *C. pneumoniae* N16 isolate harbors the 7.5 kb plasmid pCpnE1. We constructed the plasmid vector pRSGFPCAT-Cpn containing a pCpnE1 backbone, the red-shifted green fluorescent protein (RSGFP), as well as the chloramphenicol acetyltransferase (CAT) gene used for the selection of plasmid shuttle vector-bearing *C. pneumoniae* transformants. Using the pRSGFPCAT-Cpn plasmid-construct, expression of RSGFP in animal isolate of *C. pneumoniae* LPCoLN was demonstrated. Furthermore, we discovered that the human cardiovascular isolate *C. pneumoniae* CV-6 and the human community-acquired pneumonia associated *C. pneumoniae* IOL-207 could also be transformed with pRSGFPCAT-Cpn.

In previous studies it was shown that *Chlamydia* spp. cannot be transformed when the plasmid shuttle vector is constructed from a different plasmid backbone to the homologous species. Accordingly, we confirmed that pRSGFPCAT-Cpn could not cross the species barrier in plasmid-bearing and plasmid-free *C. trachomatis*, *C. muridarum*, *C. caviae*, *C. pecorum* and *C. abortus*. However, contrary to our expectation, pRSGFPCAT-Cpn did transform *C. felis*. Furthermore, pRSGFPCAT-Cpn did not recombine with the wild type plasmid of *C. felis*.

We provide an easy-to-handle transformation protocol for *C. pneumoniae* that results in stable transformants. The novel vector and technology offer a promising new approach to investigate gene function and to study all aspects of *C. pneumoniae* biology. In addition, the vector can cross the species barrier to *C. felis*, indicating the potential of horizontal pathogenic gene transfer via a plasmid.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

167/MPP

Mechanism of induction of the Toll/Interleukin-1 receptor containing protein C (TcpC) of uropathogenic *E. coli* (UPEC)

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Toll/Interleukin-1 receptor (TIR) containing proteins are present in many pathogens like uropathogenic *Escherichia coli* (UPEC). They interfere with the TLR-signaling chain and the inflammasome, which are an essential part of the innate immune system. Toll/Interleukin-1 receptor containing protein C (TcpC) from the UPEC strain CFT073 is an essential virulence factor that increases the bacterial load and causes severe organ damage. Since the production of TcpC is very energy consuming, it is only translated if necessary. This arises the question how the gene is induced. To find possible gene inductors we tested a putative promoter directly in front of *tcpC* with *gfpmut2* as a reporter gene that was measured by its fluorescence in FACS.

Since K+ efflux is the trigger for the activation of the inflammasome, UPECs might counteract by producing TcpC when the K+ concentration increases. To test this, bacteria were incubated in K+-free glucose-minimal medium (Gluc-min) and different K+ concentrations were added. The fluorescence increased significantly in three experiments. Different media that were needed for experiments with cells and bacteria might influence the promoter activity. To test this, bacteria were grown in different media over 69 h to measure the fluorescence over time. The fluorescence in DMEM ceased within 48 h, while McCoy and RPMI caused an increase in fluorescence over 69 h. In Gluc-min and EMEM, the signals were relatively low. We tested if the gene induction is dependent on the bacterial density itself. We could show that an increasing bacterial density dose-dependently induces a higher fluorescence. This effect was stronger and significant when the bacteria were grown in McCoy medium. UPECs might recognize eukaryotic cells to impair their innate immune response. To test this, bacteria were added to different concentrations of RAW, T24, THP1, and HeLa and incubated for 24 h. Higher concentrations of T24 and undifferentiated THP1 cells caused a significant increase in fluorescence, while differentiated THPs significantly inhibited the promoter. To test urine as an inductor, bacteria were incubated in different urine concentrations for 24 h. An increase in urine concentration showed a highly significant increase in fluorescence.
In conclusion, K^+, urine, high bacteria density, T24s and undiff. THP1s induce TcpC. Differentiated THP1s inhibit the expression. More mechanisms of TcpC induction and expression regulation need to be found and studied.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

168/MPP

Generation and characterisation of KDELR-KO cell lines.

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1. **Introduction**

A/B toxins such as cholera toxin, *Pseudomonas* exotoxin and yeast killer toxin K28 contain a KDEL-like motif at either subunit which ensures retrograde toxin transport through the secretory pathway of a target cell. Intoxication and host cell entry is initiated by toxin binding to plasma membrane (PM) receptors where the yeast KDEL receptor (KDELR) was recently identified as receptor of K28 (Becker *et al.*, 2016 (1)). In this context, the hypothesis of KDELR co-localisation at the PM was further confirmed in yeast and mammalian cells (Becker *et al.*, 2016 (1, 2), Henderson *et al.*, 2013). As KDELRs function as GPCRs in the regulation of vesicle trafficking in mammalian cells, a similar signalling function after cargo binding at the cell surface is assumed (Cancino et al., 2014).

2. **Goals**

To address such novel functions, CRISPR/Cas9-mediated KDELR knock-out (KO) cell lines should be generated and characterised.

3. **Material & Methods**

CRISPR/Cas9, PCR, Sequencing, qPCR, Western Blot, MTT-Assay, Scratch-Assay, Adhesion-Assay, Transcriptome analysis

4. **Results and Conclusions**

Whereas KDELR2- and KDELR3-KO HEK293 cells were successfully created, a KDELR1-KO inhibited cell survival indicating a predominant KDELR1 relevance in this cell line. Characterisation of commercially available KDELR1-KO HAP1 cells revealed a strong sensitivity under ER stress conditions and an increased secretion of PDI. Additionally, transcriptome analysis demonstrated alterations of developmental processes, processes concerning cell adhesion and extracellular matrix (ECM) functions. Disturbance of the adhesion behaviour was further confirmed by adhesion assays where KDELR1-KO cells showed poorer adhesion to uncoated surfaces and stronger adhesion to collagen- and laminin-coated surfaces. These changes of the adhesion properties might be caused by defects in protein secretion and thereby proper ECM formation, which additively influence the cellular migration abilities.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

169/MPP

Proteoglycans are important for intracellular survival of *salmonella* typhimurium

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Introduction

Proteoglycans are involved in cell-matrix interactions and activation of growth factors and chemokines, as well as adhesion of bacteria to host cells. However, the role of proteoglycans in bacterial pathogenesis is poorly understood. The *XylT2* gene encodes the β-D-xylosyltransferase initiating the glycosaminoglycan (chondroitin sulfate, heparan sulfate, heparin and dermatan sulfate) assembly on the core protein of proteoglycans. In this work, we compared *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) colonization of wild-type and proteoglycan-negative (Δ*XylT2)* epithelial cells.

Materials & Methods

Gentamicin protection assays were used to evaluate invasion and replication of *S*. Typhimurium in Chinese hamster ovary (CHO-K1) cells: wild-type CHO-K1 and xylosyltransferase-deficient mutant (psgA-745, or Δ*XylT2*). Gentamicin uptake by CHO cells was measured by ELISA. Expression of *Trpv1*, *Trpv4*, *Tnfa*, and *Cxcl1* was assessed by RT-qPCR. SPI-2 reporter strains of *S*. Typhimurium were utilized to determine intracellular location of bacteria in infected CHO-K1 cells.

Results

Adhesion to and invasion into CHO-K1 and Δ*XylT2* cells was similar. However, 24 hours after infection, CHO-K1 cells lacking proteoglycans were significantly less colonized by *Salmonella* compared to WT controls. Survival of *Salmonella* was dependent on the dose of gentamicin in Δ*XylT2* cells but not in CHO-K1 wildtype cells. Expression of transient receptor potential cation channel subfamily V member 1 (Trpv)1 and Trpv4 channels, which can mediate uptake of gentamicin was comparable between the studied CHO-K1 cell lines. In addition, intracellular gentamicin levels were similar in CHO-K1 WT and Δ*XylT2* cells. Upon infection, both CHO-K1 WT and Δ*XylT2* cells expressed similar levels of the cytokines Tnfa and Cxcl1. Thus, the decreased bacterial colonization in CHO-K1 Δ*XylT2* cells at 24 hours p.i. is not due to the increased antibiotic uptake.

Conclusions

Overall, our results indicate that proteoglycan synthesis is important for intracellular survival of *Salmonella* in gentamicin protection assay model. The effect of proteoglycans on intracellular localization of *Salmonella* in CHO-K1 cells is currently investigated.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

170/MPP

Do superoxide dismutases A and M support *Staphylococcus aureus* long-term persistence in cystic fibrosis airways?

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Introduction: Due to a genetic disorder, cystic fibrosis (CF) patients suffer from airway dehydration, leading to mucus accumulation, decreased lung function and early death. In the thick mucus, pathogens like *Staphylococcus aureus* settle down and persist for several years.

Objectives: By analyzing cytoplasmic proteome and gene expression of clonal short- (first) and long-persisting (late)

S. aureus isolates recovered from the airways of different CF patients, in an earlier study (Treffon and Block et al. J Infect Dis. 2018) we revealed several adaptation mechanisms that might support bacterial long-term persistence in the CF airways. The current project aims to elucidate the benefits of some of these adaptation mechanisms for *S. aureus* persistence in CF airways by focusing on the bacterial oxidative stress defense system.

Methods: Clonal first and late *S. aureus* isolates cultured from respiratory specimens of six individual CF patients were chosen for analysis of the bacterial survival and gene expression in different CF-related in vitro settings.

Results: While we detected a higher abundance and gene expression of the superoxide dismutase SodM in most late isolates compared to the first clones in our former study, our current study revealed that the expression of the usually more frequent SodA did not differ much among the first and late isolates. By performing neutrophil killing assays and internalization assays in human airway epithelial cells, we detected that *sodA* and *sodM* deletion mutants were eradicated faster by both cell types compared to the wild type. Interestingly, at least some of the late CF isolates were slightly more invasive and more able to survive in the epithelial cells than the first isolates. Furthermore, in internalized bacteria, both *sodA* and *sodM* were highly expressed, indicating that intracellular bacteria are exposed to oxidative stress in epithelial cells. To corroborate our findings, we plan to measure the levels of intracellular oxidative stress in these infected host cells.

Conclusion: Our results indicate that SodA and SodM might be proteins that facilitate the long-term persistence of *S. aureus* in the CF airways by protecting the bacterium against neutrophil killing and probably supporting bacterial residence in airway epithelial cells. Experiments with complemented mutants for data verification are in progress.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

171/MPP

Staphylococcus epidermidis biofilms alter the pro-inflammatory immune response in primary human macrophages

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Polarized macrophages are the first defense line of the human immune system. Since *S. epidermidis* evolved sophisticated mechanisms to escape the host"s immune response professional phagocytes are facing problems eradicating those pathogens. Biofilm formation is crucial for the bacteria in order to be protected from phagocytosis. Instead of being activated upon infection with bacteria embedded in a biofilm the macrophages" expression and secretion of pro-inflammatory cytokines such as TNF-α and Il-1ß is significantly reduced in contrast to contact with single cell bacteria. However, anti-inflammatory cytokines are highly upregulated. Along with these findings, the phagocytosis rate is dramatically decreased as soon as bacteria are protected in a biofilm. The *S. epidermidis* recognition receptor is TLR-2. By blockage of this receptor the phagocytosis rate of biofilm positive strains can be recovered as well as the induction of the pro-inflammatory defense. In order to understand the underlying mechanisms an RNAseq and validation experiments with murine TLR2 knockout cells are carried out.

Understanding biofilm related immune escape mechanisms of *S. epidermidis* and other biofilm forming bacteria may pave the way towards novel therapeutic approaches in the future.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

172/MPP

Analysis of neutrophil extracellular traps during _Streptococcus suis_ meningitis in pigs

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The formation of neutrophil extracellular traps (NET) is an innate defense mechanism against bacterial infections. Neutrophil granulocytes form NETs consisting of a nuclear DNA backbone associated with antimicrobial peptides (AMPs), histones and proteases in response to pathogens such as *Streptococcus suis (S. suis)*. This important causative agent of meningitis in pigs and humans secrets an extracellular nuclease, designated *S. suis* nuclease A (SsnA). Previous studies demonstrated that SsnA contributes to NET degradation and an improved bacterial survival *in vitro* [de Buhr et al., 2013]. However, NET formation and entrapment of streptococci in NETs was detected in cerebrospinal fluid (CSF) of pigs with meningitis [de Buhr et al., 2017].

We hypothesize that NETs can be stabilized by host factors against degradation and that *S. suis* survives entrapment in NETs due to protection against AMPs. Objectives of this ongoing study are to investigate the role of SsnA during meningitis and the described D-alanylation of lipoteichoic acid (LTA) [Fittipaldi et al., 2008] as a putative evasion mechanism against NETs and associated AMPs.

To characterize the role of SsnA, piglets were experimentally infected with *S. suis* serotype 2 wt and the isogenic deletion mutant 10ΔssnA. Animals were euthanized two and four days post infection to capture early stages of meningitis. CSF was analyzed for NET formation and bacterial entrapment by immunofluorescence microscopy.

Our *in vivo* data demonstrated formation of NETs in CSF at early stages of meningitis. However, the nuclease SsnA was not crucial for virulence of *S. suis*. Importantly, the porcine antimicrobial peptide PR39 was associated with NETs in infected CSF. This finding suggests that protection against PR39 might be crucial for survival of *S. suis* during meningitis. Accordingly, we generated further mutants deficient in the D-alanylation of LTA to test their susceptibility to AMPs such as PR39 and their putative function in protection against NETs.

In summary, our data indicates that NET formation is an important host-pathogen interaction mechanism in the pathogenesis of *S. suis* meningitis and suggests that *S. suis* expresses further factors besides nuclease SsnA for protection against NETs and its associated AMPs in CSF.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

173/MPP

The M-like protein SzM of *Streptococcus equi* subsp. *zooepidemicus* binds IgG and fibrinogen and protects against killing in equine blood

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Introduction

Streptococcus equi subsp. *zooepidemicus* (SEZ) is a very heterogeneous bacterial subspecies causing severe diseases such as pneumonia in different animals including horses. Furthermore, it is a zoonotic agent and contact to horses is a known risk factor. SEZ harbours the surface anchored M-like protein SzM. We could show in a previous study that SEZ isolates of human infections harboured distinct SzM types. Moreover, these isolates are associated with binding of equine and human fibrinogen.

Aims

In this study, we investigated the working hypothesis that the SzM proteins of SEZ are major virulence factors, involved in binding of host plasma proteins, like fibrinogen and immunoglobulin, and are important for the survival in blood.

Materials and methods

SzM proteins of human and equine isolates of SEZ were cloned and expressed in *E. coli*. Recombinant SzM (rSzM) proteins were used in Western blots and ELISAs to investigate the binding of immunoglobulins of several host species. Furthermore, the binding of equine and human fibrinogen was analysed. Two SzM in-frame deletion mutants (one human and one equine isolate) were constructed and investigated in host plasma protein binding assays and bactericidal assays.

Results

rSzM proteins of distinct SEZ isolates differed in their ability to bind immunoglobulin IgG of different host species. Furthermore, binding of equine and human fibrinogen by rSzM of SEZ isolate C33 was demonstrated. The isogenic mutants C2ΔSzM and C33ΔSzM were shown to be attenuated in fibrinogen binding in a host plasma protein binding assay. Moreover, these mutants were killed in contrast to the *wt* in equine blood *ex vivo*.

Conclusion

We show that the SzM proteins of SEZ are involved in IgG and fibrinogen binding. Furthermore, SzM-mutants loss their ability to bind fibrinogen and were attenuated in a blood survival assay. Thus, SzM of *Streptococcus equi* subsp. *zooepidemicus* binds different host proteins and most likely plays an important role in pathogenesis.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

174/MPP

Different effects of pneumolysin and pneumococcal infection on primary microglia and bone marrow-derived macrophages

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Streptococcus (S.) pneumoniae is a commensal bacterium of the human upper respiratory tract. It is the most common cause of bacterial meningitis and meningoencephalitis with a high mortality rate of 20-30 %. *S. pneumoniae* possesses major virulence factors: the capsule (cps) and the cytolysin pneumolysin (PLY). The role of both virulence factors in the pathogenesis of meningitis is still not fully understood. In the central nervous system (CNS), glial cells are considered to play an important role during inflammation and injury of the brain in bacterial meningitis. Microglia are the resident macrophages of the CNS. To characterize the response of these specific brain macrophages to pneumococcal infection, a comparative analysis of primary microglia cells and bone marrow-derived macrophages (BMDM) was performed.

Mixed glial cell cultures were prepared from neonatal C57BL/6 mice. Microglial cells were isolated and characterized by flow cytometry. Bone marrow-derived cells were prepared from adult C57BL/6 mice and differentiated into BMDM. Microglia and BMDM were exposed to different concentrations of PLY. Cytotoxicity was measured by LDH release. Furthermore, both primary phagocyte cultures were infected with wild-type *S. pneumoniae* D39 and *cps*- and *ply*-deficient mutants. The survival of phagocytes during the infection was visualized by Hoechst and propidium iodine staining, bacterial growth was determined by plating. Additionally, the intracellular survival of pneumococci was determined by penicillin-gentamicin protection assay. Treatment of cells with PLY led to a dose-dependent cytotoxic effect in both phagocyte types. However, very high concentrations of toxin were necessary for this effect. Primary microglia were killed during pneumococcal infection independently of PLY, whereas BMDM were not affected.

In conclusion, different primary phagocytes do not react to PLY and pneumococcal in a uniform manner.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

175/MPP

Exploitation of experimental *in vivo* infection models in combination with OMICs technologies to decipher pneumococcal antigens

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Introduction: *Streptococcus pneumoniae*, a pathobiont of the upper respiratory tract, encounters various host compartments in the human host causing thereby serious local and invasive infections such as pneumonia, bacteremia or meningitis. Vaccines are available and show a high efficacy, however, they protect only against a subset of the 96 pneumococcal serotypes. Thus, we initiated to identify host-compartment specific pneumococcal antigens, which alone or in combination provide protection against colonization and invasive pneumococcal infections.

Material and Methods: Pneumococci were isolated from various host compartments including the nasopharynx, the lung and their adaptation to these host compartments is investigated by proteomics and transcriptomics. A pneumococcal protein library of more than 60 proteins has been generated. Pneumococcal proteins specifically identified by *in vivo* proteomics and transcriptomics are assessed for their immunogenicity using an immunoproteome-based approach.

Results: Currently, we have identified more than 40 immunogenic antigens, which are surface-exposed and can be considered to be candidates for a serotype-independent protein-based vaccine. The humoral and cellular immunity will be characterized for selected pneumococcal antigens to identify *ex vivo* correlates of protection.

Summary: The application of *in vivo* infection models and OMICs technologies leads to select 40 candidates of pneumococcal antigens approximately. Further immunization and protection studies will be performed to generate a multi-component protein-based vaccine. This work is a part of InfectControl2020 project named VacoME.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

176/MPP

Establishing respiratory co-infections with Influenza A Virus and *Staphylococcus aureus* in mice

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Introduction: Seasonal and pandemic influenza A Virus (IAV) infections present a burden to the global community with up to 500,000 fatal cases annually. *Staphylococcus aureus* is a typical colonizer of the human upper respiratory tract. Recent studies indicate that acquisition of IAV can lead to the dissemination of *S. aureus* to the lower respiratory tract, which in turn can result in superinfections, including severe pneumonia.
Objectives: The aim of the study was to establish a natural route of co-infections by colonizing the mice with *S. aureus* before introducing the IAV to the host. This will provide the basis for future directions studying the synergistic effects of the pathogens in the host, including local and systemic effects on microbiome composition, immune responses, and tissue pathology.
Materials and Methods: First, single agent infections with H1N1 IAV and colonization with *S. aureus* strains USA300 and SA113 were established. Bacterial burden in the nasopharyngeal cavity and lungs, systemic and local immune responses, including the composition of leukocytes and the inflammatory status, were monitored over the whole period of infections. Second, following three days of colonization with bacteria co-infections will be performed.
Results: Colonization of C57BL/6J mice with *S. aureus* results in a complete clearance of the bacteria within seven days. Bacteria were only recovered from nasopharyngeal cavity during the first three days post nasal inoculation. During the first two days of colonization weight loss, increased protein concentration in the bronchoalveolar fluid as well as increased neutrophils accompanied by a drop in the lymphocytes counts were noticed systemically. Infections of mice with human derived H1N1 showed no effects during the first six days. On day seven, weight loss as well as a shift in neutrophils/lymphocytes counts were observed.
Conclusion: Although mice were only colonized, signs of systemic and local inflammation were noticed during the first two days of bacterial application. All clinical values normalized on day three. This time point will be used for subsequent viral infection in future studies.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

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Infection patterns of colonizing and invasive *Streptococcus pneumoniae* strains with human cell compartments

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Introduction: *Streptococcus pneumoniae*, the major cause of community-acquired pneumonia and bacterial meningitis, is known to cause 1.6 million deaths annually. The interaction between *S. pneumoniae* and cells of the various human lung compartments is a prerequisite for pneumococcal infection, yet the specificity of these interactions is not fully understood.
Objectives: In the present study, we aim to understand the mechanism of infection of a colonizing 19F and an invasive TIGR4 strain with lung epithelial cells, lung fibroblasts and various human immune cells.
Methodology: The interaction of the bacteria with lung epithelial cells (16HBE) and fibroblasts (MRC5) are studied by performing infection assays. The cytotoxicity of infection was determined by LDH assay. Onset of a bacterial infection is characterized by a massive infiltration of innate immune cells in the lung and the persistence of the disease can be attributed to the survival of the bacteria inside these immune cells. Therefore, the intracellular killing kinetics of the bacteria by monocytes and macrophages were also analyzed.
Results and Future prospects: The results of the present study demonstrate that the invasive TIGR4 strain invades the lung cells more efficiently compared to the colonizing 19F strain. Also, the invasive strain shows prolonged survival in professional phagocytes. Future research will include *in vitro* studies in a more complex system, e.g. 3D lung tissue model that would help enhance the understanding of the host- pathogen interplay during bacterial infections.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

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Dissecting the effect of probiotic and extraintestinal pathogenic *Escherichia coli* on an intestinal epithelial cell line at the transcriptional level

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Probiotic *E. coli* strain Nissle 1917 (EcN), uropathogenic *E. coli* (UPEC) strain CFT073 and asymptomatic bacteriuria (ABU) *E. coli* isolate 83972 are clonal and their genomes exhibit an average nucleotide identity (ANI) of > 99.8 %. While gut isolate EcN has a remissive effect on ulcerative colitis and ABU isolate 83972 is used to treat recurrent urinary tract infections (UTIs), UPEC CFT073 causes symptomatic UTI. To understand the molecular mechanisms by which probiotic bacteria can countervail inflammatory gastrointestinal conditions, we need a cell culture model that bridges the gap between the insights we obtain from 2D cell culture models and *in vivo* studies. On the transcriptional level, we want to study the bacterium-host interaction of *E. coli* at the epithelial site that includes a continuous mucus layer.
Transwell cell culture systems are used for culturing cells as polarised cell structures that better mimic the morphology and function of the gut epithelium *in vivo* than cell monolayers. Epithelial cells in the colon are covered by a mucus layer, which shields the cells from direct contact with bacteria. We culture HT29-MTX-E12 cells in a semi-wet manner under continuous shaking to trigger the secretion and formation of an adherent mucus layer[1]. This method has been described to create polarization, formation of functional tight junctions, a three-dimensional architecture and the production of an adherent mucus layer. Using the EcN human re-isolates and the above mentioned clonal *E. coli* strains with different virulence phenotypes, we tested for differential transcriptional responses of the intestinal epithelial cells upon interaction with these microbes. We saw that all *E. coli* strains tested elicited an antibacterial response in the host cells, though physically separated by an adherent mucus layer. Host cell damage caused by *E. coli* correlated to some extent with the virulence of the respective strain, while also probiotic bacterial strains affected the expression of several inflammatory markers in host cells. We believe that differential host cell response is an important factor that determines bacterial pathogenicity or probiotic traits.
[1] Reuter C, Alzheimer M, Walles H, Oelschlaeger TA. An adherent mucus layer attenuates the genotoxic effect of colibactin. Cellular Microbiology. 2018;20:e12812.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

179/MPP

Role of *Salmonella* effectors and host matrix metalloproteinases for the persistence of *Salmonella* Typhimurium

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Salmonella enterica serovars cause a variety of diseases ranging from self-limiting enterocolitis to severe systemic infections. Dependent on the serovar, 2-5% of immunocompetent individuals become chronic carriers. The factors contributing to persistent *Salmonella* infection are incompletely understood but the interaction of host defenses with bacterial virulence factors is of critical importance. *Salmonella* is equipped with type 3 secretion systems encoded in *Salmonella* pathogenicity islands (SPI). SPI-1 translocated effectors, besides their generally recognized role in invasion, are thought to participate in intracellular cytosolic replication of *Salmonella* and thus might contribute to the establishment of persistent *Salmonella* infections.
We aimed at elucidating the contribution of (1) *Salmonella* SPI-1 effectors and (2) host matrix metalloproteinase 10 (MMP-10) to *Salmonella* persistence.
(1) Murine fibroblasts (NIH 3T3) were infected with *S.* Typhimurium *ΔinvA*, a mutant unable to translocate SPI-1 virulence factors, and strains lacking specific SPI-1 translocated effectors (*ΔsipA*, *ΔsopB*, *ΔsopE*, *ΔsopE2*). Invasion and replication of the *ΔinvA* mutant was diminished in comparison to wild-type. Invasion of the triple deletion strains, *ΔsipA/ΔsopE/ΔsopE2* and *ΔsopB/ΔsopE/ΔsopE2*, was impaired, while the invasion rate of the *ΔsopE/ΔsopE2* mutant was comparable to wild-type. However, intracellular replication and survival of the triple deletion strains were better compared to wild-type. This indicates that the presence of SipA and SopB enhances invasion but might have a negative effect on intracellular survival.
(2) Infection of bone marrow derived macrophages (BMDM) results in a high upregulation of MMP-10 mRNA. Infection of MMP-10 deficient BMDM induced an increased proinflammatory response as observed by higher levels of MCP-1, IFN-β, IFN-γ, and nitrosative stress in comparison to infected wild-type macrophages. *Salmonella* showed increased survival in the absence of MMP-10 in long-term infected BMDM (3 days) while there was no difference at early points post infection (6 hours and 1 day). Filamentous growth of *Salmonella*, which might represent a survival strategy, was strongly increased in MMP-10 deficient macrophages 3 days post infection.
Conclusion: SPI-1 factor(s) are important for intracellular replication and MMP-10 plays a role in restricting *Salmonella* survival and dampening the host inflammatory response to infection.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

180/MPP

Innate immune responses to EPEC infection in the newborn mouse

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Enteropathogenic *Escherichia coli* (EPEC) is a major causative agent of infantile diarrhea in developing countries. Recently, we have developed a neonatal mouse model of infection that allows the study of EPEC pathogenesis *in vivo*. Infection of newborn mice with EPEC E2348/69 leads to a transient colonization of the intestine associated with the formation of EPEC microcolonies intimately attached to the small intestinal mucosa (A/E lesions). Here, we further describe the host response to EPEC infection *in vivo* and characterize the role played by the intestinal epithelial and hematopoietic cell compartments using specific knockout mouse lines.
Reference: Dupont A, Sommer F, Zhang K, Repnik U, Basic M, Bleich A, Kühnel M, Bäckhed F, Litvak Y, Fulde M, Rosenshine I, Hornef MW, 2016, Age-dependent susceptibility to Enteropathogenic Escherichia coli (EPEC) infection in mice, *PLoS Pathog*, 12:e1005616.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

181/MPP

The *Acinetobacter* Trimeric Autotransporter Adhesin Ata Controls Key Virulence Traits of *Acinetobacter baumannii*

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Introduction: *Acinetobacter baumannii* is a Gram-negative pathogen that causes a multitude of nosocomial infections. The *Acinetobacter* trimeric autotransporter adhesin (Ata) belongs to the superfamily of trimeric autotransporter adhesins which are important virulence factors in many Gram-negative species.
Objective: We evaluated the impact of Ata in host-pathogen interaction by analysing adhesion, invasion, host-cell-modulation and apoptosis in human endothelial and epithelial cells.
Materials and Methods: Adhesion of *A. baumannii* to human host cells was investigated using primary endothelial and epithelial cells under static and dynamic flow conditions. Furthermore, an *ex vivo* organ infection model employing human umbilical cord veins was employed. Host cell invasion was analysed by a gentamicin protection assay. Induction of apoptosis was determined using propidium iodide/annexin V staining with subsequent FACS analysis and the Caspase-Glo assay (Promega). Chemokine secretion upon infection was analysed using Multi-Analyte ELISArray Kits (Qiagen). Pathogenicity *in vivo* was evaluated using the *Galleria mellonella* infection model.
Result: Phylogenetic profiling using 3,052 *Acinetobacter spp.* genomes revealed that *ata* is present in 78% of all *A. baumannii* isolates but only in 2% of the closely related but less pathogenic species *A. calcoaceticus* and *A. pittii*. Employing a markerless *ata* deletion mutant of *A. baumannii* ATCC 19606 and complemented controls, we could show that adhesion to and invasion into human endothelial and epithelial cells under static conditions both clearly depend on Ata. This adhesion defect was even more evident when dynamic infection conditions were applied or when human umbilical cord veins were infected *ex vivo* with *A. baumannii*. Infection of primary human umbilical cord vein endothelial cells (HUVECs) with *A. baumannii* led to the secretion of the pro-inflammatory cytokines interleukin (IL)-6 and IL-8 in a time- and Ata-dependent manner. Ata induced apoptosis, but not necrosis, of HUVECs after 16 h post infection by activation of caspase-3 and caspase-7. Ata deletion mutants were furthermore attenuated in their ability to kill larvae of *G. mellonella* and to survive in larvae when injected at sublethal doses.
Conclusion: These results strongly suggest that Ata is an important multifunctional virulence factor of *A. baumannii* that triggers multiple important steps for the initiation of successful infections in different host cells.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

182/MPP

***Pseudomonas aeruginosa* microevolution in cystic fibrosis lungs**

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The airways of patients suffering from the genetic disorder cystic fibrosis (CF) are predisposed to infections with opportunistic bacterial pathogens. In most patients chronic infections with *Pseudomonas aeruginosa* are established during childhood and typically determine the clinical course. Over the years of the infection course, the bacteria undergo microevolution presumably enhancing the adaptation to the lung habitat.
At our local clinic *P. aeruginosa* isolates have been collected semi-annually from 35 chronically infected patients since the 1980s. To monitor microevolution against the CF background we investigated sequential isolates from twelve patients, six with the mildest and six with the most severe clinical course. Isolates of a persisting clone were genome sequenced (> 260 isolates in total) and interrogated for variations manifested during the infection course. The isolates were also tested for phenotypic traits such as morphology, motility and virulence factor secretion.
The sequencing data revealed various evolutionary modes from the presence of single adapted strains to the long-term persistence of co-existing clades, with mixed types in between. Courses from severely affected patients, however, were usually characterized by the persistence of one major bacterial clade during late stage of infection whereas mild courses displayed ongoing diversification to a greater extent. In total, > 4800 mutations occurred in the twelve courses, mostly nucleotide exchanges but also frame-shifting indels and accessory genome variations. Most "hotspots" of CF lung microevolution were associated with either antimicrobial resistance or surface components such as alginate. Non-neutral mutations predominantly emerged in *P. aeruginosa* genes relevant for sensing of and protection against the lung environment (antibiotic resistance, cell wall components, two-component systems). Drastic and loss-of-function mutations preferentially happened during severe courses of infection. Re-shaping of the accessory genome by uptake and loss of mobile DNA elements could be observed for all courses. The lineages from the mild courses more proficiently incorporated extra metabolic genes by these events.
In summary, the analysis of the "in host" microevolution of *P. aeruginosa* revealed hints for different evolutionary paths and modes during chronic infection depending on whether the bacterium had taken residence in a CF patient with normal or already compromised lung function.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

183/MPP

Intraclonal competitive fitness of *Pseudomonas aeruginosa* cystic fibrosis airway isolates in human precision-cut lung slices (PCLS)

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Chronic airway infections with the gram-negative bacterium *Pseudomonas aeruginosa (P. aeruginosa)* play an important role for the clinical course of patients suffering from cystic fibrosis (CF). In this case, infections of the respiratory tract cause progressive loss of lung function and reduced life expectancy. During the colonization of the patient"s lung, the bacteria undergo microevolution, presumably to adapt to the CF lung habitat. After analyzing the influence of this adaptation on the fitness of P. aeruginosa to grow together in an aquatic habitat (in vitro), we now want to explore how the competitive fitness of the bacteria looks like, if they are grown in a human lung habitat.
For this purpose, the PCLS will be used as a human based ex vivo model. The PCLS will be prepared from human explanted lung tissue, filled with agarose gel and cut into small slices. Several experiments have proven that these slices are still viable and show functional responses, like airway- and vasoconstriction or immunological and epithelial reactions*. After pretreatment with media, the slices will be infected with competitively growing serial clonal *P. aeruginosa* isolates from 12 patients (6 with severe courses and 6 with mild courses). These isolates were selected from *P. aeruginosa* positive CF-patients in the clinic at the Hannover Medical School during their whole infection period – exemplary up to 30 years. To evaluate the composition of the bacterial communities after 12 and 24 hours, the slices will be homogenized, dilutions will be made and plated on LB-agar plates. Afterwards, the bacterial DNA will be isolated and the relative quantities of each sequential isolate will be determined by sequencing of bacterial DNA-derived amplicons spanning strain-specific SNPs.

** Neuhaus, V., Danov, O., Konzok, S., Obernolte, H., Dehmel, S., Braubach, P., Jonigk, D., Fieguth, H.G., Zardo, P., Warnecke, G., Martin, C., Braun, A., Sewald, K. Assessment of the Cytotoxic and Immunomodulatory Effects of Substances in Human Precision-cut Lung Slices. J. Vis. Exp. (), e57042, doi:10.3791/57042 (2018)*

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

184/MPP

iPSC-derived macrophages as a treatment approach for multidrug-resistant Pseudomonas aeruginosa infections in cystic fibrosis patients

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According to the World Health Organization, infectious diseases of the lower respiratory tract are among the top five causes of death with 3.1 million reports in 2012. Especially infections with Pseudomonas aeruginosa (P. aeruginosa), a leading pathogen in causing nosocomial infections, are associated with substantial mortality. Since P. aeruginosa is equipped with a strong antibiotic resistance machinery, the number of infections caused by multidrug-resistant P. aeruginosa is constantly growing. In light of the emerging threat of antibiotic resistant bacteria, new and alternative therapeutic approaches are highly desirable. Similarly, new therapeutic interventions are needed for bacterial pathogens which are refractory to antibiotic treatment and commonly seen in patients suffering from cystic fibrosis (CF). In CF, P. aeruginosa is linked to progressive loss of lung function and very severe courses. In our current project, we therefore plan to investigate the phagocytic capacity of macrophages targeting different (antibiotic resistant) P. aeruginosa derived from various CF clinical isolates. We aim to use induced pluripotent stem cell technology (iPSC) to gain insights into macrophage-pathogen interaction with the aim to establish an innovative cell therapy approach for chronic P. aeruginosa infections. For this ambitious aim, we will first set up cell-based co-culture assays using the P. aeruginosa reference strain PA14 and different clinical isolates of CF patients to elucidate the phagocytic capacity of iPSC-derived macrophages. Hannover Medical School can rely on a unique strain collection of sequential clinical isolates of its patients, previously isolated right from the individual onset of infection over the whole infection period. Patient courses had been described in detail (Klockgether et al. 2018). Phagocytosis and behavior of iPSC-derived macrophages will be analyzed using selected sequential isolates in cell culture and plating experiments measuring colony forming units. Also, pro- and anti-inflammatory cytokine analysis will be conducted to evaluate the therapeutic potential of iPSC-derived macrophages.
Taken the potential to generate scalable amounts of iPSC-derived macrophages, a successful completion of this proposal will allow

assessing the capacity of these cells to phagocytose antibiotic resistant P. aeruginosa and may lay the foundation for a new cell-based therapy targeting chronic bacterial infections of the lower respiratory tract.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

185/MPP

Cysteine dependent regulation of ToxR transcriptional activity in *Vibrio cholerae*

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Introduction:
The secretory diarrhoeal disease cholera is caused by infection with *Vibrio cholerae*. There, virulence expression is coordinated by a regulatory network that has been historically referred to as the ToxR regulon. As a fundamental process, we investigate the regulation of ToxR transcriptional activity, a key player within this regulon. ToxR is located in the inner membrane exposing a transcription factor active cytosolic N- and a periplasmic located C-terminal domain. The latter contains two cysteine residues and enables ToxR to form intra- or intermolecular disulfide bonds. We recently found that also proteases respond to the redox state of the two cysteine thiol groups of ToxR leading to a rapid ToxR degradation under reducing conditions.
Objectives:
We aimed to elucidate the impact of the two periplasmatic cysteine residues of ToxR on transcriptional activity in more detail. In this context we also investigated the influence of environmental stimuli (e.g. bile salts) since the periplasmic domain has been proposed to be a sensor for abiotic signals.
Methods:
Experiments were carried out using the *V. cholerae* O1 El Tor biotype which is responsible for the most recent cholera epidemics (e.g. Yemen since 2016). Protein samples were analysed by western blotting and immune detection. Furthermore we measured transcription and performed proteolysis assays with the key players.
Results:
The ToxR^{C293S} mutation increased the transcriptional activity of ToxR and facilitated the formation of a dimer, indicating that the conserved Cys236 plays a crucial role in maintaining the activity. On the other hand, a single mutation at Cys293 or a double cysteine exchange reduced transcription of ToxR regulated genes.
Conclusion:
Cysteine residues play crucial roles in the catalysis and function of many proteins as in the case of oxygenases or cysteine proteases. This residue is redox-active and is particularly responsive to the local environment. Inhere we can demonstrate that the two cysteine residues in ToxR impair its transcriptional activity and stability by undesired disulfide bond formation and oxidation.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

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Screen for fitness and virulence factors of *Francisella* sp. strain W12-1067 using amoebae

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Francisella tularensis (*Ft*) is an intracellular pathogen causing tularemia in a variety of hosts including humans and rodents. In Germany only isolates of *Ft* subspecies *holartica* are found so far. A new *Francisella* strain not belonging to subsp. *holartica* was recently found in a water reservoir of a cooling tower in the east of Germany. This isolate (W12-1067) showed a great sequence homology to a Chinese isolate, also found in a cooling tower, which is now referred to as *Allofrancisella guangzhouensis*. Since not much is known about the German isolate W12-1067, we continue to name it *Francisella* sp. strain W12-1067. A W12-1067 Tn5 mutant bank was generated and used in a so-called Scatter Screen assay to investigate the isolate in respect to its putative virulence. The Scatter Screen method was originally established for *Legionella* to identify virulence factors. Here, the amoeba *Acanthamoeba lenticulata* was used as a host model since pretests revealed persistence of W12-1067 in this amoeba. In total, 79 Tn5 scatter clones were identified using the scatter screen method with *A. lenticulata* 45 and 118. All 79 scatter clones were further analyzed in an infection/persistence assay. Thereby, the scatter clones showed either a similar or a reduced persistence in comparison to the wildtype strain W12-1067. Clones which showed a strongly reduced persistence were checked regarding their Tn5 insertion side. Therefore we were able to identify putatively known virulence genes including *galU*, *mlaA*, *mlaD* and *relA*, but also putative new W12-1067-specific virulence factors. The majority of Tn5 mutated genes encoded for proteins which are involved in the synthesis or maintenance of the cell envelop (LPS, outer membrane, capsule). Here, we showed that the Scatter Screen method can be used for *Francisella* to identify virulence factors. Even though the strain W12-1067 exhibited various virulence factors, the pathogenicity of this strain need to be further investigated.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

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Transcriptome kinetics of *Saccharomyces cerevisiae* in response to viral killer toxin K1

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Question The A/B toxin K1 is secreted by strains of the baker's yeast *Saccharomyces cerevisiae* infected with two cytoplasmically persistent mycoviruses. This so-called killer toxin acts as an ionophore killing sensitive yeast via the disturbance of cytoplasmic membrane functions. In contrast to bacterial toxin producers, killer yeast cells need to possess unique mechanisms conferring immunity against their own toxin as they express the same receptor molecules as sensitive cells. Despite decades of research, neither the exact molecular mechanism mediating this immunity nor the toxic effect of K1 has been completely elucidated. ***Methods*** In order to investigate the toxic mechanism as well as the possibilities of a sensitive yeast to initially resist the lethal effect, we analyzed the transcriptome response of *S. cerevisiae* wild type strain BY4742 after K1 application. Therefore, a time-series experiment to dissect the kinetics of early, intermediate, and late responding genes was conducted. ***Results*** Global transcriptional profiling revealed substantial adaptations in target cells resulting in the initiation of osmotic and oxidative stress responses. Besides the induction of cellular systems for ROS detoxification, heat shock proteins as well as DNA damage repair systems, a notable up-regulation of genes involved in the tricarboxylic acid cycle, *de novo* purine synthesis, and tetrahydrofolate metabolism was detected. Remarkably, a futile cycle of the osmolytes trehalose and glycogen was identified probably representing a critical defensive mechanism upon K1 intoxication. *In silico* analysis additionally suggested several transcription factors involved in K1-triggered signal transduction. ***Conclusion*** The identified transcriptional changes implicate an evolutionarily conserved response at least initially counteracting ionophoric toxin action and provide valuable hints to elucidate the still unknown molecular events leading to K1 toxicity and immunity.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

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NGS-based phylogeny of diphtheria-related pathogenicity factors in different *Corynebacterium* species implies species-specific virulence transmission

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Diphtheria toxin (DT) is produced by toxigenic strains of the human pathogen *Corynebacterium diphtheriae*, zoonotic *C. ulcerans* and *C. pseudotuberculosis*. Toxigenic strains may cause respiratory diphtheria, myocarditis, neurological damage or cutaneous diphtheria. The DT encoding *tox* gene is located in a mobile genomic region and *tox* variability between *C. diphtheriae* and *C. ulcerans* has been postulated. In contrast, species-specific sequence analysis of the DT repressor gene (*dtxR*), occurring in toxigenic and non-toxigenic *Corynebacterium* species, has not been done yet.

We used whole genome sequencing data from 91 *tox* gene-bearing and 46 non *tox* gene-bearing isolates of different pathogenic *Corynebacterium* species of animal or human origin to elucidate differences in *tox*, *dtxR* and *tox*-surrounding regions on a large sample set. We performed de novo assembly, ordering of contigs, genome annotation and extracted translated DT, DtxR, prophages and other *tox*-surrounding pathogenicity-related mobile elements. Extracted regions were used for phylogenetic comparisons between the different species.

Sequences of DT and DtxR could be classified in four distinct, nearly species-specific clades, corresponding to *C. diphtheriae*, *C. pseudotuberculosis*, *C. ulcerans* and an atypic *C. ulcerans* nontoxigenic *tox* gene-bearing (NTTB) wildlife cluster. Average amino acid similarities within the groups were above 99% for DT and DtxR, but lower between the groups. However, for DT different subgroups could be identified, correlating with different *tox*-comprising mobile genetic elements. In most *C. diphtheriae* isolates, *tox* genes were located within known prophage sequences. In contrast, in *C. ulcerans* diverse mobile elements including the *tox* gene could be identified: either prophage sequences differing from *C. diphtheriae* prophages or, in isolates without *tox*-overlapping prophage annotations, an alternative but very homogeneous pathogenicity island (PAI) described previously. Beyond the *tox*-overlapping elements, most analysed isolates harboured a variety of additional prophages.

Our NGS data from 137 isolates indicate the existence of different genetic backgrounds of DT-mediated pathogenicity in different *Corynebacterium* species and evolution of acquired pathogenicity features with the strains. Different groups of pathogenicity-related elements imply that DT transmission pathways may differ in zoonotic *C. ulcerans* and contribute to its emerging pathogenic potential.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

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Comparative genomics and transcriptomics of *Salmonella* clinical isolates representing different cell line infecting phenotypes

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Introduction

Adhesion and invasion to various epithelial cells are among the first steps during the pathogenesis of *Salmonella* infections. *Salmonella* has evolved various strategies to adhere and invade its host cells. Moreover, different serovars have developed specific infection phenotypes. To study the cell-bacteria interaction, *in vitro* adhesion/invasion models and reference laboratory strains are often in use.

Objectives

Currently used methods are not feasible for large scale studies of cell line infectivity in *Salmonella* clinical isolates. Therefore, our objective was to develop an automated microscopy-based system for bacterial infection assays. Next, we aimed to identify genes responsible for different infecting phenotypes in clinical isolates of *Salmonella*.

Materials & methods

For screening, 127 *Salmonella* isolates from five serovars of human, cattle, swine and chicken origin were transformed with the GFP plasmid and used in infection assays on porcine IPEC-J2, human Caco-2 and avian CHIC-8E11 cell lines. Adhering/infecting bacteria were counted after 1 h and 4 h of infection with the automated VideoScan microscope. The genomes of 30 isolates with highest and lowest median adhesion/infection values and RNAs from 4 *S.* Typhimurium and 4 *S.* Enteritidis isolates were sequenced.

Results

In screening of 127 *Salmonella* isolates, the median infection value was found highest for CHIC-8E11 and lowest for IPEC-J2 cells. Among all tested *Salmonella* serovars on cell lines, *S.* Gallinarum showed lowest infection value on CHIC-8E11 and *S.* Choleraesuis had the highest infection value on Caco-2. Overall, *S.* Gallinarum displayed the lowest infection values regardless on the incubation time. Comparative genome analysis revealed differences (SNPs, deletions) in following genes: *dksA, nmpC, yidR, sanA, rfaL (waaL), sirA, aroA, lrhA, rcsD, ompW, mpl, rtsA, wza*. Analysis of transcriptomes showed disparate expression profiles of genes associated with invasion of cells (e.g. *sopA, sopE, sipA-sipD, invA-invE*) in strains with different infection phenotypes.

Conclusion

The new VideoScan module enables quantification of bacteria in *in vitro* infection assays. Each *Salmonella* isolate was characterized by a specific infection phenotype. Analysis of genomes and transcriptomes allowed identification of genetic loci influencing cell line infectivity. However, to fully understand the underlying genetic mechanisms more experiments need to be conducted.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

190/MPP

Activation of gasdermin D by *S. aureus* α-toxin

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Questions: The small β-pore forming α-toxin is a major virulence factor of *S. aureus*. At low concentrations, α-toxin induces influx of Na^+ and efflux of K^+, but only at higher concentrations was influx of Ca^{2+} and propidium iodide (PI) observed. This has led to the long standing concept that α-toxin can form pores of different size/conductance. However, the recent discovery of cytosolic membrane pore forming proteins offers an alternative explanation. For instance, the N-terminus of gasdermin D (GSDMD), a substrate of pro-inflammatory caspases, forms oligomeric trans-membrane pores which could permit the influx of PI. Moreover, pro-inflammatory caspases are activated by loss of cytosolic K^+ concentration. Using epithelial cells we here investigated whether α-toxin is able to activate GSDMD and whether caspase activation promotes PI-influx into α-toxin-treated cells.

Methods: the human keratinocyte cell line HaCaT was treated with purified α-toxin. Influx of PI was assessed by flow cytometry. GSDMD-Flag was transfected into HaCaT cells. Pyroptotic phenotypes were detected using fluorescence microsopy. Small MW inhibitors were used to block caspase activation (Z-VAD-FMK), necrosis (necrostatin-1) or membrane rupture (glycine).

Results: High concentrations of purified α-toxin trigger rapid influx of PI into HaCaT cells. At later time points signs of pyroptosis become observable in α-toxin-treated GSDMD-expressing cells.

Further, inhibition of caspases fails to prevent influx of PI. In contrast, glycine affords partial protection, suggesting that α-toxin-dependent influx of PI into epithelial cells is a consequence of membrane rupture.
Conclusions: *S. aureus* α-toxin may lead to activation of GSDM(D). However, the influx of PI early after treatment of epithelial cells with high doses of α-toxin appears to be mainly caused by membrane rupture. Current experiments address the role of GSDMD for changes of cytosolic Ca^{2+} concentration and other events downstream of membrane perforation by various pore forming proteins.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

191/MPP
A global RNA map of colorectal cancer-associated *Fusobacterium nucleatum*
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Question:
The anaerobic gram-negative bacterium *Fusobacterium nucleatum ss. nucleatum* (*FNN*) has recently garnered much attention due to its role in multiple diseases outside its natural habitat. Recent studies have found a significant association of *FNN* with colorectal cancer (CRC) or adenoma tissue for which its increased presence correlates with poor prognosis and resistance to the common chemotherapeutic. However, few genes of this species have been characterized and even less is known about the transcriptional organization in *FNN* and its possible connection to CRC.
Methods:
To obtain a better understanding of this enigmatic bacterium, we are generating a functional RNA map of *FNN* by utilizing differential RNA-seq (dRNA-seq) during different growth stages. The method has elucidated the transcriptome structure of various bacteria and allows for a precise identification of transcriptional start sites (TSS), 5'UTRs and noncoding small RNAs (sRNAs). Additionally, we are performing RNA-seq under different stress and infection-relevant environmental conditions.
Results:
Our global RNA-seq analysis identified both constitutive and growth phase-dependent TSS. This has enabled the identification of promoters important for transcriptional regulation during growth and stress. Our data has enabled us to substantially improve of the annotation of *FNN* and discover the first potential regulatory sRNAs.
Conclusion:
We report the first single-nucleotide resolution transcriptome map of *FNN* under different environmental conditions. Importantly, this also led to the identification of novel noncoding transcripts such as sRNAs. The study of these novel sRNAs in the context of different stress conditions may shed light on the physiology of this cancer-associated pathogen.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

192/MPP
Significant intra- and inter-clonal diversity in prosthetic joint infections caused by *S. epidermidis*
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Background: *S. epidermidis* colonizes the skin and nares of virtually all human beings. However, it is also the most important pathogen in foreign material associated infections. In the present study we investigate adaptation processes during infection of prosthetic joints as well as undirected micro-evolution within the infection.
Methods: We recruited 99 patients suffering from a prosthetic joint infection (PJI). 22 of these were caused by *S. epidermidis* and thus selected for analysis. Isolates from the patients" noses as well as from the infected joint were subjected to NGS and phenotypic tests to investigate inter- and intra-clonal diversity.
Results: We found evidence of marked phenotypic diversity within infecting clones. Phenotypes are stable in vitro. Micro-evolution in the infection takes place, however, does not suffice to explain the differences in phenotype. S. epidermidis from the nose differ significantly from infection isolates.
Conclusion: Intra-clonal diversity in PJI caused by *S. epidermidis* is pronounced. This is important to consider when infecting isolates are characterized as well as for the development of anti-biofilm anti-microbial agents.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

193/MPP
Protein and polysaccharide measurements in biofilms on prosthetic surfaces – a comparison of methods
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Due to the increasing life-span of the population, age induced illnesses e.g. osteoarthritis become more common. The replacement of joints with endoprosthesis like hip-, knee-, shoulder- or elbow-implants is growing accordingly. In Austria alone, an estimated 16,000 to 20,000 hip replacements are performed annually. The risk to suffer an infection after the insertion of a hip prosthesis lays approximately at 1% worldwide. A high number of so-called low-grade infections are erroneously diagnosed as aseptic, with the result that the actual incidence rate is significantly higher. Staphylococci cause about 50% of all infections of joint prostheses. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common infectious agents in this regard. Both of them are known to be biofilm inducing species. Biofilms on prosthetics are difficult to treat due to the decreased susceptibility of the bacteria in the biofilm. In order to prevent biofilm formation as much as possible, the most optimal material for prostheses is to be used. An increased surface roughness facilitates the attachment of bacteria. On the other hand, exceptionally smooth materials can enhance bacterial bonding through physical forces such as van der Waals interactions and by providing a range of molecular contact points.
The present study investigates whether different prostheses materials (titanium, PEEK and zirconium oxide) influence the biofilm formation of *S. aureus* and *S. epidermidis*. Furthermore, it was investigated whether the Pierce-BCA-Protein-Assay and the phenol-sulfuric acid method for determining the polysaccharide concentration of the biofilm could be replaced by clinical biochemical methods (Biuret, Trinder). The Pierce-BCA-Protein-Assay has already been designed for the analysis of proteins from biofilms and it is easy in handling. The replacement of the phenol-sulfuric acid-method by the Trinder-method is recommendable because of its easy handling, its being less hazardous and its results are mostly comparable with those of the phenol-sulfuric acid method for polysaccharides. Future studies are planned to get a deeper insight into the influences of the materials on biofilm formation as well as the response of the biofilms to therapeutical medication.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

194/MPP

Redox-sensing under hypochlorite stress and infection conditions by the Rrf2-family repressor HypR in *Staphylococcus aureus*

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Questions: *Staphylococcus aureus* is a major human pathogen and has to cope with reactive oxygen and chlorine species (ROS, RCS) during infections, which requires efficient protection mechanisms to avoid destruction. Here, we have investigated the changes in the RNA-seq transcriptome by the strong oxidant NaOCl in *S. aureus* USA300 to identify novel redox-sensing mechanisms that provide protection under infection conditions (1).
***Methods*:** Using mutational analysis and biochemical methods, we have studied the function and redox-sensing mechanism of HypR under NaOCl stress in *S. aureus*.
***Results*:** NaOCl stress caused an oxidative stress response in *S. aureus* as indicated by the induction of the PerR, QsrR, HrcA and SigmaB regulons in the RNA-seq transcriptome. The *hypR-merA* (*USA300HOU_0588-87*) operon was most strongly up-regulated under NaOCl stress which encodes for the Rrf2-family regulator HypR and the pyridine nucleotide disulfide reductase MerA. We have characterized HypR as novel redox-sensitive repressor that controls MerA expression and directly senses and responds to NaOCl and diamide stress *via* a thiol-based mechanism in *S. aureus*. Mutational analysis identified Cys33 and the conserved Cys99 as essential for NaOCl-sensing while Cys99 is also important for repressor activity of HypR *in vivo*. The redox-sensing mechanism of HypR involves Cys33-Cys99' intersubunit disulfide formation by NaOCl stress both *in vitro* and *in vivo*. Moreover, the HypR-controlled flavin disulfide reductase MerA was shown to protect *S. aureus* against NaOCl stress and increased survival in J774A.1 macrophage infection assays.
Conclusions: Here, we identified a new member of the widespread Rrf2 family as redox-sensor of NaOCl stress in *S. aureus* that uses a thiol-disulfide switch to regulate defense mechanisms against the oxidative burst under infections in *S. aureus*.

(1) Loi VV, Busche T, Tedin K, Bernhardt J, Wollenhaupt J, Huyen NT, Weise C, Kalinowski J, Wahl MC, Fulde M, Antelmann H. (2018). Redox-sensing under hypochlorite stress and infection conditions by the Rrf2-family repressor HypR in *Staphylococcus aureus*. *Antioxid Redox Signal*. doi: 10.1089/ars.2017.7354

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

195/MPP

Mode of action and membrane association based on structural analysis of *Legionella pneumophila* phospholipase A PlaB

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Introduction
The gram-negative pathogen *Legionella pneumophila*, transmitted by airborne water droplets, causes Legionnaires' disease, a severe and potentially fatal pneumonia. The cell-associated, highly active phospholipase A PlaB belongs to a new family of lipases first described in *L. pneumophila*. Its 3D structure is yet uncharacterized. Previous studies revealed that PlaB localizes to the bacterial outer membrane, is important for pathogenicity and exhibits phospholipase A, lysophospholipase A, and hemolytic activities, which depend on protein concentration and the state of protein oligomerization.
Aims
In order to deduce crucial domains for membrane association and activity the PlaB crystal structure was elucidated. Conspicuous motifs were subjected to targeted mutagenesis.
Methods
PlaB was recombinantely expressed and purified. Crystals were grown in sitting drop experiments. The initial model was solved by means of an anomalous dispersion experiment. Based on these data, amino acids incriminated to be important and domains for dimerization, membrane association and lipid substrate specificity were specified and mutagenized. PlaB mutants were analyzed for activity and localization.
Results
The data collected from a rod-like, triclinic crystal were truncated at a resolution of 2.0 Å. The N-terminal domain of PlaB contained the catalytic triad, situated inside the α/β-hydrolase fold and shielded by a lid structure, and two unique β-sheets which were identified as potential membrane interaction sites. These β-sheets were deleted and the mutants analyzed for enzyme activity and localization. The C-terminal domain showed a bilobed β-sandwich and cation-π interaction sites which may be important for substrate specificity or membrane association. Ongoing experiments are targeted towards specific modifications of the cation-π interaction sites to assess their effect on activity and membrane association.
Conclusion
Knowledge of the PlaB structure allows us to classify the different domains and structural elements. Furthermore a targeted investigation of important activity and localization determining domains as well as unique structures of PlaB are possible.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

196/MPP

An unusual Ig-binding protein in *Mycoplasma pneumoniae*

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Mycoplasma pneumoniae is a human pathogen that causes chronic infections and is able to survive in the host for a long time without detection. As its existence is dependent on sidestepping the host immune system, it has developed several mechanisms to evade the host immune system. In the minimal organism *Mycoplasma genitalium*, a protein (Protein M) which binds immunoglobulins (Ig) in an unconventional manner has been described. Protein M attaches to the variable region of the light chain. From this site of attachment Protein M likely blocks binding of antigens to the antigen binding site by extending its C-terminal region over the antigen binding site. In *Mycoplasma mycoides*, a two-protein system for antibody binding and degradation has been described. *Mycoplasma* Ig binding protein (MIB) first attaches to the antibody molecule in the Fab region. Then a *Mycoplasma* immunoglobulin protease (MIP) is recruited by the complex to cleave the Ig. As *M. pneumoniae* is closely related to *M. genitalium* and many of their proteins share similarity, a search was undertaken to find Ig-binding proteins in this organism. Amino acid homology indicated that protein MPN400 was a likely candidate. Further, genes from *mpn583-mpn592* are annotated as predicted proteases because they contained serine peptidase motifs and a DUF31 domain, that is associated with other proteases. MPN400 and selected putative proteases were cloned, expressed and their likely roles in the pathogenesis of *M. pneumoniae* studied. In an ELISA experiment it was shown that MPN400 binds IgA, IgG and IgM. Due to an amino acid sequence similarity of 67% with Protein M, we expect that the antibody binding mechanism is similar to that of Protein M. MPN400 also binds fibrinogen, an important component of the extracellular matrix in humans, as well as other unknown proteins in *M. pneumoniae* lysates. MPN588 seems to have proteolytic function against IgA, IgG and IgM, although it is possibly non-specific. In the MIB-MIP system used as a control, the MIB protein

was not replaceable with MPN400. To attach to epithelial cells, *M. pneumoniae* needs a mechanism to avoid detection by secretory IgA, IgG and IgM at the lung mucosal interface. Atypical binding to Ig could help to evade discovery by Igs and prevent triggering a phagocytic immune response. This mechanism could enable *M. pneumoniae* to remain undetected in the lungs, enabling carrier function in infected patients and its spread through airborne droplets.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

197/MPP

Crystallization of the virulence factor ProA from *Legionella pneumophila*

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ProA, the major secretory protein of *Legionella pneumophila*, is a zinc metalloprotease of the M4 enzyme family. It contributes to Legionnaires' disease by causing tissue destruction in the human lung, and protecting the pathogen from factors of the host immune response. After recombinant production and optimized purification via affinity, hydrophobic interaction and gel permeation chromatography ProA was successfully crystallized. The X-ray structure was determined to 1.5 Å resolution. Due to a high metal concentration in the crystallization condition, additional crystals of recombinant and native ProA were used to verify a single zinc cofactor in the active centre. ProA consists of a two domain structure with N-terminal β-sheets and C-terminal α-helices. Sequence identity of 47.7 % to pseudolysin from *Pseudomonas aeruginosa* is known, and we also approved very high structural similarities of the two proteases with a minor distance of 0.7 Å. Remaining variances to the elastase and also a proposed model of ProA in 2012 only occur in three peripheral loop structures and a second β-sheet region, which however could similarly be found in vibriolysin of *Pseudoalteromonas*. Especially the active side residues are highly conserved among the family of thermolysin-like proteases. Thus, amino acids interacting with a substrate or coordinating the central zinc ion are highly superimposable. As M4 enzymes provide a brought and similar spectrum of substrates, remaining differences in their targets may result from varying cofactors or outer loop regions somehow involved in substrate determination. Overall, revealing of a vast structural match ranks ProA among many thermolysin-like proteases, which all represent major virulence factors for their pathogen.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

198/MPP

The Chemotactile System in *Clostridioides difficile*

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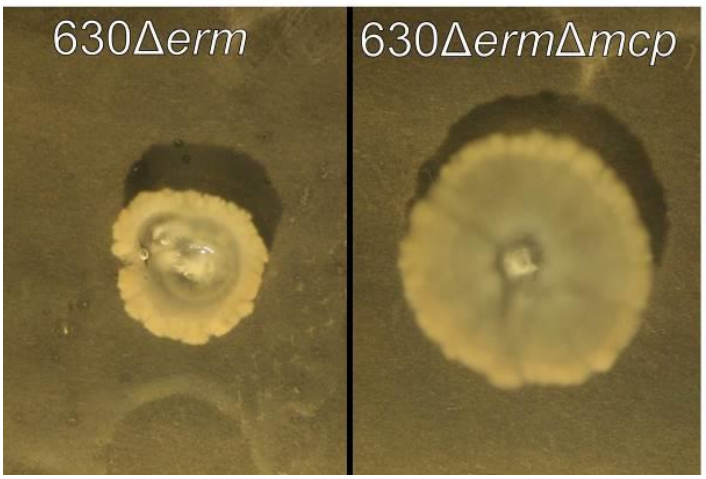
Clostridioides (Clostridium) difficile infection is an increasing problem in modern healthcare environments. The majority of *C. difficile* strains display flagellar based motility and contain a genomic region encoding for a complete chemosensory system. Even though no chemotactic behaviour has been described in *C. difficile* so far, we hypothesize that chemotaxis plays an important role for *C. difficile* in finding niches with optimal growth conditions. Within the *C. difficile* genome a single putative methyl-accepting chemotaxis protein (MCP) is predicted. The MCP has a typical structure of a four helix binding domain, which acts as a receptor, and a trans-membrane signal transducer. We are currently investigating the precise role of the MCP for chemotaxis and motility, as well as the nature of the ligand(s) that are recognised by the sensory domain. To this end we generated a Δ*mcp* mutant in *C. difficile* 630Δ*erm* and *C. difficile* R20291 by ClosTron mutagenesis[1]. Interestingly, we observed strongly increased motility for the *C. difficile* 630Δ*erm*Δ*mcp* mutant in a soft agar swimming motility assay (Figure 1), which was restored to wild type level after complementation with an intact copy of the *mcp* gene. We are currently investigating the molecular basis of this phenotype. Moreover, we are in the process of establishing a robust chemotaxis assay, in order to compare the chemotactile behaviour between parental and Δ*mcp* mutant strains. For this purpose we are comparing various chemotactic assays, for example short-term capillary-based assays and long-term soft agar based or chemical in-plug assays.

1 Heap *et al*.: The ClosTron: Mutagenesis in *Clostidium* refined and streamlined. Journal of Microbiological Methods (2010), 80(1), 49-55.

Acknowledgment: This work was funded by the Federal State of Lower Saxony, Niedersächsisches Vorab (VWZN2889/3215/3266.)

Figure 1: Comparison of growth area between *C. difficile* 630Δ*erm* and *C. difficile* 630Δ*erm*Δ*mcp*. From a mid-exponential growth phase liquid culture, 2 µl were stab-inoculated in BHIS with 0.3% (w/v) Agar. Cultures were then incubated for 48 h under anaerobic conditions.

Figure 1



Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

199/MPP

Functional analysis of the *Bartonalla bacilliformis* pathogenicity and establishment of genetic manipulation systems

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Introduction: *Bartonella bacilliformis* is the causative agent of the biphasic Carrions"s disease resulting in hemorrahagic fever (Oroya fever) and vasculoproliferations (*verruga peruana*). The prevalence of this neglected pathogen is restricted to the Peruvian Andes where it is transmission mediated by sand flies (*Lutzomyia verrucarum*).

Aims: Little is known about the pathogenicity mechanisms of *B. bacilliformis*, therefore tools and methods need to be developed to understand the complex interactions between *B. bacilliformis* and its host on a functional and molecular level. We aim to study the role of flagellin and the trimeric autotransporter adhesion (TAA) *Bartonella* repeat protein (Brp) in the infection process. Exact *brp* gene sequences, whole genome gene sequence of the strain KC584, and various genetic manipulation systems were established. Transposon instertion, promoter-trap and expression libraries were also constructed.

Materials and Methods: Infection experiments of erythrocytes and endothelial cells were performed with *B. bacilliformis* (pBBR1MCS-2-GFP, pBBR1MCS-4-GFP, pBBR1MCS-5-GFP). Bacteria and infected cells were analyzed via FACS analysis, fluorescence and electron microscopy. Dynamic data of infection experiments were evaluated via confocal spinning disc microscopy and Matlab analysis. *Brp* sequencing was performed by PCR-based Sanger sequencing. Whole genome sequencing of the strain KC584 was performed by Illumina Miseq® Nano. Deletion of *brp* was done using the NEBuilder® HIFI DNA Assembly Cloning Kit. EZ-Tn5 Tnp Transposome kit was used to generate a transposon insertion library. Fragments between 100-300 bp in length were cloned into a pANT3-GFP plasmid for generation of a promoter trap library, and pET28abc plasmids were used for the construction of an expression library in *E. coli*.
Results: The infection of human erythrocytes resulted in significant movement (average 2.00 µm/s) and agglomeration of erythrocytes compared to the uninfected cells (0.017 µm/s). Adhesion of *B. bacilliformis* to human erythrocytes and agglomerate formation was observed via electron microscopy with both, flagellated and flagellin-deficient strains. Furthermore, *brpA* (3,780 bp) and *brpB* (3,708 bp) were sequenced and deletion mutants were constructed. Also the whole genome sequence of the strain KC584 will be available soon in GeneBank.
Conclusion: Our infection experiments showed that *B. bacilliformis* adheres to human erythrocytes independent of flagellin expression. The generation of a *brp* deletion mutant will reveal whether the TAA Brp plays an essential role in adhesion to erythrocytes and endothelial cells.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

200/MPP
Identification of *Campylobacter jejuni* genes involved in adhesion, invasion and biofilm formation.
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Campylobacter jejuni, a Gram-negative, microaerophilic bacterium, is the leading cause of bacterial gastrointestinal food-borne infection worldwide. Acute *C. jejuni* infection entail watery to bloody diarrhea, with fever, nausea, vomiting and abdominal pain, and can be fatal to vulnerable individuals. Among the 18 species of Campylobacter described, 85% of human Campylobacteriosis cases are caused by *C. jejuni*. Although the infection is often self-limiting, it has been reported to lead to the development of secondary autoimmune disorder such as the Guillain-Barré syndrome. *C. jejuni* is able to adhere to and invade epithelial cells of the human gastrointestinal (GI) tract, leading to disruption of the GI epithelial layer and producing damaging pro-inflammatory host responses.
Novel pathogenicity factors involved in the ability of *C. jejuni* to adapt to the bile acid rich environment of the human gut are aimed to be identified by the generation of knockout mutants. Candidate genes were mainly chosen from proteomics data generated in our lab that resulted in the identification of differentially expressed proteins after exposure to sublethal concentrations of bile acids1.
We generated deletion mutants for 11 *C. jejuni* genes by the insertion of a kanamycin resistance cassette into the target gene via homologous recombination. Complementation was performed by insertion of an intact copy of the target gene into a noncoding conserved spacer region of an rRNA gene. Various phenotypic parameters were assessed such as adhesion and invasion into two different host cell types, soft agar motility, autoagglutination, biofilm formation and stress resistance.
We observed reduced motility in a soft agar assay for 7 of the 11 mutants. Surprisingly, several of these mutants displayed increased adhesion and invasion rates into host cells, despite their reduced motility. Biofilm formation was increased in 7 mutants. Reductions in adhesion and invasion ability were found in two mutants. Further studies are needed to understand the molecular basis of these phenotypes.

Masanta WO, Zautner AE, Lugert R, Bohne W, Gross U, Leha A, Dakna M, Lenz C. Proteome Profiling by Label-Free Mass Spectrometry Reveals Differentiated Response of *Campylobacter jejuni* 81-176 to Sublethal Concentrations of Bile Acids. Proteomics Clin Appl. 2018 Sep 24:e1800083. doi: 10.1002/prca.201800083.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

201/MPP
Influence of SigB on regulation of virulence factors of *Staphylococcus saprophyticus*
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Introduction
Staphylococcus saprophyticus is an opportunistic pathogen and a common cause of urinary tract infections. Several virulence factors have been 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. For *Staphylococcus aureus* it is known that the alternative sigma-factor SigB plays a major role in regulation of virulence factors. The physiological significance of SigB in *S. saprophyticus* and the influence of D-serine on this system was investigated by construction of a *sigB* knock-out mutant, followed by comparative physiological tests and RNA sequencing.
Methods
An erythromycin cassette and a 500 bp fragment upstream and downstream of *sigB* was amplified and transformed with the linearized 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 Conclusion
A *sigB* Knock-out mutant was successfully constructed. Expression of virulence factors was investigated by different physiological assays, such as urease activity testing, D-serine-deaminase activity testing, lipase activity testing or binding and biofilm assays and by transcriptome analysis. The sigB knock-out mutant showed a diminished D-serine deaminase activity and lower urease activity, when grown under the influence of 20 mM D-serine. However RNA-Seq showed, that D-serine had no direct influence on the regulation of the affected genes. Altogether 30 SigB target genes were identified under the chosen conditions.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

202/MPP
Role of peptidoglycan-modifying enzymes for the intracellular lifestyle of *Salmonella enterica*
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The integrity of peptidoglycan (PG) is crucial to provide bacterial cell shape and protection against external factors. Cell growth, division and adaption to changing environments are main processes in bacterial life in which the PG is modified. Many PG-modifying enzymes are responsible to achieve this PG reorganisation. The

penicillin-binding proteins (PBP) 2 and 3 e.g. are essential for cell elongation and division, respectively. Recent studies identified two PG-enzymes specifically present in the facultative intracellular pathogen *Salmonella enterica* Typhimurium (STM), but absent in non-pathogenic strains. Due to their sequence similarity to the housekeeping enzymes, they are called PBP2Sal and PBP3Sal. Expression of PBP3Sal strictly depends on acidic pH, as present in the *Salmonella*-containing vacuole (SCV) (Castanheira *et al.*, 2018).

We analyse the role of PBP3, PBP3Sal and other PG-enzymes of interest for intracellular STM. To characterise their function in the intracellular lifestyle of STM, we infected two different cultured cell lines with STM WT and mutant strains. The intracellular fitness is determined by gentamicin protection assays. To analyse the outbreak from the SCV into host cell cytosol, translocation of *Salmonella* pathogenicity island 2 effector proteins and regulator-dependent expression of PG-enzymes, reporter strains with dual-colour plasmids are used for flow cytometry. Intracellular morphologies of vacuolar and cytosolic bacteria are analysed by fluorescence microscopy.

Our data indicate that controlled expression for PG-enzyme genes affect the intracellular replication of STM in HeLa cells and murine macrophages. Controlled synthesis also leads to various effects on bacterial cell shape under different environmental conditions. Moreover, lack of PBP2 leads to decreased intracellular replication and coccoid, vacuolar bacteria in HeLa cells, while lack of PBP3 results in filamentous, cytosolic bacteria. Furthermore, we could show that the expression of the STM-specific PG-enzyme PBP3Sal depends on the virulence regulator SsrB.

Our data suggest a tight regulation of PG-modifying enzymes for the adaption to host environments during the infection process. Future analyses are necessary to decipher the contribution of the STM-specific and redundant PG-enzymes for virulence. The sub-cellular localisation, possible interactions of these and other PG-enzymes and their temporally regulated expression needs to be addressed in further studies.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

203/MPP

The small histidine-containing phosphocarrier protein HPr affects biofilm formation of *Staphylococcus aureus* on medical devices

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Central metabolism and virulence factor synthesis are often linked in pathogenic bacteria. In *Staphylococcus aureus*, this linkage is mediated among others by catabolite control protein A (CcpA), the master regulator of carbon catabolite repression in Gram-positive bacteria. CcpA is thought to respond to the glycolytic intermediates glucose 6-phosphate and fructose 1,6-bisphosphate via the small histidine-containing phosphocarrier protein HPr, which upon phosphorylation on serine-46 binds to CcpA to stimulate the interaction with its cognate DNA binding sequences called catabolite responsive elements. The non-essential protein also forms part of the phosphotransferase system that controls sugar uptake and carbon utilization in Gram-positive and Gram-negative bacteria. However, its impact on virulence factor production and infectivity of *S. aureus* has not been addressed yet.

Here we report that inactivation of *ptsH* (encoding HPr) in *S. aureus* alters the biofilm formation capacity of this pathogen on abiotic surfaces. A *S. aureus* derivative lacking HPr displayed a significantly decreased biofilm formation capacity in a static microplate based biofilm assay and in flow chamber experiments, probably due to a decreased sugar uptake and/or metabolism. Similar effects were seen when biofilm formation was monitored on catheter fragments in a non-nutrient limiting biofilm assay. When utilized in a murine foreign body infection model, the *ptsH* deletion mutant exhibited significantly reduced bacterial loads in/at the implanted catheter fragments if compared to mice challenged with the wild type strain. Notably, such an effect was not seen in mice infected with an isogenic *ccpA* deletion mutant, suggesting that HPr exerts an effect on biofilm formation and infectivity of *S. aureus* beyond activation of CcpA.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

204/MPP

The effect of the small non-coding RNA RsaE on *Staphylococcus epidermidis* biofilm communities

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Bacterial biofilms are highly organized structures which functionally emulate a multicellular organism, last but not least through exhibition of heterogeneous gene expression patterns within biofilm communities. Here we focus on factors influencing biofilm communities in the prototype biofilm-forming bacterium *Staphylococcus epidermidis* and found that RsaE, a conserved small regulatory RNA, supports polysaccharide intercellular adhesin (PIA)-mediated biofilm matrix production and metabolic heterogeneity in *S. epidermidis*.

RsaE is spatiotemporally expressed within *S. epidermidis* PIA-mediated biofilms, and its overexpression triggers a PIA biofilm phenotype as well as extracellular (e)DNA release in a *S. epidermidis* protein biofilm matrix-producing strain background. RsaE influences biofilm matrix composition by targeting mRNAs involved in eDNA release and TCA cycle activity, with RsaE undergoing processing to exploit its full target potential. Thus, full length RsaE interacts with *S. epidermidis* antiholin-encoding *lrgA* mRNA, resulting in localized bacterial lysis and release of eDNA as stabilizing biofilm matrix component. Processed RsaE (RsaEp) inhibits TCA cycle activity by targeting *sucCD* mRNAs, resulting in re-programming of staphylococcal central carbon metabolism towards PIA precursor synthesis. Additionally, RsaEp interferes with the 5' UTR of *icaR* mRNA, encoding the repressor of the PIA synthesis *icaADBC* operon, and blocks its ribosomal binding site. As RsaE itself is heterogeneously expressed within biofilms, we consider this sRNA to function as a factor favouring phenotypic heterogeneity and supporting division of labour in *S. epidermidis* biofilm communities.

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

205/MPP

Kill Em All – with Cold Plasma

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Question:

Infections caused by multidrug resistant bacteria have limited treatment options. Cold atmospheric pressure plasma may 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-, NO2). Physical treatment with tissue-tolerable plasma may be a promising alternative to antimicrobial therapy or chemical antiseptics.

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), *Enterococcus faecium* (VanA), *Candida albicans* (DSM 11948).

Cell suspensions of different cell counts were plated on CLED 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.

Results:

Among the tested strains *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 log10 levels of *C. albicans* and up to 6 log10 levels in *S. lugdunensis*. A plasma exposition of 180 seconds expended the reduction of cfu by one log10 level in *C. albicans* (5 log10 levels) and two log10 levels in *S. lugdunensis* (8 log10 levels= sterile plates). All bacterial strains were reduced by around 6 to 7 log10 levels in 60 seconds of plasma treatment.

Conclusions:

Cold plasma kills great amounts of bacterial and fungal cells on a large Agar surface in seconds. The antimicrobial activity of the new developed plasma patch was both dosage (time) 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 108 cells.

Figure 1

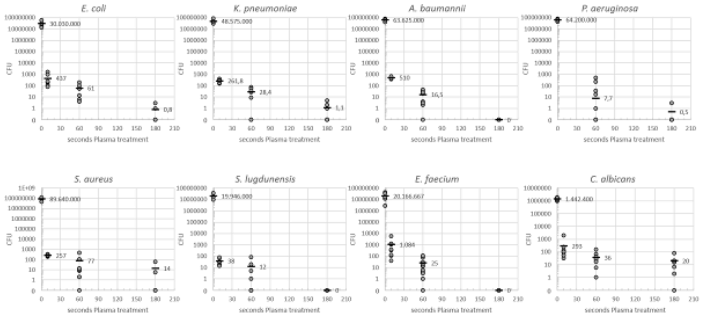

Figure 2

Presentation on Monday, February 25, 2019 from 19:00 – 21:00, upper floor.

Postersession 02
26. Febr. 2019 • 17:00-18:30

General and Hospital Hygiene (StAG HY)

206/HYP

Routine disinfection when working with free-living amoebae (*Acanthamoeba* spp., *Balamuthia mandrillaris*) trophozoites and cysts

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Introduction: Experimental research in free-living "opportunistic" amoebae (FLA) is broadly motivated. All *Acanthamoeba* species are listed biosafety level-2, *Balamuthia mandrillaris* and *Naegleria fowleri* BSL3. FLA cysts are resistant to chemical and physical conditions adverse to other eukaryotes. Formaldehyde is now considered a carcinogen (category cB).

Objectives: To develop test systems relevant to daily practice in a research laboratory, to find at least one commercial chemical disinfectant that is effective against FLA trophozoites and cysts in a "short" exposure time, with acceptable human and environmental safety characteristics and shelf life, to verify these results with different FLA species.

Materials & Methods: *A. castellanii* Neff, *A. castellanii* 1BU and *A. culbertsoni* trophozoites were cultivated in PYG 712 at 29°C. Cysts were induced in Hirukawa medium, then positively selected with SDS. Surface decontamination was tested by coating stainless steel plates with 1-5x105 amoebae and wiping once or twice (as indicate) with a tissue soaked with disinfectants. After 5 min plates were sampled with a cotton swab that was then twirled in culture medium. For decontamination in suspension, amoebae were mixed with disinfectants. After 3 min (or longer) these were either extensively diluted and washed by centrifugation, or the disinfecting agent neutralized with a mixture of L-histidine, lecithin and Tween 80. 14 d cultures revealing any viable trophozoites were rated "positive".

Results: Alcohol-based disinfectants were less effective against trophozoites and quasi ineffective against cysts. Formaldehyde-, hypochlorite-, organic peroxides-, quaternary ammonium-based disinfectants were all effective against trophozoites, but only hypochlorite and quaternary ammonium were fully cysticidal at test conditions. Additives such as detergents and chelating agents seem to play an important role, especially in surface disinfection.

Conclusions: Alcohol-based hand disinfectants cannot be recommended. Handling, human tolerability and shelf-life favor quaternary ammonium-based disinfectants for routine surface disinfection over hypochlorites and formaldehydes.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

207/HYP

Results of the surveillance focus on multidrug-resistant gramnegative bacteria

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Introduction:

Local health authorities (LHA) regularly inspect hospitals and monitor adherence to the protection against infection act (IfSG) and the national recommendations for prevention of healthcare-associated infections (KRINKO recommendations). One of the tasks of the Landeszentrum Gesundheit NRW (LZG.NRW) is to provide assistance and to support the local health authorities. Since 2015 the LZG.NRW offers support for the inspection of key aspects of hygiene within a hospital. Topics are selected by LHAs. Criteria for selection include the potential of prevention, verifiability by health authorities and recent KRINKO recommendations. In 2017/2018 the focus was surveillance of multidrug resistant gramnegative bacteria (MRGN).

Material/Methods:

The inspection focused on MRGN started with a kick-off meeting in April 2017 with 36 participants from27 LHAs. Based on a draft prepared by experts on MRGN, a common checklist for the surveillance was prepared in working groups. All 53 LHAs in North Rhine-Westphalia were invited to carry out inspections using the checklist. LHAs inspected hospitals from July to November 2017 and transmitted the protocols to the LZG.NRW. We received 72 reports from 17 health authorities. The findings were presented at a final workshop in April 2018 and the checklist was adapted based on the experiences of the health authorities.
Results:
Almost every hospital conducted MRGN screening in accordance with the KRINKO recommendations. However about one quarter of the inspected hospitals that patients were not isolated before the results were available. As no universal definition of endemic area of MRGN exists, the definitions between hospitals varied widely. Responses to some questions in the category surveillance identified large gaps in the knowledge of carbapenem resistance mechanism and the importance of carbapenemase detection.
Discussion:
The results of the project demonstrate that the KRINKO recommendations are implemented by all hospitals however several points are interpreted in different ways. Implementation of the standardized inspection protocols was able to uncover some vulnerabilities. The revised checklist is a valuable tool for the inspection of hospitals focused on MRGN.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

208/HYP

A "Masks Save Lifes" campaign in the clinical setting effectively prevents nosocomial influenza A/B: comparison of two seasons with increased influenza activity

A. Ambrosch*[1], D. Luber[1], A. Klinger[1], C. Arp[1], K. Wahrburg[1], F. Klawonn[2]
[1]*Krankenhaus Barmherzige Brüder , Institut für Labormedizin, Mikrobiologie und Krankenhaushygiene, Regensburg, Germany*
[2]*Helmholtz Centre for Infection Research, Biostatistics, Braunschweig, Germany*

Nosocomial influenza is a financial burden for the clinical health care system and associated with a high morbidity and mortality particular in elderly (1,2). Therefore, preventive strategies should be proven to reduce its impact. The present study compares two different hygiene policies focusing on a "Masks Save Lifes campaign" with regard to their effectivity to prevent nosocomial influenza in the clinical setting.
Methods: in the influenza season 2018, a "Mask Safe Lives"-campaign was introduced in the study hospital which includes the following bundle: staff from wards with > 2 Influenza inpatients has to wear surgical masks during the whole shift / in front of each ward a hygiene center was implemented with masks for use and disinfectant bottles for patients and visitors / information flyers about the campaign were provided. Data on diagnosed influenza A/B, the rate of nosocomial infections and mortality were obtained and compared to the influenza season 2015. During the influenza season 2015 influenza patients were managed according to a standard hygienic protocol including masks only in front of patients which was in line with the official recommendations (3).
Results: During the "Masks save lifes" campaign in 2018 a total of 365 patients with influenza A / B were included and compared to influenza patients from the season 2015 (n = 271) (Figure 1). Interestingly, the rate of nosocomial infections decreased for influenza A (2015 vs. 2018: 22.5 % vs. 8.4 %, $p < 0.005$, Barnard"s one-sided test) as well as for influenza B (11.1 % vs. 5.7 %, $p = 0.116$) significantly by a mean of 62 % when masks were used during the whole shift compared to the standard hygiene management. Furthermore, no one died from a nosocomial influenza infection in 2018 compared to seven patients (12.3%) in 2015 ($p = 0$ 0.048).
Discussion: Using masks during the whole shift for staffs according to our "Mask Safe Lifes" campaign is highly effective to prevent nosocomial influenza infection in the clinical setting. Our observation focuses on the significant role of hospital staffs in the transmission of nosocomial influenza which is not accounted by the official recommendations for the management of influenza.

References:

- Simmermann et al, PLosOne 209,4, e7776
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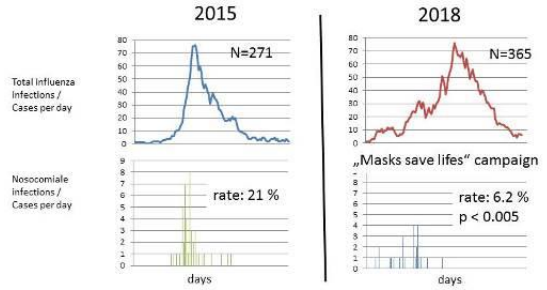
Figure 1

Figure 1: Comparison of two hygiene concepts to prevent nosocomial influenza in the clinical setting; During the „Masks save lifes" campaign in 2018 (right graph) the rate of nosocomial influenza infections is reduced by more than 60 % compared to the influenza season 2015 (left) when a standard hygiene concept was performed.



Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

209/HYP

Realization of a multicenter cluster-randomized controlled decolonization trial in Germany

L. A. Denkel*[1], F. Schwab[1], P. Gastmeier[1], C. Geffers[1], C. I. study group[2]
[1]*Charité - University Medicine Berlin, Institute of Hygiene and Environmental Medicine, Berlin, Germany*
[2]*cRCT participants, Germany*

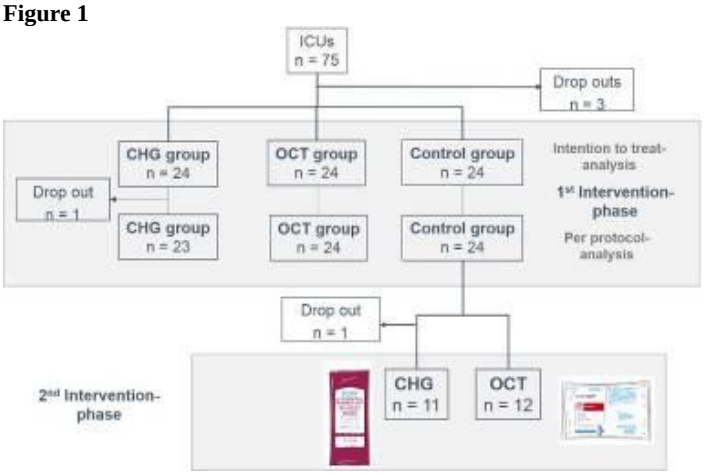
Introduction
We planned to conduct the first multi-center cluster-randomized controlled trial (cRCT) investigating the effect of daily patient bathing with chlorhexidine (CHG) or octenidine (OCT) on central line-associated bloodstream infections (CLABSI) and acquisition of multi-drug resistant organisms (MDRO) in German ICUs. Further, putative side effects of general decolonization strategies including development of resistances to CHG and OCT were addressed.
Aims
Here, we aim to present information and first data on realization of such a cRCT in Germany.
Materials & methods
Study participants (n = 75) were recruited from ICU-KISS, the German national surveillance system for nosocomial infections in ICUs. ICUs were randomized to use CHG, OCT or water and soap (control group) for daily patient bathing during a 1-year-intervention-period. Further, all ICUs received information material (posters, videos, protocols) on bathing of ICU patients and infection control measures. Primary outcome was the incidence of CLABSI; secondary outcome was the incidence of MDRO. Monthly, consumption and side effects of antiseptics were reported. Clinical isolates were collected to analyze development of resistances to OCT and CHG. After the first intervention period, control ICUs were randomized to perform daily antiseptic bathing with CHG or OCT in a second 1-year-intervention-period.
Results
Ethical approval was obtained by the ethics committee of the investigator (Charité Universitätsmedizin Berlin). This ethical approval was confirmed by 6 other ethics committees of the respective State Authorisation Associations for Medical Issues (n = 4) or the participating hospitals (n = 2). A flow chart of our cRCT can be found in Fig. 1. 71 ICUs completed the 1-year-intervention-period. During that period, report forms for side effects (n = 564) and consumption of antiseptics (n = 564); and clinical isolates before (n = 553) and at the end of the intervention (n = 434) were collected. Currently, data collection and validation of the first

intervention period as well as resistance analyses of clinical isolates are being finalized. Former control ICUs (n = 23) are going to complete their 1-year-intervention-period with CHG (n = 11) or OCT (n =12), respectively, in the 1st quarter of 2019.

Summary

This cRCT investigating the effect of daily antiseptic bathing of ICU patients with CHG and OCT is going to be completed soon. First preliminary data will be presented.

Figure 1



Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

210/HYP

Contamination of wipes dispenser buckets and disinfectant dosing devices at the Leipzig University Hospital

P. Blume*[1], J. Stahlmann*[1], I. F. Chaberny[1]
[1]*Universitätsklinikum Leipzig, Leipzig, Germany*

Question:

Surface disinfection is an accepted part of infection prevention[1]. Widely spread wipes dispenser systems, however, can be contaminated during handling and reprocessing, by the quality of drinking water used or the dosing devices[2]. For this reason, the content of wipes dispensers must be examined, especially in case of nosocomial outbreaks[3].

Methods:

Disinfectant solution of wipes dispensers as well as of dosing devices were analyzed at the University Hospital Leipzig according to the "Verbund für Angewandte Hygiene" recommendations[2,4].

Results:

Disinfectant solutions from wipes dispenser buckets (n=1069) were tested. Overall, a decline in contamination was recorded (2016: 31%; 2017: 18%; 08/2018: 8%). The microbial spectrum of positive disinfectant solutions was dominated by *Achromobacter spp.* (81%), followed by *Pseudomonas spp.* (10%), Vancomycin-resistant *Enterococci* (VRE) (4%), and other environmental apathogenic microorganisms (5%). The solutions of disinfection dosing devices (n=139) showed no microbial growth in 91.1%. In 8.2% *Bacillus spp.* was found and in 0.7% mold.

Conclusions:

As we could eliminate a contamination in our disinfectant dosing devices and our drinking water system, handling and storage of the dispenser buckets is probably the most critical point of microbiological contamination. We assume, there is not enough knowledge or rather awareness for the potential risks based on incorrect handling and a feeling of false security as disinfectant solution seems to be microbiological safe.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

211/HYP

Promoting guideline compliance to prevent surgical site infections by psychosocially tailored interventions: Protocol and status of the multi-center parallel-group cluster-randomized controlled trial WACH

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[1]*Leipzig University Hospital, Institute of Hygiene, Hospital Hygiene and Environmental Medicine, Leipzig, Germany*
[2]*Hannover Medical School (MHH), Centre of Public Health and Healthcare, Medical Psychology Unit, Hannover, Germany*
[3]*Hannover Medical School (MHH), Centre of Public Health and Healthcare, Institute of Epidemiology, Social Medicine and Health Systems Research, Hannover, Germany*

Surgical site infections (SSI) are among the most prevalent nosocomial infections in Germany [1]. Despite preventive efforts (AWMF-guideline [2]; KRINKO-recommendation [3]), evidence on compliance and interventions to promote compliance is lacking. Thus, the WACH-study (Wundinfektionen und Antibiotikaverbrauch in der Chirurgie*) has been initiated (funding: BMG, ANNIE2016-55-038; DRKS00015502). Its protocol and status are presented.

WACH expands a previous trial"s approach of psychological tailoring (DRKS00010960) [4] from hygienic hand disinfection in intensive care units of one tertiary university hospital to SSI-preventive measures in surgery in six general hospitals. The primary objective is to develop strategies to promote SSI-preventive compliance. Target groups are physicians and nurses in surgical and anesthesiological wards/operating theatres (OT). The trial has three phases. First, compliance and determinants (COM-B-model [5]) are to be empirically assessed. Second, tailored education and if feasible other interventions will be developed in and suggested to three hospitals. Third, the hypothesis will be tested that the tailored interventions lead to stronger compliance improvements and SSI-reductions than usual implementation interventions ([cost-]effectiveness).

So far, diagnostic instruments have been developed (compliance collection sheets for general, pre-, peri- and postoperative measures [2-3]; implementation questionnaire) and tested at Leipzig University Hospital on seven wards and the OT (e.g. in abdominal surgery). More than 300 opportunities were collected. Baseline assessments in the participating hospitals are being prepared.

*Approved by the Ethics Committee at the Faculty of Medicine of Leipzig University on June 12th, 2018 (034/18ek) based on the protocol developed with the Clinical Trial Centre Leipzig

[1] Behnke M et al. The prevalence of nosocomial infection and antibiotic use in German hospitals. Dtsch Arztebl Int 2017;114:851-7
[2] AK "Krankenhaus- & Praxishygiene" der AWMF. Strategien zur Prävention von postoperativen Wundinfektionen. Leitlinien-Register Nr. 029/031. www.awmf.org/leitlinien/detail/ll/029–031.html (last access: 16 October 2018).
[3] KRINKO. Prävention postoperativer Wundinfektionen. Bundesgesundheitsbl 2018; 61:448-73
[4] von Lengerke T et al. Promoting hand hygiene compliance. Dtsch Arztebl Int 2017;114:29-36
[5] Michie S et al. The behaviour change wheel. Implement Sci 2011;6:42

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

212/HYP

Using of Web-based analysis of atmospheric air pressure monitoring in storage areas for shelf life calculation of terminally sterilized items

H. Dunkelberg*[1]
[1]*Laboratory Dunkelberg, Bad Sooden-Allendorf, Germany*

Question

Medical packaging material for non-liquid terminally sterilized items is normally porous or has porous components to ensure sterilant access. Air flow into the package during transport and storage is caused by air pressure changes and can compromise product"s sterility if the filtration efficiency of the packaging material doesn"t comply with the airborne microbial challenge. But health care personnel have limited control over calculating the shelf life as the maintenance of a sterility assurance level (SAL) of ≤10-6 during the post-sterilization period. The question is how the airborne microbial challenge can be matched with the filtration efficiency of the packaging material in order to maintain sterility during the post-sterilization storage.

Methods

A real time data logger measured the barometer values in the storage room (laboratory room, University Goettingen, Germany) and was used to save the data into the SD memory card. The sampling time could be varied or are saved only when the measuring value changed (> ± 1hPa). After ending the air pressure monitoring period, the saving data file is loaded down from the SD card to the computer (Excel software). Data processing is carried out using a Web-based system. After accessing the website, the user can enter the data file with the monitored air pressure values. Following inputs are entered: the packaging volumes, the filtration efficiencies from the manufacturer"s instruction for use or from reference source and the estimated airborne microbial concentration. A software was developed and integrated in the website for the desktop monitoring application in order to plot the measured air pressure values and the calculated cumulative airflow into the packaging on a graph.

Results

The Figure shows the viewing screen of Web-based analysis after entering the relevant data (monitoring between June 26st to July 14th 2017). The upper graph shows the air pressure time course. The lower graph shows the cumulative airflow which entered a 150 ml-packaging after the beginning of the registration. The Table shows that the calculated shelf lives for the different packaging material and package volumes are in the range of 9 and 901 days.

Conclusions

The desktop application allows Web-based shelf life calculations of terminally sterilized items at the SAL based on the relevant and measured on site input data.

Figure 1

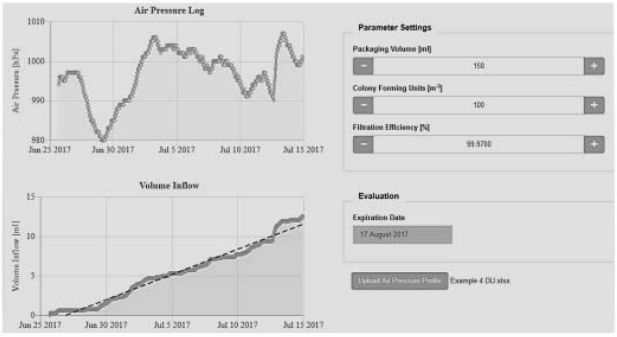

Figure 2

Web-based shelf life calculation of packagings based on air pressure recordings from June 26 to July 14, 2017 and on an airborne microbial load of 100 CFU/m³

Packaging material	Volume [ml]	Filtration eff. (%)	Shelf life (days)
Paper/film pouches (double wrapped)	150	99.97[2)]	52
High-density polyethylene packaging, pouches	150	> 99.99[1)]	157
Baskets, double wrapped medical paper	2600	99.99[2)]	9
Non-woven sheets	2600	> 99.9999[1)]	901

[1)] Data from manufacturer's instruction for use; [2)] data from reference source

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

213/HYP

Self-reported handwashing compliance in the general population in Germany: an in-depth analysis of the first BZgA-survey on hygiene and infection control

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Promoting handwashing in the general population is a key public health area not only in pandemics but also perennial infection prevention.[1] Thus, it is crucial to know how compliant the general population is, and perceives themselves to be. The Bundeszentrale für gesundheitliche Aufklärung (BZgA) has conducted three relevant representative surveys.[2] Regarding single parameters of handwashing, self-reported compliance rates of up to 96% (after using the toilet) were found.[2] However, compliance with recommendations regarding all parameters, i.e. situations, duration (≥20 sec.), and technique (using soap, washing between fingers, and drying off) has not been reported. This analysis provides such prevalence estimates using data so far publicly available,[3] and reports associations with respondents" recall of handwashing instruction signs in public restrooms.

In 2012, N=4,483 residents living in a household in Germany aged 16-85 years were surveyed by computer-aided telephone interviews (response: 49.7%). Self-reported parameters of handwashing were coded as (non-)compliant based on BZgA-recommendations. Respondents indicated whether they had ever seen instruction signs in public restrooms. Statistics were calculated and regression analyses conducted via IBM® SPSS® v24.

The rate of compliance defined as a pattern of self-reported duration of handwashing of ≥20 sec., correct technique (using soap, washing between fingers, drying off), and washing hands in ≥7 of 9 recommended situations, was 8% (men: 6%, women: 9%), while 31% were totally non-compliant (29%/33%). Compared to non-compliance, the odds of total compliance were more than twice as high among respondents recalling handwashing instruction signs in public restrooms (men: OR=2.15) and women (OR=2.08; p<.0001), however with rates still being low (e.g. 10% in women).

In 2012, self-reported handwashing compliance in the general population was low when defined by a pattern of compliant duration, technique, and situational indication. Intensified promotion is obviously needed, and may use instruction signs as a starting point.

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2. BZgA. Infektionsschutz durch Hygiene: Einstellungen, Wissen und Verhalten der Allgemeinbevölkerung – Ergebnisse der Repräsentativbefragung 2017. Köln: BZgA; 2018
3. BZgA. Infektionsschutz 2012. ZA5175 Datenfile Version 1.0.0. Köln: GESIS Datenarchiv; 2015

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

214/HYP

Bacteriological quality of indoor air in some worshipping houses (Churches and Mosques) in Benin City, Edo State, Nigeria.

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[1]*University of Benin, Department of Microbiology, Faculty of Life Sciences, Benin City, Nigeria*

Air contamination by pathogenic microorganisms which are of clinical importance due to their ability to cause infection is of public health concern. These microorganisms are ubiquitous in every environment. This study was aimed at investigating the bacteriological quality of indoor air in some worshipping homes (churches and mosques) in Benin City, Nigeria. Six churches and six mosques were selected for the study. The indoor air was sampled in the mornings (7am-10am) and in the afternoons (2pm-5pm) twice monthly, between April, 2017 and September, 2017 using the Settled Plate Method. The airborne samples were analyzed using standard microbiological methods, Polymerase Chain Reaction and 16S rRNA techniques were used for the gene sequencing. The antibiotic susceptibility pattern and plasmid profile of the characterized airborne bacterial isolates were evaluated using spread plate and agarose gel electrophoresis methods respectively while the temperature and relative humidity in the indoor air environment were determined using thermometer and hygrometer respectively.

The mean indoor air temperature and relative humidity readings recorded in the Churches ranged between 25.68 ± 0.050C to 32.68 ± 0.030C and 77.50 ± 0.29% to 91.25 ± 0.25% while in the Mosques, it ranged between 26.55 ± 0.030C to 33.33 ± 0.030C and 78.00 ± 0.41% to 90.75 ± 0.25% respectively. Eight airborne bacterial isolates were characterized and identified to include the genus; *Escherichia, Bacillus*, *Staphylococcus* and *Pseudomonas*. The airborne bacterial isolates were further characterized using the molecular biology methods to include *Endozoicomonasmontiporae* CL-33 genome, *Bacillus subtilis* strain IAM 12118, *Bacillus anthracis* strain ATCC 14578, *Staphylococcus epidermidis* strain Fussel, *Helicobacter pylori* SS, *Bacillus pumilus* strain SBMP2, *Bacillus cereus* strain JCM 2152 and *Staphylococcus warneri* strain AW 25. All airborne bacterial isolates showed multidrug resistance against the conventionally used antibiotics such as Gentamicin, Erythromycin and Augmentin. *Helicobacter pylori*, *Bacillus pumilus* and *Bacillus anthracis* were reported to be resistant to all antibiotics. The presence of these airborne bacterial isolates of public health consequence in the indoor environments of worshipping homes (Churches and Mosques) is a threat to the health of the worshippers and the environment. It therefore calls for strict regulatory measures to mitigate the public health effect of the organisms.

Keywords: Airborne, airborne bacteria, Church, Mosque and 16S rRNA sequencing.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

215/HYP

Development of large-scale peroxyacetic acid based aerosol decontamination protocols in practice

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[1]*Friedrich-Loeffler-Institut, Department of Experimental Animal Facilities and Biorisk Management, Greifswald, Germany*

Background: The adaption of airborne disinfection protocols is influenced by a variety of parameters, e.g. surface temperature, room humidity (rH), or presence of microorganisms in organic matter. Thereby, our adaption process was of upscaling nature, beginning with biosafety cabinets and ending up with complete large animal rooms.

Materials and Methods: Enveloped and non-enveloped viruses, spore forming bacteria, and mycobacteria with and without soil load were inoculated on stainless steel carriers according to quantitative carrier testing protocols (DVG, RKI guidelines). The carriers were placed at different locations within a containment animal room (245 m³). The room was aerosolized with ultrafine particles (~ 7.5 µm) of a solution containing 1.3 % peroxyacetic acid (PAA) until a high rH was reached. After an incubation time of 30 min and an aeration phase, the microorganisms were recovered and the inactivation efficacy was determined as log10 reduction. A similar procedure was chosen for the high efficiency particulate air (HEPA) filter system of the animal room.

Results: The adapted and validated protocols resulted in a ≥ 4 $\log_{10}$ reduction of spores, mycobacteria, and viruses at all selected locations within the animal room as well as the corresponding HEPA filter unit. The temperature of the surfaces as well as the soil load, respectively, had an unexpected mitigating and enhancing influence. Commercially available germ carriers might indicate false negative results. Furthermore, using the identified effective concentration of PAA, no damage of electronic devices could be observed so far.

Conclusion: PAA decontamination protocols are highly effective in rendering an area or device safe to handle. Unfortunately, each environment has to be specifically validated on relevant surfaces and at multiple locations with representative suitable surrogates for the microorganisms to be handled. Using only a small number of germ carriers or inadequate surrogates might generate misleading results.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

Antimicrobial Resistence and Drugs, Infection Prevention (FG PR)

216/PRP

***mcr-4* harbouring *Escherichia coli* isolates from food and livestock in Germany**

J. A. Hammerl*[1], K. Juraschek[1], D. Shamoun[1], S. Schmoger[1], A. Irrgang[1], M. Grobbel[1], B. A. Tenhagen[1], A. Käsbohrer[1,2]
[1]*German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany*
[2]*Institute for Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria*

Questions

Colistin is a highest priority critically important antibiotic according to WHO used only to treat severe human infections caused by multidrug- and/or carbapenem-resistant Gram-negative bacteria. In 2017, Carattoli et al. reported on the identification of a novel mobilizable colistin resistance-gene, *mcr-4*, in *Salmonella enterica* serovar Typhimurium (4,5,12:i:-). In this study, colistin-resistant *Escherichia coli* isolates from the German national monitoring programme for antimicrobial resistance in zoonotic agents from the food chain were investigated for the presence of *mcr-4* and further genetically characterized.

Materials and Methods

Antimicrobial resistance in *E. coli* was determined as recommended by Commission Implementing Decision 2013/652/EU with the broth microdilution method according to CLSI guidelines and EUCAST epidemiological cut-off values. Isolates with an MIC ≥4 mg/l were subjected to multiplex PCR for determination of the respective *mcr*-gene. S1-PFGE, Illumina MiSeq-sequencing and bioinformatical analyses were performed to identify and characterize *mcr-4* harbouring isolates in detail. The transferability of *mcr-4* harbouring plasmids was investigated by *in vitro* filter mating experiments.

Results

Up to now, in 13 out of more than 750 tested *E. coli* isolates, recovered between 2010 and 2017, *mcr-4* was detected. Sanger sequencing of PCR products revealed that two novel variants of the *mcr-4* gene (*mcr-4.2* and *mcr-4.3*) are prevalent in the German *E. coli* isolates. Genome determination and bioinformatical analysis revealed that the isolates differ in their MLST-, sero- and fim-type. However, all of them harbour a highly conserved ColE-plasmid prototype with some variability in size and genetic composition. Further genetic features of the isolates and plasmids will be presented in detail.

Conclusion

Our findings indicate that *mcr-4* is by far less frequent than *mcr-1*. However, it is more frequent than the other *mcr*-variants in German

E. coli isolates from food and livestock. Further information on the stability of *mcr-4* harbouring genetic elements, their transmission routes as well as their distribution in livestock, food products and humans are needed to assess the potential impact of this resistance determinant on public health.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Detection and characterization of NDM-1 carbapenemase-producing *Vibrio parahaemolyticus* in imported seafood in France and Germany

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Questions

Recently, a NDM-1-producing *Vibrio parahaemolyticus* isolate recovered from an imported Vietnamese shelled shrimp tail intended for human consumption in France was reported. In general, *Vibrio parahaemolyticus* is a natural inhabitant of aquatic environments, worldwide. The presence of this pathogen in food products poses a risk for humans, as the consumption of contaminated raw or undercooked seafood may cause (A) gastrointestinal infections with mild and/or strong symptoms and/or (B) septicaemia, especially in immunocompromised people.

Materials and Methods

Antimicrobial resistance in *Vibrio parahaemolyticus* was determined as recommended by 2013/652/EU with the broth microdilution method according to CLSI guidelines and EUCAST epidemiological cut-off values. S1-PFGE, Illumina MiSeq-sequencing and bioinformatical analyses were performed to identify and characterize relevant isolates in detail. The transferability of *bla*NDM-1 carrying plasmids was investigated by *in vitro* filter mating experiments.

Results

Antimicrobial resistance-testing among *Vibrio* spp. isolates from imported seafood, intended for consumption in Germany, exhibit a carbapenem-resistant *V. parahaemolyticus* isolate. Whole genome sequencing revealed the presence of a *bla*NDM-1 carbapenem gene that is embedded on a transposon, which is located on a self-transmissible plasmid. The *bla*NDM-1 carrying plasmid could be successfully transferred to *E. coli* resulting in a strong production of carbapenemases. The plasmid was determined to be 100 kb in size revealing a modular mosaic structure. The composition and organization of the plasmid genome will be given in detail.

Conclusion

As the number of reports on carbapenemase-producing *Vibrio* spp. isolates from imported fish and seafood of the South-East Asian continent increases, questions on the safety of food products from this subcontinent arises. The occurrence of carbapenemase-producing bacteria in food poses a risk for humans, as carbapenems are only used as antimicrobial substances of the last resort in the human medicine. Our findings underline that antibiotic resistance surveillance should be extended to the environment close to human activities and foods of aquatic origin.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

218/PRP

High level of resistance-associated mutations in *Mycoplasma genitalium* strains - data from two specialized STD practices in Berlin, Germany.

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Mycoplasma genitalium (M.g.) is a sexually transmitted bacterium associated with non-gonococcal urethritis in men and with cervicitis and pelvic inflammatory disease in women. Cultivation of the fastidious mycoplasmas is difficult and detection of infections based exclusively on molecular methods. According to the intrinsic resistance of the cell wall-less bacteria to betalactam antibiotics, use of macrolides (azithromycin) has been considered as first-line treatment for many years. Recently, an increasing number of treatment failures was reported. Macrolide resistance in M.g. results from mutations at positions 2058 or 2059 (*Escherichia coli* numbering) in domain V of the single copy of 23S rRNA. Fluoroquinolones have been found successful in cases with azithromycin treatment failure and are widely used as second-line antibiotics. Unfortunately, occurrence of point mutations in the quinolone resistance-determining region (QRDR) of the topoisomerase IV gene (ParC) accounts for the world-wide emergence of strains with decreased susceptibility to fluoroquinolones. Knowledge of resistance rates of M.g. strains in Germany is limited. Since September 2017, we investigated M.g.-positive DNA samples of patients (99.2% male with high proportion of MSM) attending two STD practices in Berlin. Up to now, samples (58 rectal swabs, 41 urine samples, 4 urethral swabs, one vaginal swab) of 127 patients (without follow-up samples) were investigated. Partial sequencing of 23S rRNA resulted in macrolide resistance-associated mutations in 78.0% of strains (77x A2059G; 19x A2058T; 3x A2058T). In addition, 16 patients (12.6%) harbored M.g. strains with mutations of QRDR of ParC (13x aa83: S to I or N; 3x aa87: D to N). Fifteen strains (11.8%) are macrolide- and quinolone-resistant limiting the treatment options strongly. The results of the study confirm relatively high rates of resistance among M.g. strains circulated in the MSM population in Berlin. Regarding patients of this group, azithromycin cannot longer be recommended without resistance testing. Beside the fact that further investigations are necessary to collect more nation-wide data of resistance, the results emphasize a continuous monitoring of this agent of sexually transmitted infections of humans and optimized treatment measurements.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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***Escherichia coli* producing VIM-1 carbapenemase isolated by antimicrobial resistance monitoring programs in food-producing animals**

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Carbapenems are broad-spectrum beta-lactam antimicrobials and critically important as last-line treatment options in human medicine. The mechanisms of carbapenem resistance include the production of degrading enzymes (carbapenemases). Because the genetic information is mostly encoded on mobile genetic elements, horizontal and vertical transmission between strains and species is possible.

In Germany, the monitoring of antimicrobial resistance in commensal *E. coli*, and also in selectively isolated ESBL/AmpC- and carbapenemase-producing *E. coli* (CPE), is integrated in the

national monitoring of zoonotic agents. Phenotypical resistance is determined by broth microdilution following CLSI guidelines (CLSI M07-A9). Within the specific monitoring on CPE, one isolate (17-AB01027), was detected in faeces of fattening pigs at farm. A second carbapenem-resistant isolate (17-AB02384) was found in caecum content of a fattening pig at slaughter within the monitoring on ESBL/AmpC-producing *E. coli*. The CPE selective isolation method had failed to identify the isolate in that sample. Genotype of both isolates was confirmed by PCR sequencing and characterized by PFGE, Southern Blot hybridization, MLST and NGS. Further transmission of the resistance determinant was investigated by conjugation experiments.
The carbapenem-resistance of the two isolates was related to the presence of VIM-1 carbapenemase. XbaI PFGE analysis showed that both strains differed substantially from each other. MLST results assigned 17-AB01027 to ST48 and 17-AB02384 to ST593. The first isolate was associated with phylogenetic group A, whereas the second isolate belonged to B1 and harbored an additional SHV-12 ESBL. However, sequence analyses of the plasmid showed high similarities to *Salmonella* Infantis VIM-1 plasmid pRH-R27 (LN555650.1) and *E. coli* plasmid pRH-R178 (HG530658.1) detected in 2011 in German pig production. IncHI2 plasmids of both strains were transmissible by conjugation.
The results of the characterization of the isolates suggest further spread of VIM-1 carbapenemase within the pig population. Monitoring and further characterization are necessary to identify transmission routes. Moreover, the selective isolation method needs to be improved, as one of the strains wasn´t found within the specific monitoring of carbapenemases producing *E. coli* but from the same sample within the ESBL monitoring. This improvement is one aim of the One Health European Joint Program project IMPART.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Motility is abolished at subinhibitory antimicrobial concentrations in *Acinetobacter baumannii* ATCC 17978

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Introduction: In *Acinetobacter baumannii* motility has been associated with the uptake of exogenous DNA which can facilitate the acquisition of antimicrobial resistance genes. We previously demonstrated inhibition of the RND efflux pump AdeABC and its regulator AdeRS during motility. Therefore we assumed that antimicrobial susceptibility would also be affected. This idea was supported by an *adeRS* knockout in *A. baumannii* ATCC 19606 which did not express *adeABC* and revealed significant increase in antimicrobial susceptibility to various antibiotics.
Objectives: Aim of this study was to investigate the antimicrobial susceptibility of *A. baumannii* when growing on motility plates and how antimicrobials can influence the motility phenotype.
Methods: The impact of antimicrobials on motility in *A. baumannii* reference strain ATCC 17978 was investigated with a modified agar dilution method using motility plates (0.5% agarose, 5 g/L tryptone and 2.5 g/L NaCl). Minimal inhibitory concentrations (MIC) and minimal motility inhibitory concentrations (MMIC; the concentration where motility is inhibited) for azithromycin, ciprofloxacin, gentamicin, meropenem, tetracycline and tigecycline were determined after 16 h incubation at 37 °C.
Results: The tested strain exhibited a motile and a non-motile phenotype depending upon the antimicrobial concentration. The concentration that inhibited motility was defined as the MMIC. Thereafter the cells will grow in the inoculation zone, but no longer spread from this point (Fig 1). The MIC and the MMIC differed by only a twofold dilution for tetracycline and tigecycline, whereas a fourfold dilution of gentamicin and meropenem, an eightfold lower dilution of ciprofloxacin and a sixteen-fold lower dilution of azithromycin as the respective MICs were sufficient to inhibit motility (Table 1).
Conclusion: Subinhibitory concentrations of various antimicrobials inhibited motility in *A. baumannii* ATCC 17978 while cells were still viable. This may be a direct effect of altered gene expression as for example the inhibition of *adeRS* and *adeABC*. It remains to be determined if DNA uptake is inhibited when motility is abolished
Figure 1:
A. baumannii ATCC 17978 on motility plates supplemented with 0.5 mg/L (A) and 1 mg/L (B) tetracycline. A non-motile growth zone of 5 mm corresponds to the drop size of the cell suspension used for inoculation.
Table 1:
MIC and MMIC of *A. baumannii* ATCC 17978.

Figure 1

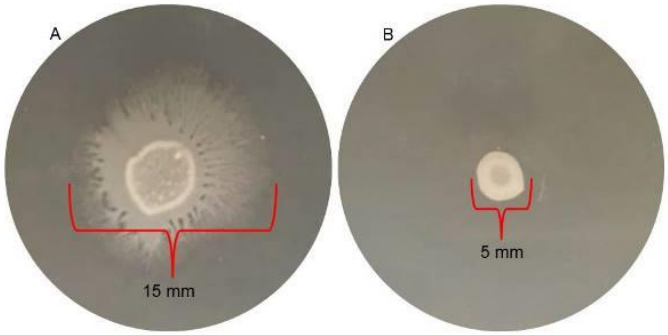

Figure 2

Antibiotic	MMIC [mg/L]	MIC [mg/L]	Fold difference between MMIC and MIC
Azithromycin	0.25	4	16
Ciprofloxacin	0.03	0.25	8
Gentamicin	0.125	0.5	4
Meropenem	0.0625	0.25	4
Tetracycline	1	2	2
Tigecycline	2	4	2

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Report of the National Reference Laboratory for Multidrug-Resistant Gram-negative Bacteria on Carbapenemases in Germany in 2018

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Question: Multidrug-resistance in *Enterobacterales*, *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 still challenging for the microbiological laboratory.

Material/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 and WGS methods allow the detection of still unknown β-lactamases.

Results: A total of 5238 isolates were investigated for carbapenemases in the National Reference Laboratory in 2018 until October 11th. Specimen sources were mostly rectal swabs (24.1 %), urinary (15.9 %) and respiratory samples (11.9 %). Carbapenemases were found in 1455 *Enterobacterales* strains, 407 of *A. baumannii* and 372 of *P. aeruginosa*. The most frequent carbapenemases in *Enterobacterales* were OXA-48 (n = 447), VIM-1 (n = 262), NDM-1 (n = 156), KPC-2 (n = 142), NDM-5 (n = 99), KPC-3 (n = 60), OXA-244 (n = 57), OXA-181 (n = 55), OXA-232 (n = 34), NDM-7 (n = 13) and VIM-4 (n = 12). GIM-1, OXA-162, VIM-2, IMI-1, IMI-2, GES-5, and and others were found in less 10 isolates each. In *P. aeruginosa*, VIM-2 was the most frequent carbapenemase (n = 252), followed by GIM-1 (n = 29), VIM-1 (n = 18), IMP-7 (n = 18) and NDM-1 (n = 14). VIM-4, GES-5, VIM-11, IMP-13, VIM-17, IMP-1, IMP-28 and others were found in less than 10 isolates each. OXA-23 was the most frequent carbapenemase in *A. baumannii* (n = 3013), followed by OXA-72 (n = 60) and NDM-1 (n = 16). GIM-1, OXA-143, OXA-58 and others were found in less than 10 isolates each.

Conclusions: A variety of different carbapenemases is detected in Germany. The molecular epidemiology in Germany differs significantly from observations made in other countries like Greece, Italy or the USA with a predominance of OXA-48. Compared to previous years, variants of OXA-48 are again on the rise, together with variants of NDM and VIM.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Vancomycin-resistant enterococci (VRE): infections and colonization over 3 years at the University Medical Center Mainz (UM)

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Introduction: Invasive infections with vancomycin-resistant *Enterococcus faecium* (VRE) are increasingly recognized. VRE-screening and contact-isolation of VRE-colonized patients is commonly used to prevent the spread of VRE. Further, the choice of an empiric therapy is often influenced by the VRE-status of the patient. However, the overall benefit of this strategy is controversial.

Objectives: VRE-epidemiology at the University Medical Center Mainz (UM) was determined to develop a rational strategy for VRE-screening and isolation precautions.

Methods: The VRE-screening results from 2014 – 2016 were retrospectively analyzed. 14431 samples from 3207 patients from 3675 stays were included. 3306 stays related to hematooncological patients. Only data of the department of hematooncology are analyzed below.

Results: The percentage of VRE-colonization increased from 10.3% in 2014 to 28.8% in 2016 ($p < 0.001$). 63% of VRE-positive patients were from the department of hematooncology. Nosocomial colonization rose from 7.2% in 2014 to 21.2% in 2016 ($p<0.001$). However, the proportion of invasive infections in VRE-positive patients was approximately 5% and did not change significantly. Considering all stays of VRE-positive patients in 3.9% (28/725) an invasive VRE-infection occurred. 13.9% (26/186) of VRE infections were linked to the department of hematooncology where VRE-screening is performed routinely.

Conclusions: The department of hematooncology was identified as a risk area for invasive VRE infections in our hospital. Despite a comprehensive screening program and contact-isolation of VRE-positive patients, the number of VRE-colonized patients increased significantly. However, a significant increase in invasive infections was not seen. Based on our data, contact isolation was stopped in 2017. It will be analyzed in the future, if this will change the VRE-epidemiology in our hospital.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Detection and characterization of plasmid-mediated quinolone resistance in *Escherichia coli* isolates recovered from livestock and food in Germany

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Quinolones are important antibiotics and belong to a family of synthetic broad-spectrum drugs. Resistance to quinolones can be chromosomally or plasmid-mediated (PMQR). One PMQR mechanism is mediated by Qnr proteins encoded by different *qnr*-genes. The horizontal gene transfer of this plasmid-mediated quinolone resistance increases the threat of fallible treatment with some antibiotics. To understand the *qnr* PMQR pathway as well as the distribution of *qnr* genes, *Escherichia coli* isolates recovered in 2016 and 2017 from livestock and food were pheno- and genotypically characterized in detail.

A total of 6,817 *E. coli* isolates from the German National Reference Laboratory for Antimicrobial Resistance were investigated. The isolates were received in the German national monitoring program for antimicrobial resistance in 2016 and 2017. The *E. coli* isolates were recovered from livestock and food (i.e. cattle, pig, poultry). Antimicrobial resistance was determined by broth microdilution according to CLSI guidelines. MIC values were evaluated using EUCAST epidemiological cut-off values. *E. coli* resistant to quinolones were subjected to *qnr*-PCR, XbaI-/S1-PFGE and WGS analysis. Six different *qnr*-PCR were conducted to identify the respective *qnr*-variants.

Out of 6,817 *E. coli* tested, more than 800 isolates were classified as quinolone-resistant (MIC NAL ≥16 mg/L and/or MIC CIP ≥0.06 mg/L). The *qnr*-variants of the isolates were identified by established PCR methods. The most abundant *qnr*-variant was *qnrS*. With the exception of *qnrD*, other *qnr*-variants were found evenly distributed within the investigated matrices. Pulsed-field gel electrophoresis with XbaI-digestion was performed to examine genetic relatedness of isolates. PFGE profiling demonstrated a rather high heterogeneity. The highly diverse PFGE pattern indicates that the screened isolates are not associated to a predominant *E. coli* clone spreading via vertical transmission. S1-PFGE plasmid profiling showed a variety of extrachromosomal elements of various sizes. Isolates, selected according their XbaI-/S1-PFGE pattern were further screened for their genetic setting through short read WGS. Sequencing of those isolates confirmed the genetic diversity of the quinolone-resistant *E. coli*.

Quinolone-resistance could not be attributed to a specific lineage of *E. coli*. Further analysis is needed for better understanding of the plasmid diversity within *qnr*-harboring *E. coli* and the prerequisites of their spread.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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***In vivo* persistence and stability of animal *S*. Infantis native *bla*VIM-1-carrying IncHI2 plasmid in a broiler infection study**

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Introduction: Antimicrobial resistance is described as one of the most urgent threat to global public and veterinary health. This is emphasized due to the rise of resistance to clinically important antibiotics, such as carbapenems, anticipated as last option in treating severe human infections. In recent years, VIM-1-producing *S.* Infantis strains were sporadically detected in swine and poultry farms and recently in minced pork meat in Germany. Therefore, we evaluated *in vivo* using a broiler chicken infection study the transferability, persistence and stability of the original *S.* Infantis (R3) native *bla*VIM-1-carrying IncHI2 plasmid (originating from a poultry farm).

Material and methods: Four experimental groups of broiler chicks (G1-G4) were designated. In G1, only VIM-1-producing *S.* Infantis donor "R3", harboring *bla*VIM-1-carrying IncHI2 plasmid was administered, whereas in G2, G3 and G4, as recipients *S.* Paratyphi B (*d*Ta+), *S.* Enteritidis and *S.* Infantis were also inoculated. The study was conducted without antibiotic treatment and lasted 29 days. For the in-depth molecular analysis, 80 *S.* Infantis re-isolates [G1 (n=25), G2 (n=18), G3 (n=21) and G4 (n=16)] were selected. This included PCR amplification of the *bla*VIM-1 gene, S1-PFGE plasmid profiling and whole-genome sequencing using MiSeq technology.

Results: *In vivo* acquisition of the *bla*VIM-1-carrying IncHI2 plasmid in gut *E. coli* and *Salmonella* recipients was not observed. In ten *S.* Infantis re-isolates, minor structural deletions (~10 kb) of this plasmid were detected. Frequency of structural deletions and plasmids acquisition in selection of the *S.* Infantis re-isolates was independent of the group origin. Nine strains acquired additional plasmids, ranging in size from <20 kb to ~100 kb.

Conclusion: This study showed that the VIM-1 producing *S.* Infantis (R3) persists in a broiler flock without significant alteration of the IncHI2 plasmids structure even without selective pressure. Although we have not detected transfer of its *bla*VIM-1-carrying IncHI2 plasmid to other recipients, as its *in vitro* conjugation frequency is also very low, acquisition of additional plasmids in VIM-1 producing *S.* Infantis re-sisolates indicates conjugal transfer events with the gut microflora *in vivo*. The *in vivo* studies are valuable as prediction tools in potential scenarios of carbapenemase-producing *Salmonella* introduction in broiler flocks as transfer of this plasmid without selective pressure seems to be less effective.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Biochemical characterization of FRI-3, a novel variant of the ambler class A carbapenemase FRI-1

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Introduction: To date, bacteria of the *Enterobacterales* are the most prevalent source of hospital-acquired infections worldwide. Especially carbapenemase producing strains have emerged as a serious threat for public health. The rapid emergence of novel carbapenemase variants is another alarming development and complicates the therapy of patients since the biochemical characteristics of these novel variants can differ substantially.Therefore, the biochemical characterization of novel carbapenemases is of utmost importance. Here we report the biochemical characterization of FRI-3, a recent variant of the Ambler class A carbapenemase FRI-1.

Objectives: The aim of this project was to characterize FRI-3 to investigate the impact of the amino acid deviations on its substrate spectrum and hydrolytic activity.

Materials/methods: The FRI-3 encoding gene was cloned into the pASG-IBA103 vector, that promotes the expression of Twin-Strep-tag®-fusion-proteins and was then expressed heterologously. The purification of the enzyme was performed by a two-step-Fast Protein Liquid Chromatography (FPLC), including affinity chromatography and gel filtration. FRI-1 was purified using the same procedure and served as a reference. The catalytic behavior of both enzymes was analyzed and compared by *in vitro* hydrolysis assays through photometrical measurement of the absorbance decrease during β-lactam hydrolysis, followed by determination of the kinetic parameters K_m and k_{cat} using the Michaelis Menten equation. The IC_{50} for FRI-3 of typical betalactamase inhibitors were determined by preincubating the enzyme with different concentrations of inhibitor for 5 min at 25 °C before 100 µM benzylpenicillin was added.

Results: The purified enzymes FRI-3 and FRI-1 hydrolyzed almost all β-lactams including penicillins, cephalosporins, carbapenems and aztreonam. Both enzymes showed the highest catalytic efficiency for cephalothin although other cephalosporins were only hydrolyzed poorly. Especially Cefoxitin and Cefepime were not hydrolyzed by both FRI-3 and FRI-1. In general, FRI-3 showed lower catalytic efficiencies than FRI-1 for all tested antibiotics and was inhibited by clavulanic acid and avibactam.

Conclusions: The biochemical characterization of FRI- 3 illustrates that it is an active carbapenemase and that the mutations in its amino acid sequence lead to a decreased catalytic activity. This emphasizes the rapidly emerging diversity of carbapenemases belonging to the same group.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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The MarR-type repressor MhqR confers resistance to quinone-like antimicrobials in *Staphylococcus aureus*.

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Question: *Staphylococcus aureus* is a major human pathogen which has to cope with reactive oxygen and reactive electrophilic species (ROS and RES). ROS and RES can cause different post-translational thiol-modifications. Quinones have antimicrobial properties and were shown to act as electrophiles and oxidants in bacteria. Methods: Here we used genetic, biochemical and phenotype analyses to characterize the novel quinone-sensing MarR-type repressor MhqR which regulates the *mhqRED* operon and confers quinone resistance in *S. aureus*. Results: Transcriptional analysis showed that the *mhqRED* operon responds specifically to methylhydroquinone (MHQ) and to quinone-like antimicrobials, such as pyocyanin and ciprofloxacin. The Δ*mhqR* mutant was resistant to MHQ, pyocyanin and ciprofloxacin. The MhqR repressor was further shown to sense quinones not via its non-conserved Cys95. Phenotype analyses of another quinone-sensing regulator QsrR revealed that QsrR and MhqR confer independently quinone and antimicrobial resistance in *S. aureus* (1). Conclusion: These studies identified a link between quinone and antimicrobial resistance via the MhqR repressor in *S. aureus*. Our current studies are directed to elucidate the quinone sensing mechanism of MhqR using biochemical and structural methods.

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Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Identification of genetic factors increasing carbapenem resistance in *Klebsiella pneumoniae* with *bla*$_{OXA-48}$

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Introduction: Carbapenemase-producing Enterobacterales have become the most clinically significant multi-drug resistant bacteria within the last years. In Germany, OXA-48 is the carbapenemase most frequently detected in Enterobacterales as shown in the annual report 2017 of the National Reference Laboratory for multidrug-resistant gram-negative bacteria (NRZ). The majority of isolates producing OXA-48 are *Klebsiella pneumoniae.* Although OXA-48 mostly mediates distinct resistance to carbapenems, some clinical isolates show low minimal inhibitory concentrations (MIC) of carbapenems and are categorized as susceptible using EUCAST breakpoints.

Aim: The aim of this study is to reveal possible genetic causations for varying MICs of carbapenems in K*lebsiella pneumoniae* with *bla*$_{OXA-48}$.

Material & Methods: 30 clinical K*lebsiella pneumoniae* isolates with *bla*$_{OXA-48}$ showing low MICs of carbapenems were chosen and put under gradually rising selective pressure of meropenem to select for mutants with elevated MICs. This was either done with disc diffusion or with broth macrodilution. Whole genome sequencing was performed with the low MIC primary isolates and the high MIC selected mutants for mutation analysis. A hybrid assembly of Illumina MiSeq and Oxford Nanopore sequencing data was carried out to generate complete genome data.

Results: Only few spontaneous mutations were found comparing genome sequences of primary isolates and corresponding mutants. The mutations possibly leading to elevated MICs were found in genes for outer membrane porins (OMP), OMP assembling enzymes, enzymes for cell wall synthesis and also transcriptional regulators. The impacts range from amino acid exchange to early termination of translation.

To further check if these mutations lead to elevated MICs of carbapenems they will be integrated into the primary clinical isolate genomes via CRISPR/Cas system. The intentionally mutated clinical isolates will be revised with phenotypical tests.

Summary: There are only few single mutations having huge impact on the MICs of carbapenems in *Klebsiella pneumoniae* with *bla*$_{OXA-48}$ as shown by sequencing data of clinical isolates and selected mutants.

To verify if these mutations lead to elevated MICs of carbapenems, they will be integrated into the primary clinical isolate genomes via CRISPR/Cas system in future analyses.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Occurrence of beta-lactamase genes in the domestic environment

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Introduction: Antibiotic resistant bacteria are not only present in clinical settings but have also been isolated from healthy carriers, animals, food and the environment [1]. Although the knowledge about antibiotic resistance (ABR) is increasing steadily, data on the domestic area remains limited [2]. Regarding ABR beta-lactamases are of great concern, since beta-lactams are considered as critically important antibiotics [3].

Aim: Aim of this study is the analysis of the domestic environment as a potential source for the transfer of antibiotic resistance, focusing on beta-lactamases. Therefore the occurring resistance genes (RG) and resistant bacteria were compared with the findings in a local wastewater treatment plant (WWTP).

Methods: Swab samples were taken from shower drains, washing machines and dishwashers. As comparison, samples from a WWTP (wastewater, sewage sludge) were collected weekly. DNA was extracted and multiplex qPCR of beta-lactamase genes was performed. Bacteria were isolated using subinhibitory concentrations of cefotaxime and ertapenem, species and antibiotic profiles were determined using Vitek 2.

Results: Beta-lactamase genes have been detected in all investigated households (n=16). While in 75 % of the dishwashers (n=12) carbapenemases of the groups KPC, OXA-48 and GES occurred, those genes were found in 53 % of the washing machines (n=15) and 44 % of the shower drains (n=16). Except for one, in all WWTP samples at least one of the examined genes was present. While in the household samples *Stenotrophomonas maltophilia* was mainly detected, *Pseudomonadaceae* were identified more often in the WWTP. Furthermore ESBL-producing bacteria such as *Escherichia coli* were isolated from five WWTP samples and one household sample. All species isolated using subinhibitory concentrations of ertapenem were resistant towards meropenem and imipenem as well or had increased minimal inhibitory concentrations. In addition, nearly all isolates showed resistance towards 2nd and 3rd generation cephalosporins.

Conclusion: Although the RGs were detected more frequent in the WWTP samples, in all households beta-lactamases occured.The results show that resistant bacteria seem to be abundant in the domestic area and therefore households might be a possible hotspot of ABR. To prove this, however, further analyses are required.

1. doi:10.1098/rstb.2014.0083
2. doi:10.1111/jam.13574
3. WHO (2017) Critically Important Antimicrobials for Human Medicine

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Knowledge on hand hygiene of children in German primary schools

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Introduction. For infection prevention, respective key knowledge and skills in the general population are mandatory; especially hand washing is of particular importance. This general aim requires basic elements for awareness that must already be set in the childhood. However, data on the level of knowledge of primary schoolchildren about infectious diseases and their prevention are missing.

Aims. Here, we investigated the level of knowledge of children attending primary schools about infectious disease prevention with focus on hand hygiene aspects.

Materials and Methods. For data collection, a questionnaire in written form was developed, pre-tested and adapted for third grade children. The final questionnaire was applied in 13 primary schools of the county of Steinfurt of the German federal state North Rhine-Westphalia enrolling schoolchildren of 27 third grade classes.

Results. So far, 289 questionnaires collected from nine schools have been evaluated. Overall, there were 288 valid cases including 138 male (47.9%) and 150 female (52.1%) pupils. The children were between 8-11 years old (median, 9.5 years). For almost all respondents (267/270 valid cases; 98.9%), it was important to wash their hands after using the toilet. About three quarters of the participants (220/288 valid cases; 76.4%) were able to order the hand washing steps correctly. Including wrong answers (e.g. cancer and allergy), 91.9% (262/285 valid cases) of the pupils possessed basic knowledge that diseases exist, which can be transferred to human beings; mostly referring to flu-like and gastro-intestinal infections. Noteworthy, most schoolchildren answered that they don"t like visiting the sanitary facilities at school (85.2%; 241/283 valid cases).

Conclusions. Most of the primary schoolchildren possessed basic knowledge on transmissible diseases and the need of prevention measures; in particular regarding hand washing subsequent to visiting sanitary facilities. However, practical details of hand washing were less well understood. Worrying, the vast majority of the enrolled schoolchildren assessed the conditions of the sanitary facilities in such a manner that they don´t like using them, thus, reflecting a situation that may thwart attempts for hygiene education in schools. Studies are warranted to investigate how educational and technology-based interventions can contribute to the improvement of the motivation and performance of hand hygiene procedures by schoolchildren.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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SurvCARE Hessen: A state-wide WGS based Surveillance Project for Carbapenem resistant Gram-negative bacteria

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Carbapenems are a mainstay for treatment of infections by multidrug-resistant *Enterobacteriaceae*. However, an increasing prevalence of carbapenem-resistant isolates is observed globally. This originates from multiple mechanisms, including the acquisition of resistance genes like KPC, OXA-48, VIM, NDM, which are often located on mobile genetic elements (e.g. plasmids). Classical surveillance of antimicrobial resistance is based on phenotypical testing and molecular typing, which does not provide the necessary depth in characterization and typing for understanding the transmission dynamics of resistance genes in the human population, which is needed for implementing targeted control measures.

With SurvCARE Hessen (Surveillance of carbapenem resistant Gram-negative bacteria), a state-wide surveillance study project of epidemiologic investigations was implemented recently in 2017. This project uses the mandatory reporting of Carbapenem-resistant Gram-negative bacteria (CRGNB), combined with isolate characterization on a whole-genome level, and therefore provides sufficient resolution on the distribution of resistance genes by space, time and person, in order to reconstruct transmission chains and source identification. Here we report preliminary results.

CRGNB were collected from patients who either reside in Hesse or a stayed in a Hessian hospital. More than 230 isolates from 35 different hospitals have already been sequenced and analyzed. SurvCARE participants receive detailed reports about the submitted isolates, including comparative analyses when multiple isolates of one species were sent in. The analyzed isolates comprise more than 11 species. Among all isolates associated with or without clinically relevant infections *K. pneumoniae*, *E. coli*, *A. baumannii* and *S. marcescens* are predominant. The identified predominant carbapenemases were KPC2, KPC3, VIM, OXA-48, OXA-23 and NDM.

Our study could identify or prove more than 15 outbreak situations by different pathogens and carbapenemases, as per example $bla_{\text{OXA-23}}$-encoding *A. baumannii* (ST218), $bla_{\text{OXA-232}}$-encoding *A. baumannii* (ST231), $bla_{\text{KPC-3}}$-encoding *K. pneumoniae* (ST101, ST512, ST307) and $bla_{\text{VIM-1}}$-encoding *K. oxytoca*.

Our preliminary data reveal that the prevalence of KPC-3-carrying *K. pneumoniae* in Hessian hospitals is currently increasing. The data also show the endemicity and circulation of a $bla_{\text{KPC-2}}$-encoding IncN plasmid in and between different pathogens from different Hessian hospitals.

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Comparative analysis of an putative *Klebsiella pneumonia* cluster on an orthopedic ward using conventional molecular diagnostic methods and whole genome sequencing

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Question

Indwelling catheters are frequently used in patients with urinary problems caused by spinal cord injuries. To detect catheter-associated urinary tract infections (CAUTI) early microbial analysis of urine samples are broadly ordered. During routine analysis we became aware of an increase of *K. pneumoniae* isolates in urine samples on an orthopedic ward with paraplegic patients. In a hygiene-follow-up relatedness these *K. pneumoniae* isolates were studied by various molecular tests and evaluated for a sensitive and timely identification of potential clusters and putative transmissions.

Methods

After the initial identification of a cluster of patients on the orthopedic ward, all patients on the station were cohorted and rectal swabs were taken. Antibiotic susceptibility testing was performed using the VITEK® 2. Molecular relatedness of the isolates was analyzed by randomly amplified polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE) and by whole-genome sequencing (WGS) using the illumina® MiSeq platform.

Results

In total 18 patients were colonized by *K. pneumoniae*. Two patients had CAUTI. The isolates were classified as 3-MDRGN (multi-drug resistant gram-negative bacteria being resistant against 3 of 4 classes of antibiotics) according to the German national infection control and prevention institute [RKI]. The molecular analysis of the isolates revealed a cluster of 12 patients that were colonized by the same strain whereas 6 patients could be shown to have unrelated strains. Additionally, none of the tested environmental sources were positive for *K. pneumoniae*.

The continued screening of new patients by rectal swabs revealed 12 additional colonized patients. None of which was related to the cluster-isolates. WGS showed the highest discriminatory resolution of the methods used. Noteworthly, RAPD proved to be fast method for an initial distinction of the isolates.

Conclusions

This report highlights on the one hand the importance of consultant surveillance and molecular typing to support routine diagnostics to timely identify nosocomial cluster/outbreak. Moreover, the detection of clusters and outbreaks depends is performed manually through the watchful eye of the responsible hygienist or microbiologist. As part of the HiGHmed Medical Informatics consortium we aim to provide an automatic tool for the detection of putative clusters within hospitals by combining i) routine bacteriological data with ii) and extended typing tools.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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The impact of antibiotic pressure on the phenotypic evolution of clinical antibiotic resistant - *Pseudomonas aeruginosa* in a Morbidostat device

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Question: Colistin is a last-resort antibiotic against *Pseudomonas aeruginosa* interacting with the bacterial cell wall. It is still unknown, whether mutations that alterate cell wall composition and lead to colistin resistance would also lead to changes in biofilm formation and virulence. Evolution towards colistin resistance in *P. aeruginosa* might furthermore be enhanced by a combination therapy with metronidazole, a drug presumably increasing the

mutation frequency. The aim of the study was to explore the link between antibiotic exposure and biofilm formation and virulence.
Methods: As a continouse culture device, a morbidostat was used to expose several clinical strains to three antibiotic conditions - colistin, colistin & metronidazole, and metronidazole - and to create samples with various colistin resistance levels. Biofilm assays were performed using the peg-lid method to detect the amount of viable cells.
To quantify the virulence, the *Galleria mellonella* infection model was used.
Results: Three clinical *P. aeruginosa* isolates were incubated in the morbidostat. Over a three week timeframe, we collected samples from the morbidostat cultures. This provided us with a total of 81 *P. aeruginosa* strains with different colistin resistance levels. These evolved strains were further investigated and compared to their baseline strains that were not cultured in the morbidostat.
The biofilm experiments showed that the number of viable cells in the biofilm measured using the peg-lid method increased heavily under colistin exposure in the highly colistin resistant samples (MIC ≥16µg/ml). There was much less or no increase in the metronidazole only conditions, and no increase in the medium run samples.
In terms of virulence, the medium run as well as the metronidazole only condition samples showed a trend of attenuation in the *Galleria mellonella* infection model. On the other hand, 9 out of 22 highly colistin resistant strains showed a significant reduction ($p<0,05$) of up to 91,5% in death rates compared to the medium run samples.
Conclusion: The morbidostat allowed us to create colistin-resistant *P. aeruginosa* strains with a wide range of MICs. Congruent to colistin resistance, the strains produced more biofilm containing more viable cells and were less virulent. Thus, these strains carry a higher risk of bacteremia for patients with clinical devices coated with biofilm but might be less pathogenic.

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Comparison of Vitek 2, three different gradient strip tests and classical broth microdilution in detecting *vanB*-positive *Enterococcus faecium* isolates with low vancomycin MICs

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Introduction. German hospital patients are challenged by an increasing number of vancomycin-resistant enterococci (VRE) from colonizations and infections. This trend is mainly driven by an increase in *vanB*-positive VRE (1). Further, there is a growing recognition of *vanB*-VRE with low level vancomycin MICs just below the breakpoint of R >4 mg/L. Uncertain diagnostic results may rely on confirmation by alternative tests such as gradient strip assays (e.g., Etest®). Importantly, in July 2018 EUCAST issued a warning regarding less reliable strip assay results for determining and confirming vancomycin resistance in enterococci (2).
Objectives. We established a strain collection of *vanB*-positive *E. faecium* isolates from all over Germany (n= 80) showing low level vancomycin MICs in previous standard diagnostic assays. We aimed at comparing performance of various diagnostic standard and confirmatory tests to identify and determine *vanB*-type vancomycin resistance.
Methods. We compared the performance in determining vancomycin MICs of bioMerieux"s Vitek II (card AST P611), of classical broth microdilution ("in house" procedure and plates) and of gradient strip assay from three providers (Oxoid, Liofilchem, bioMerieux). For the latter we compared the standard procedure vs. the "macromethod"; the latter includes a richer medium (BHI instead of MH), a higher inoculum (McFarland 2 instead of McFarland 0.5) and a longer incubation time of up to 48h. *E. faecalis* ATCC20912, *E. faecium* ATCC19434 and *E. gallinarum* BM4174 (*vanC1*) and *E. casseliflavus* ATCC25788 (*vanC2*) reference isolates were used as controls.
Results. Presence of *vanB* in all study isolates was confirmed. The collection was especially enriched with isolates demonstrating vancomycin MICs of 2 - 4 mg/L (S) and 8 mg/L (R) in classical broth microdilution. We excluded *vanB* strains with vancomycin MICs of 1 mg/L and/or below since these strains may possess defects in *vanB* resistance regulation. Preliminary results already document various MIC results in relation to the method used. Automated MIC determination in Vitek II revealed the highest number of isolates with an MIC of ≤4 mg/L ("S"). Generally, the "macromethod" was more sensitive than the standard gradient strip protocol. Strips of the three producers performed differently. (study will be completed and results evaluated by December 2018).
1) Klare et al. Epid Bull 2017;46:519 – 527
2) http://www.eucast.org/ast_of_bacteria/warnings/

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ESBL-Producing *K. pneumoniae* and *E. coli* in an Urban African Rat Population

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Introduction
High-risk ESBL-producing Enterobacteriaceae (ESBL-E) have been described in wildlife worldwide. Rats are of special interest not only due to their indicator role for environmental pollution with multi-resistant bacteria but also as possible infection source in urban environments.
Methods
Twenty-nine animals from three different rat (*Rattus*) species were captured in the city of Conakry (Guinea, West Africa) in 2015. Rectal swabs were analyzed for ESBL-E using selective media. Species typing and phenotypic antimicrobial resistance analysis to broad-spectrum beta-lactams and other classes of antimicrobials was performed for Enterobacteriaceae-like isolates using the VITEK®2 system (BioMérieux, Germany). Confirmed ESBL-producing *E. coli* and *K. pneumoniae* were whole-genome sequenced and resistance genes, phylogenetic background and genes related to bacterial fitness and virulence were analyzed.
Results
In total, six of twenty-nine rats (20%) carried ESBL-E (*K. pneumoniae* and *E. coli*). All ESBL-producers were multi-drug resistant with *bla*CTX−M−15 as the dominating ESBL-type. Interestingly, ESBL-associated clonal lineages *E. coli* ST38 and *K. pneumoniae* ST307 were found. The ESBL-plasmid in *K. pneumoniae* ST307 revealed high sequence similarities to pKPN3-307_TypeC, a >200 kbp IncFII plasmid originating from a human clinical ST307 isolate. This was in contrast to the core genome: the rat isolate was distantly related to the human clinical ST307 isolate (27 SNPs/Mbp). In addition, we identified π-fimbrial, capsule 2, and glycogen synthesis clusters in the rodent ST307 isolate, whose involvement in the adaptation to survival outside the host and in human urinary tracts has been suggested.
Conclusion
Our results demonstrate the presence of clinically relevant, ESBL-producing *K. pneumoniae* ST307 and *E. coli* ST38 clonal lineages in an urban West African rat population. The human community is likely the initial source of ESBL-E however, rats might function as infection source and transmission hub, accelerated by frequent interactions at a human-wildlife interface.

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Generation of colistin resistant *Pseudomonas aeruginosa* strains in a morbidostat device and subsequent colistin susceptibility test evaluation

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Question:

Colistin is commonly administered against XDR *Pseudomonas aeruginosa* due to limited treatment options and is thus an important last resort antibiotic. However, accurate determination of colistin susceptibility in *P. aeruginosa* is still not comprehensively investigated. This is mostly because evaluation of antimicrobial susceptibility testing (AST) methods is hampered by the lack of resistant isolates and those around the susceptibility breakpoint. The aim of this investigation was to transform colistin susceptible clinical strains into resistant ones by using a morbidostat device, and as a second step to use these strains for AST method evaluation.

Methods:

A morbidostat device was used to transform susceptible clinical *P. aeruginosa* strains into isolates with a wide range of colistin MICs. Following this transformation, five commercial AST methods were compared against the gold standard broth microdilution (BMD) method: MICRONAUT-S, SensiTest, Sensititre, Rapid Polymyxin Pseudomonas and Etest.

Results:

A total of 131 *P. aeruginosa* isolates were included for colistin susceptibility test evaluation (100 genetically distinct colistin susceptible and 31 colistin resistant strains). The 31 colistin-resistant isolates developed resistance in the morbidostat and reached different MICs (ranges: 4-512 mg/L, 100% resistance generation efficacy). The categorical agreement (CA) rates for MICRONAUT-S, SensiTest and Rapid Polymyxin Pseudomonas were 94.7%, 93.9% and 92.4%, respectively. The Sensititre had the highest CA score (96.9%), whereas the Etests had the lowest CA score (84%). The very major discrepancy (VMD) rates for all tests were between 3.2% and 67.7%.

Conclusions:

The morbidostat device can efficiently provide laboratories with a sufficient number of colistin-resistant strains for test evaluation, even in a setting with low colistin resistance prevalence. Although CA rates were high for commercial AST methods except for Etests, none met the ≤1.5% CLSI limit for VMD rates. Performance was generally limited when using isolates with low-level resistance around the clinical breakpoint.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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WASA – risk perception of the development of antibiotic resistances and outcome expectancies

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Background:

Antibiotic resistance reduces effectiveness of treatments for serious bacterial infections and is associated with an increased risk of fatal outcomes. It constitutes a serious threat to global public health. Evidence-based antibiotic prescriptions can decelerate this. Germany has detailed guidelines for antibiotic prescriptions. Various studies indicate physicians" adherence to these needs improvement. Multiple training initiatives address this challenge. Yet, studies on the effectiveness of trainings in primary healthcare are sparse.

Aim:

WASA aims to investigate the effectiveness of interactive trainings for general practitioners (GP) on their antibiotic prescriptions.

Methods:

Trainings on guideline-based antibiotic management for respiratory and urinary tract infections (UTI) among GP (intervention) are provided in South-East-Lower Saxony in workshops moderated by trained course leaders. Presentation documents were provided and checked by independent field experts. Regional GP receive invitations. Those who attend are asked to fill a pre- and post-training questionnaire. A 3rd one will collect information from trained GP a year later. These data will be connected to health insurance data. The questionnaires are based on the HAPA (Health Action Process Approach) model and cover items like risk perception (development of resistant pathogens) and outcome expectancies (own prescription behavior); both part of the phase in which behavioral intentions are formed. For the first 8 trainings on UTI, a Wilcoxon signed rank test was used to detect differences before and after the training on 3 HAPA items.

Results:

Of the 122 physicians having attended trainings, 71 were deemed eligible and 40 filled in both, pre- and post-training questionnaire. More than 80% of the GP fully or strongly agreed that the guidelines provide evidence-based recommendations for selecting the right antibiotic; 60% considered the guidelines to be merely an aid to decision-making during treatment. There is a difference ($p < 0.05$) observed for outcome expectancies with a shift from IQR (2 – 3) towards IQR (1 – 2) in a positive direction (7-point Likert-scale), but not for risk perception.

Conclusion:

Trainings seem to evoke a change in outcome expectancies. According to the HAPA model this might contribute to forming an intention to change the prescribing behavior. Further analyses including data that are still to be collected will provide additional conclusions.

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Synergy of a Twort-like Myovirus and Oxacillin against MRSA

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Introduction:

Extensive use of antibiotics over the last decades has increased the problem of multi-drug resistant bacterial strains. To control bacterial pathogens, such as *Staphylococcus aureus* (MRSA) new or rather forgotten approaches, such as the use of bacteriophages, should be considered.

Aim:

This study investigated the potential of phage/antibiotic combinations to control clinical isolates of *S. aureus*.

Methods:

Twenty-four clinical isolates of *S. aureus* strains (nineteen methicillin-resistant *S. aureus* (MRSA) and five methicillin-susceptible *S. aureus* (MSSA)) as well as four reference strains were categorized into clonal clusters using a genomic fingerprinting analysis. Phage P1, isolated from a phage cocktail obtained from the George Eliava Institute of Bacteriophages, was used as a single agent or in conjunction with oxacillin against the bacterial strains. Antibacterial efficiency was measured with planktonic cultures for 16 h in 20 min time intervals.

Results:

Phage P1 as single agent showed already a strong antibacterial effect against 75% of *S. aureus* strains. As expected, oxacillin alone was only effective against MSSA, but not against MRSA. A significant stronger bacterial suppression (MSSA and MRSA) could be observed in most of the cases, when oxacillin and phage were combined. True synergistic interactions were observed with 13 *S. aureus* strains. Additive effects were seen with ten strains, while facilitation occurred with two strains. Only five strains showed no improved effect compared to the best acting single

agent. Phage/antibiotic combination also delayed the emergence of phage resistant variants.

Conclusion:

This study shows proof-of principle that antibiotics, such as oxacillin, can still be used against multi-drug resistant bacteria when applied together with phages. The meaningfulness of this dual approach warrants further investigation in additional infection models such as bacteria growing in biofilms or under *in vivo* conditions. In addition, improved therapeutic effects can be expected with analogous phage/antibiotic combinations against other multidrug-resistant bacteria.

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Effects of C/N ratio and moisture content on the survival of ESBL-producing *Escherichia Coli* during chicken manure composting

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The high prevalence of extended-spectrum-ß-lactamase (ESBL)-producing *Escherichia (E.) coli* in European broiler farms leads to the possible dissemination of antibiotic-resistant strains into the environment using contaminated feces as organic fertilizer. The aim of the present study was to determine the effects of the carbon/nitrogen (C/N) ratio and the moisture content on the reduction kinetics of an artificially added ESBL-producing *E. coli* strain during lab-scale composting of chicken manure in bioreactors.

Four bioreactors were filled with four different compost mixtures consisting of chicken manure, wheat straw and water. The two initial C/N ratios of 10:1 and 40:1 were each combined with an initial dry matter content (DM) of 40 % and 80 %. The compost mixtures were spiked with a commensal ESBL-producing *E. coli* strain that was isolated from chicken feces and carries the blaCTX-M15 gene. Initial levels of 107 colony forming units (cfu)/g in the mixtures were achieved. The bioreactors were then incubated at 40°C and the number of *E. coli* was determined daily for ten consecutive days. In addition, the temperature profile within each bioreactor was recorded. All samples were also analyzed for total ammoniacal nitrogen, volatile fatty acids and pH. The experiment was conducted twice.

The fastest reduction of *E. coli* numbers occurred for both compost mixtures with a C/N ratio of 10:1. After 48 hours, the number of *E. coli* was below the detection limit (< 102 cfu/ml). Additionally, the dry mixtures led to a faster reduction compared to the moist mixtures despite lower maximum temperatures. The temperatures within the bioreactors were lower in mixtures with a C/N ratio of 40:1 compared to the mixtures with a C/N ratio of 10:1. After 10 days, *E. coli* were only still detectable in the mixture with a C/N ratio of 40:1 and 40 % DM.

In conclusion, composting of chicken manure is a suitable method to reduce antibiotic-resistant *E. coli* in chicken manure. Especially mixtures typical for chicken manure with a low C/N ratio and a high DM content lead to the rapid reduction of ESBL- producing *E. coli*.

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Risk assessment of antibiotic resistance development by antibiotic-loaded bone cements – is it really a clinical concern?

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The presence of foreign material in orthopaedic implant surgeries increases the operational risk of deep infections due to the easy bacterial colonization & biofilm formation on implant surfaces. Perioperative antibiotic prophylaxis (PAP) is widely accepted as a potent anti-infective measure in joint replacement surgeries. A strategy which relies on systemic & local antibiotic prophylaxis via antibiotic-loaded bone cement (ALBC) establishes two "independent" antibacterial frontlines and increases the probability that the intrusers become eradicated, even if one of both fails.

There is an ongoing controversy whether the observed benefit of infection risk reduction by ALBC outweighs the risk of possible antimicrobial resistance development. The scientific & clinical literature in PubMed, Medline and Embase has been systematically reviewed with the keywords "antibiotic resistance", "antibiotiic loaded bone cement", "local antibiotics", "bacterial colonization" and "joint infection". In total 27 publications were found with the majority reporting laboratory results. Only 8 papers focused on clinical septic situations & patient data.

Results

1. Because of the huge peak antibiotic concentrations in situ, antibiotic susceptibility or resistance criteria based on systemic antibiotic use cannot be full applied to locally applied antibiotics. Even resistant bacteria in the antibiogram may be killed at such high local antibiotic levels if not belonging to the high level resistance type
2. Bacterial growth or re-colonization of ALBC has been observed, both experimentally & clinically in those cases where infections were not eradicated by the combination of systemic & local antibiotics. This was most notable for CoNS, reflecting a selection process of high-level resistant strains.
3. Impregnation of bone cement with a combination of at least two antibiotics in septic implant revision cases greatly reduces the amount of resistant CoNS bacteria in the bone bed and leads to a higher rate of infection eradication.
4. There is no evidence for a widespread increase of antibiotic resistancies in the orthopaedic ward because of routine use of gentamicin-loaded bone cement.
5. Bacteria resistant against the antibiotic in bone cement are in vast majority still susceptible to the clinically most important antibiotic therapy options

Conclusion

The benefit of a lower infection probabilityout weighs the risk of more resistant bacteria if still an infection occurs.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Identification of the novel class D β-lactamase OXA-679 involved in carbapenem resistance in *Acinetobacter calcoaceticus*

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Introduction: Species of the *Acinetobacter calcoaceticus/baumannii* complex are opportunistic pathogens with the ability to cause severe nosocomial infections. They are characterised by their extended antibiotic resistance, which is due to either intrinsic resistance determinants such as the *Acinetobacter*-derived cephalosporinases (ADC) and certain oxacillinases (OXA) and/or acquisition of new resistance determinants

Objective: To characterize the clinical *Acinetobacter calcoaceticus* isolate AC_2117 with the novel carbapenem-hydrolysing class D beta-lactamase (CHDL) OXA-679.

Materials and Methods: Species identification and detection of beta-lactamases were done by genome sequencing (Illumina and PacBio platforms) and phylogenetic analyses. Antibiotic susceptibility of AC_2117 and transformants harbouring cloned $bla_{OXA-679}$ was evaluated by E-test and microbroth dilution. OXA-679 was purified heterologously and kinetic parameters were determined using spectrometry or isothermal titration calorimetry. The impact of OXA-679 production during imipenem therapy was evaluated in the *Galleria mellonella* infection model.
Results: Sequencing of the complete genome of AC_2117 identified a novel CHDL, which was assigned to as OXA-679. This beta-lactamase shared sequence similarity of 71% to the families of OXA-143 and OXA-24/40. Phylogenetic analyses revealed that OXA-679 represents a member of a new OXA family. Cloning and expression of $bla_{OXA-679}$ resulted in reduced susceptibility to carbapenems in *E. coli* and *A. calcoaceticus*, whereas expression in *A. baumannii* resulted in high-level carbapenem resistance (e.g., 256-fold MIC increase for meropenem). The purified enzyme showed an efficient hydrolysis of penicillins (more than 100-fold higher than for OXA-143) and carbapenems. Turnover numbers (*kcat*) for ertapenem and doripenem were the highest compared to the main OXA families OXA-23, OXA-24 and OXA-58. Infection of larvae of *G. mellonella* with a sublethal dose of $bla_{OXA-679}$ expressing *A. baumannii* could not be cured by high-dose imipenem therapy indicating carbapenem resistance *in vivo*.
Conclusion: We identified $bla_{OXA-679}$ in a clinical *A. calcoaceticus* which represent a member of the new OXA-679 family and which conferred high-level carbapenem resistance *in vitro* and *in vivo*.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Mobile colistin resistance *mcr-1* gene in *E. coli* from slaughterhouse wastewater in Pakistan

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Question: The mobile colistin resistance gene *mcr-1* has been identified worldwide in human and animal sources, however its presence in the environment is largely unknown. In this study, we report on the detection and molecular characterization of *mcr-1*-carrying *E. coli* from slaughterhouse wastewater in Faisalabad, Pakistan.
Methods: Raw wastewater samples from slaughterhouse were collected from January to June, 2018. After enrichment in 2 mg/L colistin supplemented broth, samples were streaked on MacConkey agar supplemented with 2 mg/L colistin. PCR was used to detect *mcr-1* gene. Whole Genome Sequencing was performed to determine genetic characteristics of *mcr-1* positive *E. coli.* In-silico analysis WGS data were performed on web service of the Center for Genomic Epidemiology.
Results: Six *E. coli* isolates carrying *mcr-1* gene were found in the slaughterhouse wastewater. Antibiotic resistance profile showed that all the isolates were MDR and most common co-resistance was seen against ceftriaxone, gentamicin and ciprofloxacin. WGS showed most strain carried plasmid resistance genes for beta-lactam (*blaCTX-M-15*), aminoglycosides (*strB, strA, aadA1, aadA2*), quinolones (*qnr*) genes. PlasmidFinder showed IncH12 as common plasmid replicon type. Of 6 *mcr-1* positive *E. coli*, 4 strains belonged to MLST type ST10 whereas 2 isolates belonged to ST632.
Conclusion: This study showed a high occurrence of mcr-1 in slaughterhouse wastewater. Widespread dissemination of *mcr-1* could be linked with colistin use in food producing animals in Pakistan.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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High diversity of 3rd Generation Cephalosporin resistant *Escherichia coli* in Schleswig-Holstein urban wastewater

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Introduction Multiresistant Gram-negative organisms are a major threat with increasing therapeutic challenges in many regions of the world. In Germany, multiresistant *Enterobacteriaceae* are frequently detected in patients as well as in environmental sources. In this study, we investigated the clonal relationship of 3rd Generation Cephalosporin resistant *Escherichia coli* (3GCREC) as marker organisms in urban sewage water.
Methods In eight urban wastewater treatment plants in Schleswig-Holstein wastewater was sampled continuously for seven subsequent days using Bühler 3010 automatic water samplers for 24 hours at different stages of the treatment process. Samples were investigated for 3GCREC by culture on ChromAgar ESBL (bioMérieux, Marcy L´Etoile, France) for 24 h at 42° C. From samples of influent and effluent water up to ten colonies were isolated. For 98 representative isolates whole genome sequencing was performed and the genomes were analyzed using the Finder tools of the Center of Genomic Epidemiology (DTU, Lyngby, Denmark). Clonal relation between the Isolates was determined by MLST (Warwick scheme) and cgMLST using the SeqSphere software (Ridom GmbH, Münster, Germany).
Results MLST revealed 41 different sequence types in 90 isolates and for 8 isolates no sequence type could be determined. Only sequence types ST10 (n=12), ST38 (n=11), ST131 (n=7), ST69 (n=5) were identified in at least four different locations. Analysis using cgMLST and the determination of resistance and virulence genes as well as potential plasmids revealed even in these common sequence types a high diversity. CTX-M-15 (n=38) and CTX-M-1 (n=23) were the most often detected resistance mechanisms against beta-lactam in 3GCREC. Two isolates displayed the virulence genotype of typical (*agg*R-positive) enteroaggregative *E. coli* (tEAEC), whereas for other isolates a relative low abundance for virulence genes was observed.
Conclusions 3GCREC are frequently observed in urban wastewater. A high diversity with only few clonal lineages appearing in different wastewater treatment plants was observed. Two isolates could be assigned as tEAEC whereas no other definitely pathotypes could be assigned. This data indicate, that tEAEC might be of more relevance as currently supposed in Germany. The examination of the possible sources of 3GCREC in wastewater should be forced to identify appropriate measures for the reduction of the dissemination of resistance genes in the water circuit.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Fragment-based carbapenemase inhibitors

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Carbapenemases are serine and metallo- β-lactamases (SBL and MBL) that mediate resistance of bacteria against all types of β-lactam antibiotics. The latter are the most commonly used class of antibiotics and represent the cornerstone for the current treatment of bacterial infections. The increasing spread of carbapenemases leads to higher incidence of antimicrobial resistance and consequently represent an enormous threat to human health and

lives. Carbapenem resistant microorganisms presenting NDM-1 β-lactamases can oft express resistance to other classes of antibiotics and thus reduce considerably treatment options. Therefore, the development of novel treatment options against Gram negative carbapenemase resistant pathogens is urgently needed.
We, therefore, aimed to discover carbapenemase inhibitors for clinical use in combination with last resort ß-lactam antibiotics such as Meropenem and Imipenem. Previously, we have developed an assay platform for testing of carbapenemase inhibitors, which uniquely reflects the pathophysiology of infection and therapy. By utilizing this screening platform, we identified two novel fragment classes with inhibitory activity in the micromolar range against selected metallo-carbapenemases (fragment class I) and metallo- as well as serine-carbapenemases (fragment class II), respectively. Biological activity of both fragment classes *in vitro* against carabapenem-resistant clinical isolates in MIC shift experiments is currently under investigation. Preliminary results suggest that at fragment class II is capable of increasing the susceptibility of NDM-producing clinical isolate *E. coli* to meropenem. Both fragment classes bind NDM-1 in a specific but distinct manner as shown by NMR studies. X-ray structure analysis of a co-crystal with this fragment class II and IMP-13 is a valuable starting point for structure-based fragment optimization. Furthermore, we have established first SAR analysis for both fragment classes. These fragment inhibitors are promising basis for the development of innovative pan-carbapenemase inhibitors, which will help restore efficacy of carbapenem antibiotics in clinical settings.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Year after year – A proposal for influenza infection control in hematology and oncology patients

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Introduction
Influenza is a relevant healthcare risk for patients with oncologic disease. Influenza infection is typically community-acquired, however nosocomial onset occurs as well. A multimodal infection control concept is needed to address influenza in hospitals.
Methods
An infection control concept regarding isolation, barrier precautions and handling of contact patients was established. Moreover, we implemented in winter 2016/17 a RT-PCR influenza screening program for respiratory asymptomatic patients on a pediatric and an adult oncologic ward [1].
Results
During the 2016/17 season, the following infection control measures were used. All symptomatic patients were isolated in single rooms and immediate diagnostic was conducted. Strict isolation of patients with influenza was enforced. To end solation two negative PCR based test results were required. In principle cohorting of patients with the same influenza subtype was allowed, in practice, however, it was avoided whenever possible. Patients were trained in hand hygiene, and wore a surgical mask outside their own room. Contact patients were put in quarantine and stayed there for 72 hours. Healthcareworkers were urged to wear a protective mask and a gown when entering the room of an influenza infected patient. Visitors were encouraged to wear masks at any time on the ward during the influenza season. From December 2016 to April 2017, 251 patients admitted to either of the wards were screened by PCR. We detected 11 respiratory asymptomatic patients with influenza infection (10 adults and 1 child). 5 of these patients were presumably detected during incubation period as indicated by follow-up samples showing increase of virus load. Infection control measures for influenza positive patients without symptoms were the same as for symptomatic patients with influenza.
Summary
To control influenza transmission in high risk patients a well-accepted and multimodal infection control concept is necessary. Our concept included a screening program targeting asymptomatic shedders of influenza (e.g. incubation phase, long term shedders due to immunosuppression) and helped to identify 11 patients for which infection control precautions were established to reduce risk of nosocomial spread.
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Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Attitude and knowledge towards disinfection of gloved hands

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Background
Chemical resistant single use gloves allow the practice of glove disinfection (1). However, different recommendations for their use exist.
Material and Methods
We conducted a standardized survey during an infection control meeting and an occupational health symposium in order to gain data regarding knowledge, experiences and attitudes of hygiene team members and occupational health physicians in hospitals towards glove disinfection by group comparison (2).
Results
558 out of 1000 questionnaires were returned, among them 246 (44.1%) from infection control practitioners, 63 (11.3%) from link nurses, 39 (7.0%) from hospital epidemiologists, 97 (17.4%) from link physicians, and 46 (8.2%) from occupational health physicians. The remaining 67 participants worked in multiple other entities.
75% of infection control professionals, 35% of occupational health physicians and 25% of link nurses and link physicians were contacted regarding glove disinfection within the last 12 months by fellow health care workers.
In many institutions, glove disinfection is not allowed; 67% of respondents voted against it. Large knowledge and practice deficits regarding single glove use were reported.
Discussion
The topic of glove disinfection is primarily an issue for the infection control team. However, a close cooperation with occupational health would be warranted but is not practiced. There is relevant uncertainty regarding the presupposition of disinfectable gloves and the practical indications and procedures for the desinfection of gloved hands.
Keywords
Single use gloves, disinfection, hand hygiene, material compatibility, infection control

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Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Implementation interventions to improve compliance with clinical interventions to prevent surgical site infections in abdominal surgery: first results of a systematic review

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Surgical site infections (SSIs) are among the most common nosocomial infections in Germany (1). Their prevalence is especially high in abdominal surgery (2). There are several measures to prevent SSIs (clinical interventions) (3). However, compliance is often suboptimal (4), even though there are many implementation interventions to increase compliance (5). This systematic review aims to identify implementation interventions used to promote compliance with measures to prevent SSIs in abdominal surgery, and to determine interventions especially effective in promoting compliance and reducing SSI rates.

The literature search was conducted in PubMed and Web of Science Core Collection in April 2018. Two reviewers (IT & NRH) screened the records independently. Implementation interventions identified were classified using the "Implementation Strategies"-part of the EPOC taxonomy (5) as a category system by two reviewers (IT & TvL). Compliance- and SSI-rates were taken or calculated from the studies.

N=40 studies were included. Implementation interventions varied among studies, with most studies using a combination of different interventions (range: 1-10, mean: 4.6, std. dev.: 2.5). Mostly used were audit and feedback (80%), organisational culture (70%), monitoring the performance of the delivery of healthcare (65%), reminders (53%), and educational meetings (45%). Compliance rates were incompletely reported, while 30 studies reported pre- and post-intervention SSI-rates. A first descriptive analysis showed SSI-reduction was larger in studies with 3 or more implementation interventions (-8%) than in those with fewer interventions (-4%).

In the field of abdominal surgery, mostly standard implementation interventions are used to promote compliance with measures to prevent SSIs, with a descriptive tendency towards larger effects given more implementation interventions.

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Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Systematic selection-procedure for surgical-site-infection-prevention-measures for a bundle approach with particular regard to implementation aspects

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Introduction

In the HYGARZT project the implementation and effectiveness of infection prevention measures (IPM) in traumatology and orthopedics by infection prevention link practitioners will be investigated. In the context of intervention-preparation a systematic selection procedure for surgical site infection (SSI) prevention measures, under special consideration of implementation aspects was developed.

Aim

The aim was to establish a systematic, practical workflow, for composing IPM bundles and planning their implementation, based on known, theoretical models.

Materials & Methods & Results

Starting point was the building of an interdisciplinary team (IDT), which consisted of motivated representatives of the relevant occupational groups. All members committed themselves to aim of lowering the local infection rate. The IDT resolved the establishment of an infection surveillance system, to identify crucial problems. After the accomplishment, IDT identified SSIs as the main problem on the participating wards (PW) and conducted a systematic literature review to summarize possible prevention and implementation measures. The measures were ranked according to level of evidence and study quality. Additionally a situation analysis of the currently performed IPM and the compliance to them was accomplished on the PW. The IDT evaluated those IPMs, considering the results of the literature review and decided to dismiss, adjust, or unalteredly continue with them. Furthermore the IDT analyzed the highly ranked and currently not performed IPM and estimated the proportion of local patients, who may benefit from them. After that a provisional bundle (PB) consisting of adjusted currently performed and new IPM was conducted. During the first step of implementation planning, economic resources (materials, infrastructure, staff) and human factors (reactance and possible countermeasures) were calculated by IDT to estimate the feasibility of the PB. After that the bundle was modified accordingly to find the most efficient, but at the same time feasible, evidence based approach under local conditions. Then the second step of implementation planning started by assembling smaller work groups (WG) for detailed planning of the IPM. The results of these WGs were discussed, and adopted by the IDT and implemented afterwards.

Summary

We present a systematic workflow for the selection and implementation-planning of IPM, which may serve as a blueprint for future projects.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Standardized observations to identify non-adherent hand hygiene compliance during the dressing change process in a pre-intervention phase of a planned intervention study

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Introduction

The project "HygArzt" (ZMVI1-2516FSB111), funded by the Federal Ministry of Health (BMG), intends to reduce the infection rate of nosocomial infections, especially postoperative wound infections, in the long term by introducing evidence-based infection prevention measures. In the pre-intervention phase, hygiene-relevant processes in orthopedics and trauma surgery were examined. The dressing change is an essential hygiene-relevant process to avoid nosocomial postoperative wound infections. During the process, non-disinfected hands of clinical staff are the most important carrier of pathogens to the patient. To identify hygiene-relevant deviations, the dressing changes were examined more closely by standardized observations.

Methods

In the pre-intervention phase of the study, 337 dressing changes were observed on three orthopedics and trauma surgery normal wards as part of the morning visit and morning patient care. A checklist with 26 items was compiled for this purpose. Particular attention was paid to hand hygiene compliance before and after the dressing change as well as to the transition between the impure and pure phase of the dressing change.

Results

A total of 1348 indications for hand hygiene during dressing changes were observed during the rounds and during normal ward routine. With 80%, the highest total compliance rate was, as expected, at hand disinfection after dressing change (ASH indication after contact with potentially infectious material). A value of 63% could be determined for the compliance rate before the start of dressing change. This is composed of the indication before the start of dressing change (ASH indication before aseptic activity) with a compliance of 36% and the indication immediately before the start of dressing change with a compliance of 14%. The total compliance of 63% has a higher value than the two individual indications, since persons who performed both hand disinfections correctly were additionally considered. The lowest compliance rate (42%) could be determined between impure and pure phase of the dressing change. Based on this data, the pre-indications and the transition between pure and impure phase could be identified as problem areas. The most frequently observed errors were incorrect storage of sterile dressing materials, incorrect disposal of dressing waste.

Summary

Through the observations in the pre-intervention phase, the largest non-adherencies in the dressing change process could be identified. Based on these findings, a new dressing change concept was developed, which will be implemented in the following intervention phase of the HygArzt study.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

Microbiota, Probiota and Host (FG PW)

249/PWP

Exploring fungal-bacterial interactions in the mammalian gut

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The human microbiota comprises members of all three domains of life, *i.e.* bacteria, archaea and eukaryotes. Yet little is known about the biology of the non-bacterial constituents of this microbial community and even less about how they interact with cohabiting microbes. We are investigating the interplay between the fungus *Candida albicans* and the bacterium *Bacteroides thetaiotaomicron*, two of the most prevalent species of each phylum in the human gut. By co-colonizing germ free mice with *C. albicans* and *B. theta*, we have established that these two species reach colonization levels (in colony forming units per gram of stool) similar to those achieved by each microbe in monocolonized animals. Immunohistochemistry analyses of colon sections of mono- and co-colonized animals indicate that both microbes co-exist in close physical association in the gut and that *B. theta* determines, to a large extent, the localization of fungal cells to the outer mucus layer. Finally, screening a *B. thetaiotaomicron* transposon mutant library in gnotobiotic mice co-colonized with the fungus uncovered several bacterial genes that potentially contribute to the *in vivo* fitness of the bacterium in the presence of *C. albicans.*

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Mechanism of *E. coli*-mediated colonization resistance against *Salmonella* Typhimurium in Oligo-MM^{12} mice

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The mammalian gut microbiota fulfills many beneficial tasks for its host, such as nutrient degradation, training of the immune system and protection against enteric pathogens, a phenomenon termed colonization resistance (CR). Due to the high diversity of the gut microbiota, it is challenging to pin down the contribution of individual bacteria to CR and to identify protective species. Therefore, we used a gnotobiotic mouse model (Oligo-MM^{12}) with reduced microbial complexity to investigate the functions of individual bacteria during enteric *Salmonella* Typhimurium (*S.*Tm) infections. This minimal microbiota exhibits intermediate CR against an avirulent *S.*Tm strain in comparison to mice colonized with the Altered Schaedler Flora (ASF) and mice with conventional microbiota. By genome-informed design, an improved version of the Oligo-MM^{12} was created by adding three facultative anaerobic bacteria (*Escherichia coli, Streptococcus danieliae* and *Staphylococcus xylosus*) and this consortium provided conventional-like CR (Brugiroux et al., Nature Microbiology 2016). We further dissected the role of facultative anaerobic bacteria in CR and found out that *E. coli* is solely responsible for the restored CR against *S.*Tm in this model, while *S. danieliae* and *S. xylosus* are dispensable. The future aim is to unravel the mechanism underlying *E. coli* mediated CR in Oligo-MM^{12} mice.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

251/PWP

Gnotobiology Unit within the Priority Program SPP1656 Intestinal Microbiota

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The Institute for Laboratory Animal Science and Central Animal Facility of the Hannover Medical School (Ztm) has served as a core gnotobiotic unit within the DFG funded Priority Program "INTESTINAL MICROBIOTA" (SPP1656). The availability of gnotobiotic models was crucial for this program, as these models have been recognized as a valuable tool for studying complex host-microbiota interactions.

During the SPP1656 the gnotobiotic unit expanded in the number of isolators and strains kept germ-free (GF). Today it counts 37 Hannover type isolators and maintains more than 25 different gnotobiotic animal models. Main goals of this unit were to generate and maintain gnotobiotic animal models, support collaborative experiments and provide training to scientists working with these models. For GF re-derivation mice have been sent in a SPF stage to Hannover, where these animals have been re-derived GF by hysterectomy. Upon re-derivation mice have been maintained as GF colonies at the gnotobiotic unit or/and sent to laboratories of respective scientists. Currently, the gnotobiotic unit provides several wild-type strains, but also multiple genetically modified murine lines and disease models. Furthermore, the unit established isolators housing mouse strains carrying defined bacterial consortia such as Oligo Mouse Microbiota12. Moreover, the gnotobiotic team of the Ztm supported many collaborative gnotobiotic experiments over the past 5 years. For members lacking the infrastructure to perform gnotobiotic experiments in their own animal facilities, Ztm offered experimental isolators and microisolator cages as well as expertise in preforming gnotobiotic experiments. Performed experiments included a broad spectrum of approaches ranging from dietary intervention studies to infection trials and colonization studies with commensals. Additionally, the re-derivation of various animal models into GF conditions allowed analysis whether and to which degree this phenotype is microbiota-driven. Furthermore, for collaborators that had available infrastructure or for final experiments gnotobiotic animals have been sent to their facilities by using Han-gnotocages, which are designed to preserve gnotobiotic status during transport and allow shipment of gnotobiotic animals Germany-wide.

Overall within last 5 years Ztm gnotobiotic unit offered its expertise and techniques in gnotobiology field and accomplished Germany-wide collaboration with research groups within SPP1656.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Dietary sulfonates are efficiently converted by the human gut microbiota

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Introduction: Diet is a major factor influencing the gut-microbiota composition. Recently, the colitogenic bacterium *Bilophila wadsworthia* was reported to proliferate in IL10-/- mice in response to a high-fat diet rich in saturated fatty acids. This was found to be due to *B. wadsworthia´*s ability to utilize the sulfonate moiety of taurine-conjugated bile salts as an electron acceptor for its growth.

Aim: We hypothesize that sulfonates other than taurine also stimulate the proliferation of *B. wadsworthia* and related bacteria. The objectives are, first to investigate the conversion of relevant sulfonates by the human gut microbiota including taurine, isethionate, cysteate, 2-mercaptoethanesulfonate (CoM), sulfoquinovose (SQ), 2,3-dihydroxypropane-1-sulfonate, and 3-sulfolactate to the final product sulfide, second to identify SQ and taurine-converting gut bacteria and third to examine the conversion of the sulfolipid sulfoquinovosyldiacylglycerol (SQDG), present in chloroplasts of leafy vegetables, in conventional C57BL/6J mice.

Material & Methods: Fecal slurries from healthy human subjects (n = 4) were incubated with individual sulfonates under strictly anoxic conditions (N2/CO2, 80:20) for 96 hours, using formate or lactate as electron donor. Isolates were obtained from fecal slurries after repeated enrichments. C57BL/6J mice (10 - 12 weeks, male, n = 8) were fed for three weeks a semisynthetic diet containing *Arthrospira platensis* (Spirulina), which is rich in SQDG.

Results: Human fecal slurries converted all tested sulfonates, except CoM, to sulfide. Sulfonate-converting isolates were identified based on their 16S rRNA and dissimilatory sulfite reductase gene (dsrA) sequences and Gram staining. Mice fed the Spirulina diet had an increased body weight and a higher fat accumulation compared to the control mice. Macroscopic characteristics of the organs did not indicate any differences. Sulfonate conversion depending on the gut microbiota composition in mice will be examined in future studies.

Summary: Our studies indicate members of the human gut microbiota are capable of utilizing a whole range sulfonates for their growth under strictly anoxic conditions. Mice fed an SQDG-containing diet had an increased body weight and adipose tissue accumulation. My investigations help to characterize the bacterial conversion of various dietary sulfonates in the intestine and will lead to the identification of involved bacteria.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Molecular analysis of the immune-modulatory properties of colitogenic intestinal microbiota

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Question

Inflammatory bowel disease (IBD) is widely accepted to be caused and/or significantly influenced by at least three factors, i.e. a. predisposing genetic alterations, b. acquired environmental triggers and c. dysregulated immune responses. In IBD, many studies revealed a substantial alteration of the bacterial and viral composition, i.e. dysbiosis where especially the alpha-diversity of the microbiome is reduced in colitic vs. non-affected tissues. While dysbiosis is hence a characteristics frequently found in the gut of IBD patients, its functional contribution to hampered intestinal tissue homeostasis during colitis remains mainly unresolved and was hence in the center of the current study.

Methods

To shed light on the molecular and functional properties of colitis-mediating intestinal microbiota, we took advantage of the CD4+CD25- naive T cell transfer and anti-CD40-driven colitis models. Besides clinical, endoscopic and histopathological evaluations of the colitis phenotype, flow cytometric analyses of the intestinal immune cell subpopulations were performed. To assess the intestinal microbiota, co-housing experiments were performed and bacterial communities were assessed by 16S rRNA gene sequencing analyses.

Results

By 16S rRNA gene sequencing analyses of colon feces we identified the composition of intestinal microbiota within one of our lines to be dysbiotic. Functional studies revealed that the intestinal microbiota selectively aggravates T cell-driven transfer colitis. This phenotype was completely dependent on the composition of the intestinal microbiota and transmittable through co-housing while e.g. Th17 cells were rather dispensable for colitis formation. In line with this, results of co-housing experiments also suggest that the presence and/or functionality of conventional dendritic cells 1 (cDC1s) or 2 (cDC2) are not required for the manifestation of the accelerated colitis formation.

Conclusions

Here we provide an example for colitis induction that is rather driven by the intestinal microbiota than by a specific immune

mediated pathway previously implicated to be a mainstay in IBD pathogenesis. Hence, specific therapeutic strategies to directly modulate e.g. the microbial ecology or microbiota-host-interaction underlying the development and shaping of disease-mediating T cell populations emerges as an option to harness intestinal inflammation.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Age-dependent microbial selection in the neonate intestine determines adult microbiota composition

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The enteric microbiota plays a critical role for immune maturation and host metabolism. Alterations of the microbial composition have been associated with inflammatory and immune-mediated diseases of the gastrointestinal tract, skin and lung as well as obesity and diabetes. The postnatal period appears to play a critical role in the establishment of the life-long host-microbial interaction and immune homeostasis. However, the underlying molecular mechanisms of this neonatal "window of opportunity" have not been defined. Here we report on the identification of a host-mediated regulatory circuit of bacterial colonization that acts solely during the early neonatal period but influences the life-long microbiota composition and disease susceptibility. We demonstrate age-dependent expression of the flagellin receptor toll-like receptor (Tlr)5 in the neonatal gut epithelium. Using competitive colonization experiments in neonate mice we show the critical role of epithelial Tlr5 expression and Reg3g production for the counter-selection of colonizing flagellated bacteria. Comparative cross-breeding, cohousing and microbiota transfer experiments in neonate and adult wildtype and Tlr5 deficient animals reveal that neonatal Tlr5 expression is both, sufficient and necessary to shape the life-long microbiota composition. Thus, the beneficial microbiota in the adult host is determined during early infancy. This might explain why environmental factors that disturb the microbiota establishment during early life influence the host"s immune homeostasis and health in adulthood.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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The Impact of Dietary Fibers on Intestinal Microbiota and Homeostasis

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Health benefits of dietary fibers are mainly mediated by the intestinal microbiota, e.g. by the production of short-chain fatty acids. The interaction of the microbiota and the immune system is vital for intestinal homeostasis and disturbance of this complex relation may lead to intestinal as well as extra-intestinal diseases.
The aim of this study was to analyse the mechanism by which cellulose, an insoluble dietary fiber, affects the gut microbiota and intestinal homeostasis. For this purpose, animals were kept on chemically defined diets with or without cellulose.
Animals on cellulose-free diet gained body weight comparable to controls and showed no signs of impaired fitness. However, they were highly susceptible to DSS-induced intestinal inflammation, characterized by enhanced expression of pro-inflammatory cytokines and a leaky epithelial gut barrier. 16S rRNA amplicon analysis of the intestinal microbiota revealed that cellulose dramatically influences the development (diversity) of the intestinal microbiota. At young age (eight weeks), a similar diversity of the cecal microbiota was seen, independently of the diet. Interestingly, between week eight and twelve a massive increase in the microbial diversity was exclusively found in mice that were fed a diet containing cellulose. Moreover, the altered microbiota lead to alterations in the cecal metabolome. To study the mechanism of increased DSS susceptibility in the absence of cellulose, we analysed the impact of the altered microbiome and metabolome on central key players of intestinal homeostasis, especially intestinal immune and epithelial cells.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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MicrobIEM – a user-friendly and interactive analysis tool to explore the microbiome

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Introduction: Recently, multiple microbiome studies have been performed generating high amounts of amplicon sequencing data and meta-information. Overall, most microbiome studies were initiated by scientists with expertise in other fields than bioinformatics. Hence the analysis and interpretation of microbiome data is still a bottleneck for research projects. Microbiome analysis is time consuming and harbors many pitfalls even for experienced scientists. This especially holds true for studies with low microbial biomass, such as skin microbiome samples. Therefore, there is a great demand for user-friendly computational tools for non-bioinformatics experts.
Aims: Our tool, microbIEM enables the filtering of relevant species and the removal of biological and technical contaminants. We established this tool as a collaboration project between bioinformaticians and microbiologists. This approach helped us to develop meaningful features that scientists need to explore their datasets.

Methods: microbIEM is an in-house tool, which is implemented in the scripting language R. It uses a Java-based front-end to remove operational taxonomic units (OTUs) based on filters that account for the total and relative amount of reads. Alternatively, it removes OTUs based on negative and positive controls. microbIEM also allows the definition and omission of low quality samples. Furthermore, an option to filtering for artifacts (technical filtering) and for relevant OTUs (biological filtering) is provided. All filters can be used either fully or semi-automatized.
Results: We tested microbIEM with different data sets, including intestinal- and skin microbiome studies. microbIEM allows filtering for relevant OTUs and samples. It generates overview tables of amount of reads in total per OTU. It also depicts on the species level, which OTUs were lost by filtering. microbIEM allows computing alpha- and beta-diversity efficiently. Furthermore, it is possible to select and de-select samples or sample groups and calculate alpha- and beta-diversity. This allows scientists to explore microbiome data sets, for example by selecting a specific genotype or treatment. In contrast to other tools, microbIEM can be easily used by non-experts. Additionally, results can be compared to state-of-the art tools such as QIIME or RHEA by providing input files.
Conclusion: For the future, we plan to further develop microbIEM and include methods to correlate microbiome composition with gene expression.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Ageing impacts the microbial community along the gut

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During ageing the overall body functions and organs tend to be altered and being also associated with changed intestinal microbiota composition. Intestinal microbiota has been linked to the maintenance of gut homeostasis and health as it is related to the development of the immune system, metabolic regulation, nutrient digestion and absorption. Therefore, impairments of these processes during ageing could be linked to the changes occurring in the microbial ecology of the intestine. Up to now, microbiota research mainly targeted the large intestine or fecal samples, representative of the lower colon and rectum. Limited information is available related to the small intestine microbiota; however, it is one important intestine section responsible for nutrient absorption, with may also impact health status during ageing. Our aim was to identify changes of intestinal microbial ecology occurring duringageing and herein to compare the duodenal and colonic samples. Duodenum and colon samples were obtained from healthy C57Bl/6J male mice fed with a standard diet at 2, 15 and 30 [B1] months old. DNA was extracted using a modified Trizol protocol, bacterial DNA was targeted for the library preparation of the V1-V2 16s rRNA gene region. Libraries were sequenced using Illumina MiSeq platform using 250 bp paired-end sequencing chemistry. Microbial changes were observed between the different ages ($P < 0.05$) and sites ($P < 0.01$). In colonic samples abundances of *Firmicutes* were lower compared to duodenum samples at all ages, while the abundance of *Proteobacteria* was statistically higher in colon increasing significantly at 30 months compared to 2 months old mice. *Bacteroidetes* abundances declined in the colonic samples of the 30 months old mice compared to the younger ages. At species level, we could see an age-related increment of *Helicobacter hepaticus*, an identified pathosymbiont, in the colonic samples, as well as a decrease in *Propionibacterium acnes*. In the duodenum two species of *Lactobacillus*, *L. reuteri* and *L. johnsonii*, were lower in abundance at 30 months compared to the 2 months old. In summary, our data suggest that in mice aging is associated with marked changes of the microbial composition of the duodenum and the colon which may also impact the development of age-related diseases.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Intestinal microbiota and permeability during aging: Is NO a critical modulator?

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Introduction: As many populations around the world are rapidly aging and life expectancy is raising, the prevalence of aging-associated impairments is increasing, too. Studies suggest that alterations of intestinal barrier function and microbiota composition may be of importance in modulating healthy aging. However, mechanisms involved in the interaction of intestinal microbiota and barrier have not yet been clarified.
Aim: The aim of the present study was 1) to determine if changes of bacterial endotoxin levels found in healthy elderly and old aged mice are associated with alterations of intestinal barrier function and microbiota composition and 2) to assess the role of NO-synthesis herein.
Methods: In healthy 23-33 years and 85-97 years old men as well as in 3 months and 24 months old male C57BL/6J mice markers of intestinal permeability were assessed. Furthermore, besides intestinal microbiota composition, markers of nitric oxide system were determined in mice. Moreover young and old mice were treated with the arginase inhibitor nor-NOHA (10 mg/kg BW).
Results: In both, old healthy men and mice bacterial endotoxin levels were significantly higher than in young men and mice. In mice this increase in bacterial endotoxin was associated with a loss of tight junction proteins and alteration of intestinal microbiota composition in proximal small intestine. Furthermore markers of the nitric oxide system like citrulline plasma concentration or NO_2^- levels and iNOS mRNA expression in proximal small intestine were also markedly lower in old animals compared to young. Additional treatment with nor-NOHA in old mice improved markers of intestinal permeability.
Conclusion: Taken together, our data suggest that aging is associated with impairments of intestinal barrier function accompanied by an increased translocation of bacterial endotoxin and that in mice this is associated with a dysregulation of the intestinal nitric oxide system (DFG FKZ: BE2376/8-1).

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Dietary sulfonates do not promote bacteria-induced gut inflammation

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Introduction: The interplay between the intestinal microbiota and the host has increasingly been recognized as a major factor impacting health. Diet is probably the most influential determinant affecting the gut microbiota. A diet rich in saturated fat was shown to stimulate the growth of the colitogenic bacterium *Bilophila wadsworthia* by enhancing the secretion of the bile acid taurocholate (TC), whose sulfonated taurine moiety is utilized as a substrate by *B. wadsworthia* (Devkota *et al*., Nature 2012). This bloom of *B. wadsworthia* was accompanied by an increased incidence and severity of colitis in interleukin (IL)-10-deficient mice, which are genetically prone to develop intestinal inflammation.
Aims: Therefore, we aim to find out whether the intake of dietary sulfonates also stimulates the growth of *B. wadsworthia* or other

sulfite-reducing bacteria thereby promoting intestinal inflammation in genetically susceptible mice.

Methods: Dietary sources of sulfonates include chloroplasts in green vegetables and cyanobacteria, which contain the sulfolipids sulfoquinovosyl diacylglycerols (SQDG) in considerable amounts. In our study, we fed specific pathogen-free IL-10-deficient mice either a diet supplemented with the cyanobacterium *Arthrospira platensis (*also called Spirulina) or a control diet. Additional mouse groups were gavaged with the SQDG metabolite sulfoquinovose, TC as positive control or water. During the experiments, the body weight of mice was monitored, fecal samples were collected and gut permeability was determined. After three weeks of treatment, mice were killed and their intestinal tissues scored histopathologically. The abundance of *B. wadsworthia* and other sulfite-reducing bacteria was determined in fecal samples.

Results: None of the mice treated with the above mentioned sulfonates showed weight loss or macroscopic signs of inflammation. Mice fed the Spirulina diet even gained weight. The histopathological assessment revealed no signs of colitis. Colon barrier integrity was not affected by sulfonate feeding and fecal cell numbers of *B. wadsworthia* remained low in all mice.

Conclusion: In summary, neither the tested dietary sulfonates nor TC led to intestinal inflammation in the IL-10-deficient mouse model.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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The Role of Arginases in the maintenance of mucosal immune homeostasis

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Introduction

Arginase 1 (Arg1) and the inducible nitric oxide synthase (iNOS) compete for the common substrate L-arginine and exhibit various, sometimes opposing effects on immune responses, tissue regeneration and intestinal microbiota. In mucosal tissues of patients with inflammatory bowel disease (IBD), however, the expression and activity of both Arg1 and iNOS are simultaneously enhanced.

Aims

As the biology of intestinal Arg1 and iNOS in IBD is not understood, we aimed to characterize the interactions of both enzymes with the intestinal microbiota. Furthermore, we assessed the cellular compartments and microevironmental parameters, which mediate the induction, perpetuation and resolution of intestinal inflammation.

Material and Methods

The expression of Arg 1 and iNOS, the composition of the intestinal microbiota, bacterial replication and dissemination as well as the extent of inflammation was assessed in IBD patients and in mouse models of dextran sodium sulfate (DSS) and *Salmonella typhimurium* induced colitis using immunohistochemistry, conventional plating assays, 16S rRNA sequencing, high resolution endoscopy, immunofluorescence laser scanning microscopy, intravital 2-photon microscopy and conditional Arg1-knockout mice.

Results

Tie2-Cre x Arg1fl/fl mice that lack Arg1 in hematopoietic and endothelial cells unexpectedly developed less severe colitis than wild type littermates upon DSS application or infection with *Salmonella typhimurium*. The protection from colitis correlated with compositional changes of the intestinal microbiota. Furthermore, the endothelial permeability, vessel density, systemic bacterial dissemination, leukocyte adhesion and the inflammatory immune response were significantly reduced in Tie2-Cre x Arg1fl/fl mice compared to wild type littermate controls. Fecal transfers into broad-spectrum antibiotic-treated B6 recipient reconstituted this phenotype suggesting that an altered microbiota in Arg1-deficient Tie2-Cre x Arg1fl/fl mice decreases the susceptibility of mice to DSS- or infection induced intestinal damage.

Summary

Arg1 promotes an accumulation and a systemic dissemination of intestinal bacteria and subsequent inflammation due to the induction of endothelial dysfunction and of dysregulated myeloid cell responses. Thus, Arg1 represents a novel therapeutic target for clinical intervention in IBD patients and a clinical progression parameter for the accumulation of an inflammatory microbiota.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Colitogenic or beneficial: functional relevance of opportunistic pathogen *Enterococcus faecalis* in chronic colitis is determined by the microbial environment

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Inflammatory bowel diseases (IBD) are associated with intestinal microbiota alterations, but the contribution of individual bacteria to the pathogenesis remains largely unknown. The commensal *Enterococcus faecalis* (*E.f*) has been linked to IBD and induces colitis in susceptible IL10 KO mice. We aim to unravel the relevance of *E. f* in chronic colitis with regard to gene expression and the microbial environment.

E.f function in a bacterial community was addressed by colonizing mice with a colitis-inducing microbial consortium (SIHUMI). Surprisingly, colonization of IL10 KO mice with SIHUMI in the absence of low abundant *E.f* not only induced inflammation, but resulted in an aggravated phenotype. This suggests that the colitogenic activity of *E.f* is compensated by other bacteria and that the presence of a colitogenic strain can be protective in a certain community. A massive response of reactivated MLN cells to *E. coli* stimulation and a positive correlation between colitis activity and *E. coli* abundance points to this bacterium as driver of SIHUMI mediated colitis.

The transition from a colitogenic to a protective activity of *E.f* might be the result of altered gene expression profiles in a more complex microbial environment. *E.f* disease-associated transcriptome was assessed via RNA sequencing of bacteria isolated from monoassociated and SIHUMI colonized mice. Under inflammatory conditions, the majority of regulated genes was not shared between *E.f* from monoassociated and SIHUMI colonized mice, indicating that the microbial environment has a strong influence on *E.f* gene expression. Among the highest upregulated genes in inflamed monoassociated mice, genes of the ethanolamine utilization (eut) locus were identified. Ethanolamine (EA) is known to play a role in host adaption and virulence for diverse pathogens. In contrast, *E.f* isolated from SIHUMI mice showed increased expression of eut genes in healthy and mildly inflamed compared to severely inflamed animals, suggesting rather an anti-inflammatory role of EA utilization in this context. In line with this, deletion of *E.f* eut genes resulted in exacerbated colitis in SIHUMI colonized mice, but had no influence in monoassociation.

Our data show, that the microbial environment can not only influence the transcriptome, but also the overall relevance of an opportunistic pathogen in chronic colitis. Furthermore, EA utilization seems to be important for the protective effect of *E.f* observed in SIHUMI colonized mice.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

Infection Epidemiology and Population Genetics (FG MS)

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Detection and lysogenic conversion of a P1-/P7-like prophage mediating transmission of an incorporated transposon comprising a *bla*$_{\text{CTX-M-15}}$ resistance gene

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Questions

To characterize the genetic basis of ESBL-producing *E. coli* isolates and its mechanisms for the transmission of the cephalosporin resistance, *Escherichia coli* isolates recovered from wildlife in Germany between 2015 and 2017 were characterized in detail. Within this study, an *E. coli* isolate was identified harboring a P1-/P7-like prophage comprising a cephalosporin resistance determinant.

Materials and Methods

The isolate was further studied by S1-PFGE, DNA-DNA hybridization and WGS analysis to determine the genetic basis of the cephalosporin resistance. Restriction profiling, transformation, *in vivo* filter-mating and antimicrobial resistance testing was performed to characterize the properties of the isolate and its mobile genetic elements. Mitomycin C inductions were conducted to assess the activity of the prophage and its impact for the transfer of antimicrobial resistances.

Results

Genome determination of the *E. coli* isolate revealed a *bla*CTX-M-15 carrying sequence contig exhibiting significant homologies to known P1-/P7-like plasmid prophages. Induction and plaque tests indicated that the phage possess a broad host range. The phage showed a morphology of typical myoviruses and is able to infect various *E. coli* strains of different serotypes. Due to the incorporation of the phage in novel recipient *E. coli*, lysogenic conversion of the bacteria was observed by the production of extended-spectrum beta-lactamases.

Conclusion

Based on the current knowledge on P1-/P7-like prophage-plasmids we suppose that these phages may be efficient vehicles for the incorporation and transfer of a broad range of antimicrobial resistance determinants. As the resistance genes are often associated with transposon sequences the dissemination of the resistances is further forced by their activity and specificity. The transfer of antimicrobial resistances by phages may represent an evolutionary adaption to extend the number of possible intra- and interspecies hosts. Thus, phages may support an initial interspecies transmission of antimicrobial resistance genes.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Whole genome sequence based resistance prediction and molecular typing of *Mycobacterium tuberculosis* complex (MTBC) strains in BioNumerics

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Question

Tuberculosis (TB) is one of world"s deadliest infectious diseases caused by *Mycobacterium tuberculosis*. Whole-genome sequencing (WGS) has become an important tool for epidemiologic studies of TB, including outbreak detection and elucidation of infection sources and transmission routes. In contrast to the time-consuming phenotypic drug susceptibility tests (DST), WGS enables rapid prediction of associated antibiotic resistance. In this study, we present a new MTBC genotyping tool. This plugin for the BioNumerics software can predict associated resistance to first and second line antibiotics, but also determines the species, the lineage and spoligotype of the strains under consideration

Materials and methods

All functionalities within the MTBC plugin are based on sequence reads generated by WGS technologies. **Species decomposition** is based on the sequence of the 16S rRNA. The **lineage determination** component classifies the MTBC strains in 8 known lineages and 55 sublineages based on 62 single nucleotide polymorphisms. In addition, *in silico* **spoligotyping** is performed using the nucleotide sequences of 43 spacers as references in a local mapping analysis. Lastly, **resistance prediction** for 12 antibiotics is based on known mutations in 28 resistance genes. Additional resistance markers can be implemented in the plugin on a regular basis. The pipeline is integrated in the BioNumerics software, and implemented on a scalable high-throughput calculation environment. Results were validated on 161 MTBC samples (PRJNA187550)

Results

The majority (98%) of the strains were correctly identified by the plugin as being MTBC species and the predicted lineage of all samples corresponded with previous reports. For 99% of the MTBC isolates, the tool predicted the same spoligotype as the SpoTyping program. The sensitivity for isoniazid (INH), rifampicin (RMP), ethambutol (EMB), kanamycin (KAN) ranged between 80 and 95%. For ethionamide (ETH) this was only 50%. The specificity for INH, RMP, ETH, EMB and KAN, ranged between 70 and 100%. All samples were predicted to be resistant to streptomycin (SM) while this was only true in 52% of the cases according to phenotypic DST. The high amount of false positive and negative drug resistance predictions indicated the need for a revision/extension of the current genotype-phenotype correlations, currently being implemented

Conclusion

The BioNumerics genotyping functionality for MTBC strains is an effective and user-friendly tool for species identification, strain typing and prediction of resistance and can thus be useful for high-throughput molecular surveillance and control of TB

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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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 *Mycobacterium tuberculosis* complex (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.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

265/MSP

Sub-cluster analyses of lyssavirus full genome sequences with affinity propagation clustering.

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Introduction
Rabies caused by rabies lyssavirus (RABV) is one of the oldest known zoonotic diseases. Numerous viral variants within the RABV species cause tens of thousands of human deaths annually on a global scale. Multiple studies on sequenced RABV isolates were conducted. So far, the clusters defined in the belonging analyses were most commonly defined by visual inspection of the phylogenetic dendrograms. This could lead to different results due to a lack of criteria for cluster definition.
Objectives
Next generation sequencing enables scientists to generate a huge amount of RABV sequences, approximately 21,000 datasets, including full genome sequences. Phylogenetic methods in combination with affinity propagation clustering (AP Clustering), a mathematical method that uses similarity matrices as input, revealed four generic main clusters, named "Arctic", "Cosmopolitan", "Asian" and "New World", on basis of RABV full genomes. Further analyses with AP clustering and phylogenetic methods should enable the division of the four main clusters into sub-clusters.
Materials & Methods
In order to identify sub-clusters of the RABV full genome tree the defined main clusters were used and subdivided by affinity propagation clustering. The results were spatially correlated with the geographic spread of included isolates with the help of ArcGIS.
Results
The sub-clustering of the existing four main clusters with AP Clustering gained a total of aprox.12 sub clusters. The main "Arctic" and "Asian" clusters could be subdivided into three sub-clusters respectively and reasonable. The "Cosmopolitan" cluster also splits up into three sub-clusters. The resulting AP sub-cluster distribution shows discrepancies compared to the phylogenetic results, but is reasonable in the spatial validation. For the "New World" cluster AP Clustering showed the same tendencies to split up into three or four clusters. Unfortunately, because of the limited metadata the results cannot be resolved spatially thus cannot be validated.
Conclusion
The AP Clustering method was applied to validate the presence of spatially-dependent sub-clusters of the four predefined full genome RABV main clusters. In total, we defined aprox.12 new and uniquely distinguishable sub-clusters. The application of AP clustering, phylogenetic analyses and the combination of both revealed concordant results for RABV sub-cluster demarcation.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

266/MSP

Emergence of carbapenem-resistant *Enterobacteriaceae* in chickens and fish raised in close contact

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Carbapenems (CAR) are broad-spectrum b-lactam antibiotics of critical importance in human medicine. They are considered as the last-line therapy against multidrug-resistant Gram-negative bacteria. However, the efficacy of CAR is threatened worldwide by the emergence of CAR-resistant bacteria. We have previously found a high prevalence of CAR-resistant *Klebsiella pneumoniae* in chicken farms. Therefore, the aim of the present study is to examine the occurrence of carbapenem-resistant *Enterobacteriaceae* in fish raised in an integrated system with chickens. Samples were collected from the chickens (n =45), fish (n=45), fish ponds (n=15), and humans (n=30) working at these farms. A high number of *Enterobacteriaceae* was isolated from the chickens (n=28), fish (n=27), fish ponds (n=10), and the workers (n=30). The isolates were examined for susceptibility to CAR using disk diffusion test which showed that rate of resistance is higher in fish (n=21, 77%) and fish ponds (n= 6, 60%) than that of chickens (n=6, 25%) and humans (n=15, 50%). The CAR resistant isolates were subjected to PCR for identification of the presence of blaKPC, blaNDM, and blaOXA-48 resistance determinants. The 21 CAR-resistant fish isolates carried blaKPC either alone (n=3) or with blaNDM, and blaOXA-48 (n=18). Similarly, the 6 CAR-resistant isolates from the fish ponds carried blaKPC alone (n=3) or with blaNDM, and blaOXA-48 (n=3). Out of the 6 CAR-resistant isolates from chickens, 4 were blaKPC and blaOXA-48 positive and 2 were blaKPC, blaNDM, and blaOXA-48. Furthermore, among the 15 human CAR-resistant isolates, 12 carried blaKPC, blaNDM, and blaOXA-48, and 2 harboured blaKPC. In conclusion, carbapenemase-producing *Enterobacteriaceae* occurred at relatively high frequency among fish, fish ponds and workers at fish farms integrated with chickens. However, the chickens showed CAR resistance, the rate was lower than that in fish, suggesting that resistance in fish is not attributed to that in chickens. Further studies are needed to understand the source of resistance found in fish and the fish ponds

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

Reference and Consutling Laboratories (StAG RK)

267/RKP

Combination of microbiome analysis and serodiagnostics to assess the risk of pathogen transmission by ticks to humans and animals in central Germany

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Question: Arthropod-borne diseases remain a remarkable health-threat for humans and animals worldwide. To estimate the distribution of pathogenic agents and especially *Bartonella* spp., we conducted tick microbiome analysis and determination of the

infection status of wild animals, pets and pet owners in the state of Hesse, Germany.
Methods: In total, 189 ticks collected from 163 animals were tested. Selected ticks were analyzed by next generation sequencing (NGS) and confirmatory PCRs, blood specimen of 48 wild animals were analyzed by PCR to confirm pathogen presence and sera of 54 dogs, one cat and 11 dog owners were analyzed by serology.
Results: Bartonella spp. were detected in 9.5% of all ticks and in the blood of 17 roe deer. Further data reveal the presence of the human and animal pathogenic genera *Spirochetaceae* (including *Borrelia miyamotoi* and *Borrelia garinii*), *Bartonella* spp. (mainly *Bartonella schoenbuchensis*), *Rickettsia helvetica*, *Francisella tularensis* and *Anaplasma phagocytophilum* in ticks. Co-infections with several genera were detected in nine ticks. One dog and five dog owners were seropositive for anti-*Bartonella henselae*-antibodies and one dog had antibodies against *Rickettsia conorii.*
Conclusions: This study shows the current image of pathogens circulating in ticks in central Germany. A broad range of tick-borne pathogens are present in ticks and animals, especially wild animals with possible implications for animal and human health. However, a low incidence of *Bartonella* spp. especially *Bartonella henselae* was detected. Ticks might serve as an excellent sentinel to detect and monitor circulating pathogens.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

268/RKP
Microbiome analysis reveals the presence of *Bartonella* spp. *and Acinetobacter* spp. in deer keds (*Lipoptena cervi*)
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Question: The deer ked (*Lipoptena cervi*) is distributed in Europe, North America and Siberia and mainly infests cervids as roe deer, fallow deer and moose. From a one health perspective, deer keds occasionally bite other animals or humans and are a potential vectors for *Bartonella schoenbuchensis.* This bacterium belongs to a lineage of ruminant-associated *Bartonella* spp. and is suspected to cause dermatitis and febrile diseases in humans.
Methods: In this study, we analyzed the microbiome from 130 deer keds collected from roe deer, fallow deer and humans in the federal states of Hesse, Baden-Wuerttemberg and Brandenburg, Germany and confirmed the results by conventional PCR methods.
Results: Endosymbiontic *Arsenophonus* spp. and *Bartonella* spp. represented the biggest portion (~90%) of the microbiome. Most *Bartonella* spp. (n=93) were confirmed to represent *B. schoenbuchensis*. Furthermore, *Acinetobacter* spp. were present in four samples, one of those was confirmed to represent *A. baumannii*.
Conclusions: These data suggest that deer keds harbor only a very narrow spectrum of bacteria which are potentially pathogenic for animals of humans.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

269/RKP
Identification of a novel botulinum neurotoxin subtype (BoNT/F9) which exhibits unique catalytic properties and substrate specificities
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In the recent past, about 40 botulinum neurotoxin (BoNT) subtypes belonging to serotypes A, B, E, and F pathogenic to humans were identified among hundreds of independent isolates. BoNTs are the etiological factors of botulism and represent potential bioweapons; however, they are also recognized pharmaceuticals for the efficient counteraction of hyperactive nerve terminals in a variety of human diseases. The detailed biochemical characterization of subtypes as the basis for development of suitable countermeasures and possible novel therapeutic applications is lagging behind the increase in new subtypes. Here, we report the primary structure of a ninth subtype of BoNT/F. Its amino-acid sequence diverges by at least 8.4% at the holotoxin and 13.4% at the enzymatic domain level from all other known BoNT/F subtypes. We found that BoNT/F9 shares the scissile Q58/K59 bond in its substrate vesicle associated membrane protein 2 with the prototype BoNT/F1. Comparative biochemical analyses of four BoNT/F enzymatic domains showed that the catalytic efficiencies decrease in the order F1 > F7 > F9 > F6, and vary by up to a factor of eight. KM values increase in the order F1 > F9 > F6 » F7, whereas kcat decreases in the order F7 > F1 > F9 > F6. Comparative substrate scanning mutagenesis studies revealed a unique pattern of crucial substrate residues for each subtype. Based upon structural coordinates of F1 bound to an inhibitor polypeptide, the mutational analyses suggest different substrate interactions in the substrate binding channel of each subtype.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

270/RKP
Azithromycin – an antibiotic suitable for the post-exposure prophylaxis of *Neisseria meningitidis* in Germany
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Introduction
In most countries, post-exposure prophylaxis (PEP) is recommended to close contacts of invasive meningococcal disease (IMD) cases to avoid secondary cases by eradicating supposed meningococcal colonization. Currently, ciprofloxacin, rifampicin, and ceftriaxone are recommended in Germany by the standing committee on vaccination (STIKO). Azithromycin has been shown to eradicate *Neisseria meningitidis* colonization [1], has been used as PEP in some countries and is approved for adults as well as children and may even be used in pregnancy. Because of less toxicity and easier application, it might be an alternative to rifampicin and ciprofloxacin. It Furthermore, resistance to ciprofloxacin and rifampicin has been reported [2][3].
Aim of the study
To assess the prevalence of azithromycin resistance in invasive meningococcal isolates in Germany.
Methods
Minimal inhibitory concentrations (MIC) of invasive meningococcal strains isolated in Germany were determined using bioMérieux ETEST® gradient agar diffusion tests. We randomly selected 97 strains submitted to the NRL for Meningococci and *H. influenzae* between 2006 and 2010. As there are no published EUCAST clinical breakpoints for azithromycin in *N. meningitidis*, the CLSI breakpoint of 2 mg/l was applied for the interpretation as susceptible.
Results
The azithromycin MIC of the 97 isolates ranged between 0.094 and 1 mg/l (median: 0.19 mg/l, Q75: 0.38 mg/l). All isolates were categorized as susceptible to azithromycin.

Discussion
We identified no isolate that was resistant to azithromycin. A further series covering 2013 to 2018 will be reported in this presentation. Azithromycin might be an eligible antibiotic for PEP of IMD close contacts and is easy to handle. Of note, azithromycin resistance, which most likely originated from gonococci, has been reported in a urethritis isolate [4]. Therefore, careful monitoring of the emergence of resistant strains is also necessary for meningococci. Due to the low number of isolates tested until now, a comparison to resistance levels found for ciprofloxacin (3/1648 from 2006 to 2010) and rifampicin (1/1648) is not yet possible.

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Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

271/RKP

Hedgehogs as a new reservoir of zoonotic diphtheria toxin bearing *C. ulcerans* strains in Germany

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Question *Corynebacterium (C.) ulcerans* is an emerging zoonotic pathogen in several industrial countries. Toxigenic strains may cause wound infections and classical respiratory diphtheria in humans. About 50 years ago *C. ulcerans*-caused infections were associated with consumption of dairy products or contact to cattle. Today companion animals are recognized as an important reservoir with documented transmission to humans. Meanwhile *C. ulcerans* has been isolated from a wide variety of animal species. Here we report on the unusual finding of 4 toxigenic *C. ulcerans* strains in 4 hedgehogs with cutaneous diphtheria and pneumonia, one of them with previous contact to humans.

Methods Strain identification was performed by biochemical differentiation, MALDI-TOF analysis, Fourier-transform infrared spectroscopy and cluster analysis. Toxigenicity was verified by real-time PCR and a modified Elek test. Susceptibility testing was performed according to the CLSI and EUCAST guidelines. Multilocus sequence typing based on seven housekeeping loci was done using the NGS-data and the respective MLST database (http://pubmlst.org/cdiphtheriae/).

Results Lung, heart and soft tissue materials obtained from the hedgehogs grew 4 *tox*+ *C. ulcerans* strains, 3 of them were toxigenic tox-bearing strains. All isolates were resistant against penicillin and clindamycin, but susceptible against erythromycin, cephalosporins and sulfonamides. NGS-derived MLST revealed 3 different sequence types. Phylogenetic minimum spanning trees, built from cgMLST results of NGS data showed that the genetic similarity of the 4 *C. ulcerans* isolates from hedgehogs was much lower compared to the German NTTB wildlife cluster than to human samples from different geographic regions.

Conclusions The observation of toxigenic *C. ulcerans* in hedgehogs, a species known to reside in urban and suburban environments in close proximity to humans, highlights potential transmission risks and should raise the public health awareness towards zoonotic infections. The analyzed dataset indicates closer genetic similarity of the hedgehog-derived isolates to human isolates than to those from wild animals, although no close relationships between the hedgehog isolates to any other isolate was detected.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

272/RKP

Borreliella? Borreliophila? or simply Borrelia? Diagnostic microbiology in the taxonomic jungle

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In recent years taxonomic reclassifications have been made for several bacterial families and genera including the genus *Borrelia*. Many of these reclassifications were based on comparison of Average Nucleotide Indices (ANI) or Average Amino acid Indices (AAI), the presence of insertions and deletions and phylogenetic clustering of species. The genus *Borrelia*, originally described by Swellengrebel in 1907, contains tick- or louse-transmitted spirochetes belonging to the relapsing fever (RF) group of spirochetes, the Lyme borreliosis (LB) group of spirochetes and spirochetes that form intermittent clades.

In 2014 it was proposed that the genus *Borrelia* should be separated into two genera; *Borrelia* Swellengrebel 1907 emend. Adeolu and Gupta 2014 containing RF spirochetes and *Borreliella* Adeolu and Gupta 2014 containing LB group of spirochetes.

In this study we conducted an analysis based on a method that is suitable for bacterial genus demarcation, the percentage of conserved proteins (POCP). We included RF group species, LB group species and two species belonging to intermittent clades, *Borrelia turcica* Güner et al. 2004 and *Candidatus* Borrelia tachyglossi Loh et al. 2017. These analyses convincingly showed that all groups of spirochetes belong into one genus and we propose to emend, and re-unite all groups in the genus *Borrelia*.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

273/RKP

First invasive meningococcal isolate with beta-lactamase in Germany

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Introduction
Antimicrobial resistance is rare in invasive meningococci. Nevertheless, reduced susceptibility towards penicillin increased in the last decade (1,2). It is based on mutations in the chromosomally encoded penicillin-binding protein 2 (PBP2). In contrast to gonococci only few meningococcal strains harbouring plasmid-encoded beta-lactamases have been reported (3). Mostly, these plasmids were identical to gonococcal plasmids. Newer reports demonstrate chromosomal integration of a ROB-1 beta-lactamase gene in serogroup Y meningococci of sequence type (ST) 3587 (4,5).
Objective
Description of the first German invasive meningococcal isolate with a beta-lactamase
Methods
Antimicrobial susceptibility was estimated by gradient agar diffusion. Beta-lactamase production was detected with nitrocefin disks. Standard sequence typing procedures were applied.
Results
In March 2017, an invasive meningococcal isolate from a 10-year-old girl suffering from Waterhouse-Friderichensen syndrome was submitted to the reference laboratory. The strain was finetyped as

Y:P1.5-2,10-2:F4-1:ST-3587 (serogroup:PorA-VR1,VR2:FetA:ST). Susceptibility testing revealed a penicillin MIC of 12 µg/ml. Beta-lactamase production was positive. A ROB-1 beta-lactamase gene was identified. The isolate was susceptible to cefotaxime.

Discussion

This is the first beta-lactamase positive meningococcal isolate identified at the reference laboratory. It´s MIC is dramatically higher than those based on mutation in the PBP2 (max. 1 µg/ml). Similar isolates were recently found in France and Canada, respectively (4,5). Whole genome sequencing revealed a chromosomal located ROB-1 beta-lactamase gene with a high homology to that on the Haemophilus influenzae plasmid pB1000. A genome sequence of the German isolate will be obtained to analyse clonal identity to the French and Canadian isolates.

Although disease caused by the strain is obviously rare, careful surveillance of a spread of ROB1-positive strains is needed. Due to the yet sporadic nature of the observation and the widely used empiric cephalosporin therapy of invasive meningococcal disease, safety concerns regarding current therapeutic regimens do not exist.

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Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

274/RKP

Laboratory surveillance report: invasive *H. influenzae* in Germany 2009-2017

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Introduction

Vaccination against *H. influenzae* (Hi) serotype b (Hib) has reduced the incidence of invasive Hi infections significantly. However, case numbers are steadily increasing again. The role of non-typeable *H. influenzae* (NTHi) for the rising incidence of invasive Hi infections especially in elderly patients is well documented for all countries with a general Hib vaccination programme.

Objectives

To provide an update on the laboratory surveillance for invasive Hi infections 2009-2017 conducted by the NRL for meningococci and Hi.

Materials and Methods

Hi was confirmed in strains submitted to the NRL on a voluntary basis. The strains were serotyped phenotypically and by PCR. Antibiotic resistance to ampicillin was tested using gradient agar diffusion test and β-lactamase tests.

Results

From 2009 to 2017, about 3400 submissions were processed including bacterial isolates from 3270 patients from Germany with invasive infections. The NRZMHi analysed 3229 viable Hi isolates. Among these isolates, 2539 derived from blood, 197 from cerebrospinal fluid (CSF) only. We received Hi both from blood and CSF from 30 patients. The number of processed cases rose from 109 in 2009, over 284 in 2013 to 586 in 2017, reflecting a steady increase.

The majority of blood or CSF isolates were NTHi (2291/3229; 70.1 %), followed by Hif as the most frequent capsular serotype (n=321;9.9%). Hib (n=77; 2.4%) and Hie (n=68;2.1%) showed third highest frequency among the serotypes. Of the rare serotypes, eight Hia (0.2%), and no Hic or Hid were isolated. The age group most affected was > 40 years (71.5% of all cases). The percentage of patients aged ≥ 65 years was 54.5%. In addition, a significant proportion of cases (n=252; 7.8%) was found in children aged

Four hundred and seventy-eight isolates (14.8 %) were ampicillin resistant (MIC > 1 µg/ml), of which 339 (70.9 %) showed β-lactamase production. Thus, 29.1% were classified as β-lactamase negative ampicillin resistant isolates (BLNAR).

Conclusions

Invasive infections caused by Hi in Germany reflect the typical epidemiology in the post-Hib era. Whereas most infections are found in elderly patients due to NTHi, invasive infections in children, especially neonates, presumably to vaginal carriage by the mothers, need further attention. Likewise, rising antibiotic resistance rates, even though still at a moderate level, warrant close monitoring.

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275/RKP

Colonization by Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumonia, and Staphylococcus aureus in healthy elderly people

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Introduction

Individuals older than 65 years represent an increasingly important part of the population in Western countries. Besides other susceptible groups, such as neonates or immunocompromised patients, elderly people are at risk for invasive infections. The bacteria *H. influenzae*, meningococci, and pneumococci as well as *S. aureus* are important causative agents with mortality rates being highest in elderly individuals. Asymptomatic carriage is the epidemiological basis of invasive bacterial infections. Whereas surveillance is in place to monitor infections due to *H. influenzae*, meningococci, and pneumococci as well as of MRSA in the elderly, there is a remarkable lack of data on colonization rates in elderly people.

Objectives

This study aimed at providing prevalence data for *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumonia*, and *Staphylococcus aureus* in asymptomatic elderly people. Risk factors associated with colonization were assessed.

Materials and Methods

The multi-centre cross-sectional study conducted in two German regions included 677 adults aged ≥ 65 years that were free of infectious diseases. The volunteers were independently living or from nursing homes. *N. meningitidis*- and *H. influenzae*-carriage was investigated in 474 individuals. Nasal and oropharyngeal swabs as well as questionnaires were collected from October 2012 to May 2013 and analysed statistically using multiple logistic regression models.

Results

Colonization by *H. influenzae* was found in 2.5% ([95%CI: 1.3-4.4%]; 12/474). Only two individuals showed *N. meningitidis* carriage (0.4% [95% CI: 0.1-1.5%]). No pneumococci were detected (0% [95% CI: 0-0.5%]). However, 28.5% of the individuals ([95% CI: 25.1-32.1%]; 193/677) were *S. aureus* carriers, and 0.7% ([95% CI: 0.02-1.7%]; 5/677) were positive for methicillin-resistant *S. aureus*. In subjects living independently, colonization with *S. aureus* was significantly associated with higher educational level (adjusted OR: 1.905 [95% CI: 1.248-2.908]; p = 0.003). Among nursing home residents, colonization was associated with being married (adjusted OR: 3.367 [1.502-7.546]; p = 0.003). No other factors associated with *S. aureus* carriage were identified.

Summary

The prevalence of *N. meningitidis*, *H. influenzae*, and *S. pneumoniae* was low among older people in Germany. The *S. aureus* carriage rate was consistent with data from previous studies.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

Zoonoses (FG ZO)

276/ZOP

Zoonotic Parasites in Wild Animals in Germany – First Studies in Brandenburg

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Question:
Toxoplasma gondii, Alaria alata and *Cryptosporidium* spp. are parasites with zoonotic potential that are prevalent in wildlife. To estimate the public health risk emanating from these three different parasites in game, more data on their presence in wild animals in Germany is needed. In this study the prevalence of *Alaria alata, Cryptosporidium* spp., and *Toxoplasma gondii* in wild animals in Brandenburg, Germany was assessed.
Methods:
During the hunting season 2017/2018, driven hunts were organized in eight different hunting grounds in Brandenburg (Germany) in cooperation with the Frankenförder Forschungsgesellschaft and the German Bundesforst. In the framework of these hunting events, wild boars, roe deer and red deer were sampled. For the direct detection of the three parasites, samples of faeces, tongue, abdominal fat and muscle tissue of diaphragm, foreleg, masseter muscles and myocardium were examined. For serological analysis, blood samples were taken from the abdominal cavity. Direct detection of *T. gondii* and *Cryptosporidium* spp. was conducted using molecular methods, while *Alaria alata* was detected using the *Alaria* spp. mesocercariae migration technique (AMT).
Results:
Serological examination of 194 serum samples of roe deer, red deer and wild boar revealed *T. gondii*-specific antibodies in 3.7-23.3% of the samples. Mesocercariae of *A. alata* could be found in 27% of 171 tested wild boars. 18SrRNA of *Cryptosporidium* spp. could be detected in 25.5% of 235 feces samples by nested polymerase chain reaction.
Conclusions:
These results indicate a high exposure of game to *Toxoplasma gondii, Cryptosporidium spp.*, and *Alaria alata* in the monitored hunting areas in Brandenburg and a possible transmission route of these parasites to humans.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Real-time interaction analysis of Shiga toxin with *lipid raft*-associated glycosphingolipids of human brain endothelial cells

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Introduction: Infections of enterohemorrhagic *Escherichia coli* (EHEC), releasing Shiga toxins (Stxs) as their main virulence factors, result in the worst case in the development of hemolytic-uremic syndrome (HUS). Stx preferentially damages endothelial cells of human kidney and brain. The B-subunits of the AB5 toxin preferably bind to the glycosphingolipid (GSL) receptor globotriaosylceramide (Gb3Cer) and to less extent to globotetraosylceramide (Gb4Cer). Both GSLs are common constituents of the plasma membrane of human brain microvascular endothelial cells (HBMECs) [1], where they are believed to cluster in *lipid rafts*. However, the precise mechanisms underlying the initial attachment of Stx to the membrane surface is still poorly understood.
Objective: We aimed at producing *lipid raft*-analogous detergent-resistant membranes (DRMs) from HBMECs, identifying *lipid raft*-associated Stx GSL receptors, and performing Stx-GSL-interaction analysis with DRMs in real time.
Materials and Methods: DRMs were prepared from HBMECs by solubilization of the cells in Triton buffer. DRM and nonDRM fractions were obtained from discontinuous saccharose gradients upon ultracentrifugation. The Stx receptors were determined in GSL preparations of the gradient fractions using thin-layer chromatography (TLC) overlay immunostaining. The various Gb3Cer and Gb4Cer lipoforms were structurally characterized by means of electrospray ionization mass spectrometry (ESI MS). Label-free real-time interaction measurements of Stx with DRMs were recorded with a surface acoustic wave (SAW) biosensor.
Results: TLC immunostaining using anti-Gb3Cer and anti-Gb4Cer antibodies revealed accumulation of Gb3Cer and Gb4Cer in DRM *versus* nonDRM fractions indicating their association with *lipid rafts*. Gb3Cer and Gb4Cer lipoforms with saturated fatty acids dominated in the DRMs, whereas GSLs with unsaturated fatty acids prevailed in the nonDRM fractions. Real-time interaction analyses evidenced attachment of Stx to DRMs in contrast to nonDRMs, which gave negative binding results. SAW binding kinetics allowed for calculation of the binding strength between Stx and *lipid raft*-associated Stx receptors in the DRM preparations.
Conclusion: In this study we could show specific binding of Stx with HBMEC-derived DRMs suggesting *lipid raft* association of the Stx receptors Gb3Cer and Gb4Cer.
[1] Legros N, Pohlentz G, Steil D, Müthing J. (2018). *Int. J. Med. Microbiol*., pii: S1438-4221(18)30362-X.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Epidemiology of *Leptospira* spp. in Germany

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Background: In 2007 and 2014 leptospirosis outbreaks were reported among strawberry harvesters in Germany with *L. kirschneri* infected voles as the most likely outbreak source (Desai et al. Clin Infect Dis 2009; 48 691-697, Dreesman et al. Gesundheitswesen 2016; 78). In the One Health approach of the RoBoPub consortium, *Leptospira* spp. will be investigated together with hantaviruses to understand the epidemiology in one comprehensive and synergistic study. In this joint project the German Federal Institute for Risk Assessment will investigate the prevalence of pathogenic *Leptospira* spp. in rodent populations in Germany. Additionally, seroprevalence of human leptospirosis will be assessed in different risk populations. The sensitivity and specificity of commercial kits for the rapid and robust diagnosis of leptospirosis in comparison to an "in house" ELISA will be evaluated. Furthermore, studies on the tenacity of *Leptospira* spp. will be performed and are. Here, the results of the survival studies on *Leptospira* spp. on strawberries are presented.
Materials and methods: Strawberries were spiked with *L. kirschneri* serovar Grippotyphosa strain Moskvain and were subsequently exposed to four temperatures over different incubation periods. After washing the strawberries, the recovery and viability of *Leptospira* in the washing suspension was examined using dark field microscopy as well as bacterial culture. Leptospiral growth was examined each week over one month.
Results: *L. kirschneri* was able to survive for 2 hours on strawberries at a temperature of 25°C. After incubation over 4 hours at 21°C and 25°C, viable leptospires could be detected in >50% of the replicate experiments. Viable *Leptospira* were examined <50% of all temperatures for the incubation time 6h. All cultures were negative at an incubation time of 8 hours irrespective of the temperature.
Conclusion: The obtained data indicates that *L. kirschneri* serovar Grippotyphosa is able to survive on strawberries for up to 6 hours under supporting temperature (>21°) and moisture conditions. The generated survival data as well as further data on seroprevalences in risk groups and occurrence of *Leptospira* strains in rodent reservoirs will support future microbial risk assessments on the occurrence leptospirosis in the German population.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.

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Monitoring of zoonotic pathogens in wildlife in Brandenburg, Germany

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Question:

As game represents an important reservoir and plays a key role in the epidemiology of many zoonoses (*Kruse et al.*, 2004), the investigation of wildlife is an important step to fully assess and evaluate the public health risk emanating from certain zoonotic pathogens. With wildlife population and game meat consumption on the rise, monitoring zoonotic pathogens in wildlife is of high relevance.

Thus, in cooperation with the German Bundesforst, the German Federal Institute for Risk Assessment will conduct a monitoring study on zoonotic pathogens in wildlife.

Methods:

In this study, prevalence of parasitic, bacterial and viral pathogens, specifically *Toxoplasma gondii*, *Alaria alata*, *Cryptosporidium* spp., *Campylobacter* spp., *Yersinia* spp., rotavirus and hepatitis-e-virus, will be investigated.

Samples will be taken from wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), foxes (*Vulpes vulpes*), raccoons (*Procyon lotor*) and raccoon dogs (*Nyctereutes procyonoides*) collected during the hunting season of 2018/2019 in different areas (Havel Oder Spree and Westbrandenburg) in the federal state of Brandenburg, Germany.

Blood, heart, tongue, larynx, diaphragm, masseter muscle, liver, tonsils, and feces of the aforementioned pathogens will be tested using specific direct and indirect methods (e.g. bacterial culture, PCR, qPCR, ELISA, Spot-Test).

Results:

Based on previous and estimated prevalences of the described pathogens as well as on data provided by the German Bundesforst, a sampling scheme was proposed and agreed among the stakeholders. Hunting participants were trained in sampling techniques and leaflets with detailed information were distributed among hunters to ensure proper handling of samples. A more detailed study design and preliminary results will be presented.

Conclusion:

This study will give new insights into the prevalence of various zoonotic pathogens in wildlife and will deepen the understanding of their epidemiology. Monitoring generates baseline information that is essential for the risk assessment of zoonotic pathogens.

Kruse, H., Kirkemo, A.-M., & Handeland, K. (2004). Wildlife as Source of Zoonotic Infections. *Emerging Infectious Diseases*, *10*(12), 2067–2072.

Presentation on Tuesday, February 26, 2019 from 17:00 – 18:30, upper floor.