Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

DVP01

"Inactivated mycobacteria bead preparation" method for identification of mycobacteria by MALDI-TOF MS

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Introduction: Next to the clinical important *Mycobacterium tuberculosis* complex (MTC) Nontuberculosis mycobacteria (NTM) are pathogens especially in immunocompromised and senior people. Species identification is important for diagnosis and optimal treatment. MALDI-TOF identification of some *Mycobacterium* strains is hampered by low quality of acquired mass spectra from samples processed by standard procedures. Therefore, a new preparation method for Mycobacteria was established and influence of an additional preceding heat inactivation step was evaluated.
Methods: 14 *Mycobacterium* strains were prepared using standard ethanol/formic acid extraction procedure. The new established Mycobacteria bead preparation protocol was tested with these 14 and additional 22 strains. An extended protocol named inactivated Mycobacteria bead preparation method was tested with 10 strains. Mass spectra were recorded in a microflex LT MALDI-TOF mass spectrometer and identification was done applying MALDI Biotyper 3.0 software (Bruker Daltonik, Germany).
Results: Out of 83 *Mycobacterium* spp. strains tested (data not shown) 14 strains were hard to analyse by MALDI-TOF and therefore selected for this study. Consequently, standard ethanol/formic acid extraction procedure failed to acquire mass spectra from 12 strains. A significant improvement of quality of mass spectra was achieved by using Mycobacteria bead preparation method with glass beads for cell suspension and disruption. Now, 11 out of 14 *Mycobacterium* strains resulted in good quality mass spectra and three ones in average quality. In addition, 22 further *Mycobacterium* strains were processed according to this new protocol and all resulted in good quality spectra.
An additional heat inactivation step was introduced for safety reasons. Mass spectra of 10 *Mycobacterium* spp. strains processed by Mycobacteria bead preparation method with and without heat inactivation step were compared. Mass spectra revealed only minor differences, just higher peak intensities were obtained in general with the heat inactivation procedure.
Conclusion: A fast, secure and easy to perform method was developed for mycobacterial protein extraction. These extracts are highly accessible to MALDI-TOF analysis and good quality mass spectra are a precondition for identification of Mycobacteria by this technique. The new preparation method was successfully tested for 36 different strains representing 30 species. A supplemental heat inactivation step was introduced for minimizing hazards by handling living MTC cells.

DVP02

MALDI-TOF MS for functional detection of ß-lactam resistance of positive blood cultures

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Background: The main mechanism of bacterial resistance against ß-lactam antibiotics is the expression/overexpression of ß-lactamases which destroy the ß-lactam ring of ß-lactam antibiotics by hydrolysis. This reaction results in a mass shift of plus 18 Da easily detectable by mass spectrometry. A rapid MALDI-TOF MS based assay was set up to detect ß-lactam resistance of bacteria from blood cultures.
Methods: Blood culture bottles were inoculated with different *E. coli* and *Klebsiella pneumonia* strains and incubated at 37°C until they were indicated as positive. Subsequently, 1 ml fresh positive blood culture was processed according to a slightly modified protocol of the MALDI Sepsityper kit. The washing step was repeated once and subsequently bacteria were incubated with the corresponding antibiotics solution for 3 h at 37°C under agitation. Ampicillin was tested with the *E. coli* strains. Ertapenem was tested with the *Klebsiella pneumonia* strains. After incubation of the bacteria with the corresponding antibiotics and subsequent centrifugation, the supernatant was directly applied to a MALDI sample carrier. Dried spots were overlaid with MALDI matrix (HCCA). MALDI-TOF MS spectra were acquired on a microflex LT benchtop mass spectrometer. Inhibition of hydrolysis was performed in the presence respective ß-lactamase inhibitors.
Results: The MS spectrum corresponding to DH5alpha revealed the molecular peak of Ampicillin (350 Da) and the respective sodium adducts. The spectra derived from the ß-lactamase producing strains show peaks at 368, 394, 412 and 324 Da corresponding to the hydrolyzed form, its sodium adducts and the hydrolyzed, decarboxylated form of ampicillin, respectively. A slight spontaneous hydrolysis was also observed in the spectra of DH5alpha. Successful inhibition of Ampicillin hydrolysis was demonstrated by incubation in the presence of calvulanic acid.
Incubation of Ertapenem with *Klebsiella pneumonia* strains revealed a clear difference between the carbapenem sensitive strain and carbapenamase positive strains. For the carbapenem sensitive strain the molecular peak of Ertapenem $[M+H]^+$ at 476 Da was clearly detectable. Incubation of Ertapenem with the carbapenemase positive strains resulted in the disappearance of the molecular peak of Ertapenem. In contrast to Ampicillin, the hydrolyzed form of Ertapenem was very instable and immediately broke down further. Therefore, only the decrease of the molecular peak of Ertapenem can be employed as indicator for resistance against carbapenems at the moment. Additional peaks appear in the spectra of the carbapenemase positive strains but these do not directly correspond to the hydrolyzed form of Ertapenem and have not been identified so far.
Discussion: The developed approach provides a rapid method for the detection of beta-lactamase activity of bacteria within less than 4 h from positive blood cultures.

DVP03

Molecular typing of *Stenotrophomonas maltophilia* from patients with cystic fibrosis by repetitive sequence-based PCR

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Objective: The purpose of this study was to investigate the genotype distribution of *S. maltophilia* isolates from patients with cystic fibrosis (CF) by repetitive sequence-based PCR (rep-PCR) using DiversiLab System.
Methods: Sputum samples (n=623) from 165 CF patients were inoculated onto a selective medium for improved isolation of *S. maltophilia* over a period of two years. Suspected isolates were identified by conventional standardized methods, as well as by sequencing. A total of 65 *S. maltophilia* isolates from 33 CF patients were detected. For genotyping, DNA extraction was performed with the UltraCleanTM microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA), according to the manufacture´s instructions. DiversiLab bacterial DNA fingerprinting kit (bioMérieux, Marcy I´Etoile, France) was used for typing following the protocol of the manufacturer. The relatedness was determined by cluster analysis using Pearson´s Correlation coefficient. Isolates with a similarity of ≥ 95% were considered as clinical related, and isolates with a similarity >98% were considered as indistinguishable.
Results: Rep-PCR demonstrated that *S. maltophilia* isolates from CF patients exhibited a high rate of genetic diversity. A small proportion of isolates were clonally related (12%), suggesting either cross-transmissions or a common source of exposure.
Conclusions: *S. maltophilia* from CF patients showed a wide variation of the molecular profiles. Further epidemiological studies such as source tracking are required to elucidate the route of colonization/infection in CF patients.

DVP04

Photochemical inactivation allows papid diagnostics of Alpha- and Poxviruses

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The objective of this study was to determine whether photochemical inactivation of viruses could be accomplished with high efficiency while preserving the molecular integrity of viral targets allowing subsequent diagnostic tests to be performed at a lower level of containment and cost. We studied the effect of either 5-Iodonaphthyl 1-azide (INA) and amotosalen (AMO, also known as S-59), that are known to target either viral proteins or viral nucleic acids. We found that Vaccinia virus (VacV), an orthopox virus with a DNA genome, and Pixuna virus (PIXV), an alphavirus with an RNA genome, were stable when irradiated with UVA alone or when exposed to either INA or AMO in the dark. AMO followed by UVA exposure was at least one-thousand-fold more virucidal than INA/UVA on VacV and on PIXV treated under similar conditions. Photoinactivation with either INA or AMO at conditions that abolished viral infectivity resulted only in minimal impairment of subsequent ELISA and PCR testing. The presented data could assist in developing methods to inactivate in the field environmental and forensic samples suspected of contamination with high virulence virus, thus limiting the need for costly security and safety operations after an accidental or intentional viral release.

DVP05

Rapid detection and molecular differentiation of toxigenic *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* strains by LightCycler PCR

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The systemic symptoms of diphtheria are caused by the *tox*-encoded diphtheria toxin (DT) which is produced by toxigenic *Corynebacterium* spp. In recent years, the emerging zoonotic pathogen *C. ulcerans* has replaced *C. diphtheriae* as the major agent for diphtheria in several industrialized Western countries. The reliable detection of toxigenic *Corynebacterium* spp. is substantial for both diphtheria surveillance in the public health sector and the clinical work-up of a patient with diphtheria-like symptoms. Since the respective *tox* genes of *C. diphtheriae* and *C. ulcerans* differ in both DNA and amino acid sequence from each other, both *tox* genes should be covered by novel real-time PCR methods. We describe the development and validation of a LightCycler PCR assay which reliably recognizes *tox* genes from both *C. diphtheriae* and *C. ulcerans* and differentiates the respective target genes by fluorescence resonance energy transfer (FRET) hybridization probe melting curve analysis.

DVP06

Use of recombinant proteins for optimized serodiagnosis of M. pneumoniae infections

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Mycoplasma pneumoniae (M.p.) is the third most frequent pathogen responsible for community-acquired pneumonia (CAP). Infections are most prevalent in children and in young adults showing epidemic peaks every 3 to 7 years. M.p. infections are often diagnosed by PCR but mainly by serology in clinical routine laboratories.
In this study, we evaluated more than 35 recombinant M.p.-specific proteins to develop a serological line-immunoassay (Line). Out of these, seven proteins proved to be useful in the detection of specific IgG, IgA and IgM antibodies. In addition, the I-protein of human erythrocytes, an autoantigen, which induces cold agglutinins of the IgM-type in 50 to 70% of acute M.p. infections was included. Furthermore, the integration of genotype-specific and conserved regions of the main P1 adhesin enables us to differentiate between infections with the two most common M.p. subtypes 1 and 2 and to estimate the prevalence for specific IgG.
To establish the Line, we tested 200 sera (46 sera with possible M.p. infection, 52 blood donors, 38 Bordetella pertussis or Legionella pneumophila positive sera, 37 sera of patients with acute non-respiratory infections and 27 sera of children; all sera were pretested by ELISA and Western Blot using the whole M.p. antigen preparation) in the Line. When compared to a consensus result of ELISA and Western Blot the following sensitivities and specificities were found: 93%/98% (IgG), 100%/93% (IgA) and 98%/94% (IgM). In addition, we determined a M.p.-specific IgG-prevalence of 53% for our sera panel.
A positive PCR result as reference test for confirmation of M.p. infection is well established. Therefore, we analysed 70 sera from the network for community-acquired pneumonia (CAPNET) with positive M.p. PCR result in the corresponding respiratory specimen. Furthermore, 30 sera of M.p. PCR-negative healthy persons and 30 sera from patients suffering from CAP caused by other agents than M.p. were analysed for sensitivity and specificity. In reference to the PCR results the Line achieved sensitivities for IgG, IgA and IgM of 53, 65 and 83%, respectively. When accumulating the results for IgA and IgM and for IgG, IgA and IgM, sensitivities of 91 and 92% were obtained. The results demonstrate the practical usefulness of a new line blot based on selected proteins as a highly specific and sensitive tool for serodiagnosis of M.p. infections.

DVP07

MALDI-TOF-MS analysis of bacteria difficult to identify by standard procedures

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Background: The standard procedures for the identification of bacteria are based on the detection of distinct metabolic properties (biochemical test systems such as API®, VITEK®). In addition, gas chromatographic determination of species-specific patterns of long-chain fatty acids is used in some laboratories.
Bacteria isolated from tissues and fluids of patients under antibiotic treatment and, specifically, from the viscous bronchial mucus in the respiratory tract of patients with cystic fibrosis often have changed their characteristic metabolic properties and therefore elude the identification by conventional procedures.
We examined whether the analysis of bacterial protein patterns by MALDI-TOF-MS (Matrix-assisted laser desorption/ionization time of flight mass spectrometry) results in an unequivocal identification in those cases.
Methods: Isolates obtained from tissues and fluids of patients in the clinical routine which could not be unambiguously identified by API® or VITEK® or by gas chromatography, respectively, were analyzed by MALDI-TOF-MS.
A small number of bacterial cells from the culture plate was directly deposited onto a sample plate. The bacteria were inactivated and intracellular, mostly ribosomal proteins were liberated by exposure to 0.3 µl 2.5-dihydroxybenzoic acid used as organic matrix.
The spectra of these proteins were compared with the entries of a database (SARAMIS®, AnagnosTec, Germany). The whole process of sample preparation, measurement and database matching takes only a few minutes.
Results: By using MALDI-TOF-MS analysis we could identify nearly 80 % of all isolates which previously had not given a clear result when standard identification procedures were applied. In most cases the bacteria could be identified at the species level, in some cases only at the genus level.

DVP08

Carbapenemase detection by phenotypic methods

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Background: Detection of *Enterobacteriaceae* carrying carbapenemases is challenging since the MIC can be in the susceptible range and because differentiation between carbapenem resistance due to carbapenemases or other mechanisms is not possible by routine susceptible testing.
Methods: *Enterobacteriaceae* with elevated carbapenem MICs were sent to the German National Reference Laboratory for multidrug-resistant gramnegative bacteria. For this study 80 strains were used, for which carbapenemases were excluded by a bioassay based on cell-free extracts and up to 15 specific PCRs. In addition, 80 strains with carbapenemases characterized by sequencing were studied. In all strains the MIC for carbapenems was determined by Etest, the modified Hodge-Test (MHT) was performed on different media and combined disk tests with EDTA and boronic acid were performed.
Results: The collection comprised *K. pneumoniae* (n = 82), *E. coli* (n = 24), *E. cloacae* (n = 23), *E. aerogenes* (n =12) and other *Enterobacteriacea* species. Strains with OXA-48 (n = 24), OXA-162 (n = 4), KPC-2 (n = 12), KPC-3 (n = 11), VIM-1 (n = 15), VIM-2 (n = 2), VIM-4 (n = 4), NDM-1 (n = 4) and GIM-1 (n = 3) were included.The sensitivity of the MHT on Mueller-Hinton agar (Oxoid) was 98.8%, 88.8% and 93.4% for imipenem,meropenem and ertapenem, respectively, whereas the specifity was 71.3%, 91.3% and 86.3%. For the subgroup of metallo-betalactamases (n = 27) the MHT was performed on four different agar media with the lowest sensitivity for the MHT with meropenem performed on MH agar by Merck (7.4%). Addition of 20 µl 50 mM zinc to the disk increased the sensitivity only to 30%. The sensitivity of the MBL Etest was 74.1%, whereas the combined disk test with EDTA had a sensitivity of 96.3%.
Conclusions: In contrast to recommendations by CLSI the MHT should also be performed for imipenem. Despite a lower specifity the importance of carbapenemase detection justifies a higher sensitivity. Caution should be used in choosing the test media since huge differences in metallo-betalactamase detection are seen. Due to a better sensitivity the combined disk test with EDTA should substitute the MBL Etest in *Enterobacteriaceae*.

DVP09

Direct identification of uropathogens by MALDI-TOF-MS and interfering factors

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Introduction: Urinary tract infections (UTI) are the most common nosocomial infections worldwide. Standard procedures for the identification of uropathogens are based on the cultivation on agar plates and the subsequent biochemical differentiation (e.g. with VITEK™, API™ etc.) and often require more than one day so that the initial antibiotic treatment is non-specific for the species. We developed a protocol for a fast (< 30 min) direct identification of the species of pathogens in urine samples based on matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS).
Methods: We analysed urine samples from inpatients sent to the Institute for Medical Microbiology for routine diagnostic. We separated the uropathogens from the sediment consisting of leucocytes, erythrocytes, cell detritus and protein cylinders by centrifugation and filtration protocols. The filter residue was then eluated and transferred onto the spot of a metal plate. After inactivation with 2.5-dihydroxybenzoic acid as organic matrix the protein spectra were acquired by a MALDI-TOF-mass spectrometer (Voyager-DE™ STR, AppliedBiosystems). For identification the spectra were compared with a database (SARAMIS™, AnagnosTec, now BioMérieux). The whole process was completed within 30 minutes.
Results: Direct identification of uropathogens is already successful in > 60 %. In ≈ 90 % of the unidentifiable samples a very intense peak triplet occurs corresponding to the human alpha-defensins 1, 2, and 3 as interfering factor at m/z values of around 3440 Da. These proteins are produced naturally in the body and cannot be removed simply by washing the filter residue because they closely interact with the bacterial membrane. Therefore we have to improve our protocol for purification of the separated bacteria.

DVP10

Comparison of different methods for the isolation of tick DNA

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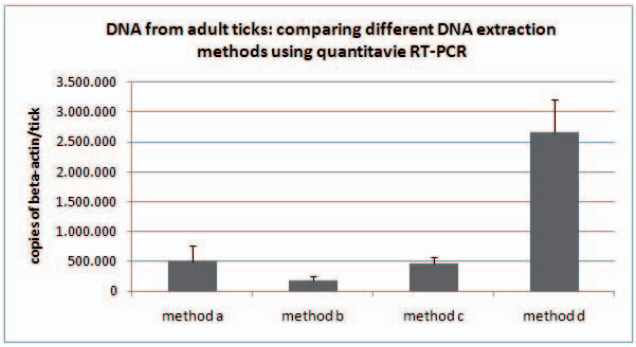
Introduction: In ticks different pathogens can be found that may cause serious infections in humans and domestic animals. For the reliable detection of these pathogens by PCR, isolation of nucleic acids is one of the most important prerequisites influencing the whole diagnostic procedure. Therefore an optimized protocol for DNA isolation from ticks is required. Since the question regarding optimal DNA extraction from ticks is only poorly addressed so far, the present study compared different methods for isolation of DNA from ticks.
Material and Methods: Four different methods for the DNA extraction from field-collected, unfed ticks were compared. DNA-quantification in all experiments was done by 1) NanoDrop-spectrometer and 2) quantitative PCR measurement of tick beta-actin. In experiment 1 DNA was isolated from each 15 single homogenized nymphs per method: a) high-salt DNA isolation protocol (PeqLab, Germany) b) MagBeads DNA extraction protocol (Carl-Roth, Germany) c) column-based DNA extraction protocol (PeqLab, Germany) and d) a modified TriPure-isolation protocol (PeqLab, Germany). Experiments a-c were done according to the manufacturer's instructions. In experiment 2 the best DNA extraction method of experiment 1 was used for an additional enrichment of DNA. Again 15 single nymphs per treatment were used, each pre-treated e) with liquid nitrogen or f) without pre-treatment prior DNA isolation. These experimental runs were combined with different additional steps of proteinase K treatment prior to experiments e and f based on differences in the treatment duration. Finally the best method of experiment 2 was used for the DNA-isolation from adult ticks in comparison to the methods a-c.
Results: In experiment 1 the highest DNA-yields were obtained by the modified TriPure DNA isolation protocol with up to 15 times higher DNA-amount ($p<0.001$). In experiment 2 the pre-treatment with proteinase k overnight combined with an additional liquid nitrogen treatment showed the highest DNA recovery ($p<0,03$). Finally these findings were transferable to the DNA isolation results from adult ticks ($p\leq0,001$) (fig. 1).
Discussion: For the detection of different pathogens in ticks a reliable and repeatable method for the DNA-isolation is an important prerequisite. Our experiments clearly show a significant different performance of various DNA extraction methods when applied to ticks. Furthermore, modifications clearly enhanced the quantity of isolated DNA. Using this optimized method, studies with a new qualitative and quantitative PCR are on the way to measure the amount of borrelia in single ticks in Germany.

Figure 1
DNA from adult ticks (n=15 per treatment) isolated by different extraction protocols: (a) high-salt DNA isolation protocol, (b) MagBeads DNA extraction protocol, (c) column-based DNA extraction protocol, and (d) modified TriPure-isolation protocol.



DVP11

Analytical comparison of the BD GenOhm Cdiff Assay to the Ridascreen *C. difficile* Toxin A/B ELISA for detection of *C. difficile* associated disease (CDAD)

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Rapid detection of toxin producing *Clostridium difficile* is essential in CDAD patients. This study compares the performance of a new PCR assay (BD GenOHM Cdiff Assay) and a conventional ELISA (Ridascreen *C. difficile* Toxin A/B) for rapid detection of toxin-producing *C. difficile* strains directly from stool.
531 stool samples obtained from 411 patients with suspected CDAD from a single centre were analysed by real-time PCR and ELISA. Only samples in accordance with the criteria for diarrheal stool (Bristol Score) were eligible for the study. Anaerobic cultures were also performed (George´s agar). The study was approved by the institutional review board.
Using the ELISA as an analytical gold standard PCR yielded 106 concordant positive results (93,8%) and 352 concordant negative results (84,2%). In 73 stool samples both assays showed discrepant results. Including culture results into the discrepant analysis, PCR resulted in 165 (31,1%) true positives and 359 (67,1%) true negatives. One false positive and 7 false negative PCR tests were observed yielding an analytical sensitivity and specificity of 99,4 respective 98,1% compared to 64,5% and 98,4% for the ELISA. Positive and negative predictive values for PCR testing were 95,9% and 99,7%, respectively.
Although, studies dealing with cost effectiveness of PCR in the management of CDAD patients are sparse, the BD GenOhm Cdiff assay (PCR) has greater analytical sensitivity than a conventional toxin ELISA maintaining comparable specificity in samples from CADA patients.

DVP12

Evaluation of Broth microdilution susceptibility testing, disk diffusion test and brilliance ESBL® Chromogenic Agar for detection of extended-spectrum, β-Lactamases-Producing *E. Coli*

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Introduction: Extended-spectrum, β-lactamases-producing (ESBL) *Enterobacteriacea* show resistance against various β-lactam-antibiotics like penicillins, cephalosporins, and aztreonam. As minimal inhibitory concentration (MIC) of these strains can be increased but below the standard CLSI-breakpoints, further confirmatory tests are required. This study compared ESBL-screenig results of MIC-determination, disk diffusion confirmatory test and growth onto a chromogenic ESBL-detection agar.
Material and Methods: Within the German Resistance Monitoring Program 1378 *E. coli* from diseased food-delivering and companion animals were collected from 2006 to 2007. MIC determination by broth microdilution to β-lactam-antibiotics (penicillin G, cefotaxime, cefoperazone, cefquinome, ceftiofur), and disk diffusion confirmatory test for ESBL (ceftazidine (30µg)/ ceftazidine-clavulanic acid (30µg/10µg) and cefotaxime (30µg)/ cefotaxime-clavulanic acid (30µg/10µg) were performed in accordance to CLSI document M31-A3. Brilliance ESBL® chromogenic agar (Oxoid) was applied corresponding manufacturer's constructions. ESBL-suspicious strains were verified by detection of $bla_{\text{CTX-M}}$-, bla_{OXA}-, bla_{TEM}-, and bla_{SHV}-genes.

Results: At broth microdilution testing, 49 *E. coli* (3.55%) exhibited MIC values of ≥2 mg/L for cefotaxime (=CLSI ESBL-screening concentration). Simultaneously performed disk diffusion test and screening onto chromogenic agar resulted in 27 (1.96%) and 26 (1.87%) ESBL-suspicious strains respectively. By PCR 26 ESBL-positive *E. coli* could be confirmed. All these strains were characterized by MIC values of ≥16 mg/L for cefotaxime, a ≥5mm increased zone diameter in disk diffusion ESBL confirmatory test and blue growth onto chromogenic ESBL-detection agar.
Conclusions: Overall, disk diffusion ESBL confirmatory test as well as chromogenic ESBL-detection agar were suitable to detect ESBL-positive *E. coli*. Both tests showed a sensitivity of 100%. The chromogenic agar further resulted in a specifity of 100%, whereas by disk diffusion test one false-positive strain was detected. However, use of disk diffusion tests is often more staff- and time-intensive.

DVP13

Outbreak of Carbapenem resistant *Klebsiella pneumoniae* in a University Hospital: Can MALDI-TOF be helpful for initial epidemiological analysis?

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Background: Enterobacteriaceae resistant to carbapenems are a growing problem in an increasing number of countries worldwide. In Germany, carbapenemase resistance is still rare, but appears to be rising, and several hospital outbreaks have been described. As many infections with carbapenem resistant strains are acquired abroad, special precautions have to be undertaken to control infection and local spread. Rapid isolate typing is a cornerstone for infection control.
Outbreak description and results: In our institution, no carbapenemase producing enterobacteriaceae have been isolated previously. In January, 2011, a Russian patient was admitted, and screening for multiresistant microorganisms revealed colonization with a *Klebsiella pneumoniae* strain of increased MIC for imipenem and meropenem. Carbapenemase production was demonstrated by modified Hodge Test and confirmed by genotyping for OXA-48 type carbapenemase. Subsequently, four additional patients were found to be colonized with *K. pneumoniae* strains of identical resistance patterns and later as OXA-48 producers. It is suspected that transmission occurred during the time span between admission and initiation of infection control measures on the index patient. Random PCR (RAPD) was attempted to prove clonal origin of the isolates, yet, in case of these *K. pneumoniae* isolates the method was not discriminative. As an alternative for rapid typing, MALDI- TOF spectra of isolates were compared yielding an excellent spectrum identity between outbreak strains and a good discrimination towards other unrelated isolates, yet, automated calculation of the dendrogram by the biotyper software was not applicable for intra-species differentiation. Results were subsequently confirmed by pulsed-field gel electrophoresis (PFGE).
Conclusion: In conjunction with or instead of other rapid typing methods (e.g. RAPD), the diagnostic use of MALDI-TOF spectra may be helpful for preliminary epidemiologic analysis of clinical outbreaks within few hours. The values and limitations of the method need to be further validated for different microorganisms by a standard procedure (e.g. PFGE, MLST), yet, ease of applicability and low running costs make mass spectra analysis a promising tool for first line clinical outbreaks analysis.

DVP14

Performance of WASP, an Automated Specimen Processor, during a State-wide Universal MRSA Screening Campaign and in routine bacteriology

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Background: Universal screening for methicillin-resistant *Staphylococcus aureus* (MRSA) is a valuable tool to estimate MRSA prevalence, yet, for a single institution, the laboratory challenges of a large campaign requiring quality-assured handling and processing of samples can be enormous. For a two-month universal MRSA admission prevalence screening project, >20.000 samples needed to be analyzed. To reduce the manual burden for processing 500-1000 additional specimens workdaily, the Walk Away Specimen Processor (WASP, Copan, Italy), a fully automated, robot-based instrument managing all steps of tube handling, vortexing, medium inoculation, and plate labelling was used. Here we report on our experience using WASP in this setting and its parallel use in processing other routine samples.
Methods: All samples were collected with eSwabs (Copan). For the MRSA project, a modified eSwab system consisting of two flocked collection swabs (for throat, and both nares, respectively) and one liquid Amies medium-containing tube was used. Samples were inoculated on chromogenic S. aureus / MRSA biplates (Mast, Germany), which were incubated at 37°C for 48h and read at 24h and 48h. Other routine samples like urines were processed according to standard procedures using different universal and selective media plates.
Results: During an initial pilot phase, 800 MRSA screening samples were plated in parallel both by WASP and manually. Initially, important differences in recovery rates between both methods appeared, with manually inoculated plates yielding about ten times elevated colony counts compared to parallel plates inoculated by WASP. Subsequently, several modifications on the WASP specimen processing protocol (various vortexing times, quantities of medium plated or streaking patterns) were carried out with support of the readily available WASP technical team both on-line and on-site. A specific optimized streaking pattern was found to be crucial for optimal colony counts. After optimizing the WASP protocol, additional testing of specimen processed either by WASP or manually now revealed that even at very low colony numbers, the sensitivity of WASP was comparable to manual plating. The readily achieved WASP connection to our LIS MLab (Dorner, Germany) further allowed for processing of a variety of different samples types at the same time. As a basis for fast and reliable work flow, streaking quality and yield of single colonies was consistently of high quality. Especially for urines, use of a one quadrant streaking pattern allowed an easy and fast semiquantitative estimation of colony count by showing a fir tree like growth pattern on agar plates.
Conclusion: Integration of WASP in the laboratory procedures both for a large universal screening program and for processing of routine samples allowed for considerable reduction of manual workload and quality-conform specimen processing.

DVP15

Prospective evaluation of commercial real-time PCR assay for *Pneumocystis jirovecii* detection in respiratory samples

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Introduction: *Pneumocystis jirovecii* pneumonia (PCP) is a relevant opportunistic disease in immunocompromised patients. Recommended diagnostic procedures involve direct microscopic examination using Giemsa, fluorescence and immuno-fluorescence stained respiratory samples. Until recently, no commercial PCR assay has been clinically validated.
Materials and Methods: We prospectively evaluated a commercial real-time PCR (MycAssay™ Pneumocystis, Mycognostica, Manchester, UK) targeted against the mitochondrial large subunit rRNA gene of *P. jirovecii* (analytical detection limit, ≤3.5 copies/µl of sample) in a single-centre tertiary care hospital with a lung-transplant program. We included all deep respiratory samples sent to us for *P. jirovecii* diagnostics from January 2011 until June 2011. Samples were stained with calcofluor-white, Giemsa, IFT (MONOFLUO™ KIT P. jirovecii, Bio-Rad, Hercules, CA, USA), and quantitative real-time PCR was performed with a defined sample volume. Patient′s charts and radiological images were reviewed for clinical signs of PCP.
Results: We included 131 respiratory samples in our trial (94.6% of which were bronchoalveolar lavage specimes [BAL]) from a total of 68 subjects with several underlying diseases: solid organ recipients (lungs and/or kidney), HIV-positive patients, and patients treated with TNFα-inhibitors for rheumatological disorders and chronic inflammatory bowel disease. Clinically, four subjects had a PCP. In all patients *P. jirovecii* was detected at least by one technique. Microscopic examination (calcofluor, Giemsa and IFT) missed 1/4 case; the real-time PCR assay was positive in a patient without clear signs of PCP infection (low copy number). BAL brush samples were a less reliable specimen for the detection of PCP.
Discussion: PCP diagnostics lacks a sensitive, specific, and quantitative tool that enables laboratories to rapidly detect relevant amounts of the opportunistic pathogen in respiratory samples. In clinical cases of PCP infection, we found a high concordance of cost and time efficient microscopic techniques and the highly sophisticated DNA extraction and PCR. Results of a quantitative PCR assay should be interpreted in dependence of clinical signs and symptoms given unknown dilution factors in bronchioalveolar samples and presence of *P. jirovecii* life forms in healthy volunteers. In addition, positive PCR results in patients without PCP may be explained by low-level infection or colonization.

DVP16

False positive candida and cryptococcal antigen detection in serum as a result of cross reactivity with different therapeutic iv solutions

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Background: Fungal antigen detection in serum is a useful diagnostic tool in cases with suspected invasive yeast infection and helpful in monitoring haematological patients e.g. after bone marrow transplantation. Positive test results usually lead to prompt therapeutic intervention. In two recent cases positive test results were attributed to cross reactivity with components of simultaneously administered infusion therapy.
Methods: Suspected cross reacting components and other iv infusion solutions were tested with CE-labeled antigen detection kits for candida, cryptococcal and aspergillus antigen in different dilution steps with antigen free serum.
Results: The following components (among others) were tested positive or equivocal for candida and/or cryptococcal antigen (all not diluted): NaCl 1 molar, CaCl 1 molar, KCl 1 molar, Glucose 5%, Glc 20%, Glc 40%, Aqua dest. All samples were negative for aspergillus antigen. After a single dilution step (1:1) with antigen free serum none of the components were cryptococcal antigen positive any more. Magnesiocard® remained candida antigen positive up to a dilution of 1:4. None of the diverse solutions were positive in culture.
Conclusion: Following the manufacturer's instruction, the test kits are suitable only for serum and not for undiluted iv solutions. However, if preanalytic standards are not observed (sample collection from infusion lines in serious ill paediatric patients to prevent repeated venous puncture , previous to collection flushing with normal saline or aqua) may result in considerable contamination of serum samples with cross reacting components. In this respect positive antigen test results should be interpreted with caution by the lab and the clinician.

DVP17

recomWell EBV test system is an effective tool for comprehensive EBV serodiagnostic

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The Epstein - Barr virus (EBV) is a virus of the herpes family, and is one of the most common viruses in humans. Most people become infected with EBV at an early age, which is often asymptomatic but may cause infectious mononucleosis. Symptoms of infectious mononucleosis are fever, sore throat, and swollen lymph glands. Although the symptoms of infectious mononucleosis usually resolve in 1 or 2 months, EBV dormant or latent in a few cells in the throat and blood for life. Periodically, the virus can reactivate and is commonly found in the saliva of infected individuals. This reactivation usually occurs without symptoms of illness.
The novel recomWell EBV -test system combined with the confirmatory assay recomLine EBV IgG (Avidity), IgM is an effective tool for comprehensive EBV serodiagnostic. The system is based on four separate ELISA (enzyme linked immunosorbent assay); recomWell EBV EBNA IgG, recomWell EBV VCA IgG, recomWell EBV EA IgG and recomWell EBV IgM. The standardized conception design of the EIA test kits enables implementation of all assays by using a single sample dilution and standard incubation times. The system performance was evaluated in four sample collections; healthy blood donors, EBV seronegative samples und samples representative of acute and subsided EBV infections.
The assays were characterized by high specificity and sensitivity. As compared with other commercially available EBV EIA test systems, the recomWell EBV tests showed considerable advantages; compared to the other assays tested, the primary EBV infections were detected in very early phase of an infection due to the high sensitivity of the recomWell EBV VCA IgG, recomWell EBV EA IgG and EBV IgM assays. Additionally, due to the high specificity of the recomWell EBNA IgG assay false positive anti-EBNA IgG findings among the acute infections were utterly excluded.
The high specificity of Anti -EBNA IgG assays is an important aspect of EBV diagnostic, as the differentation of a primary recent infection from a subsided past infection is primarily achieved through detection of anti -EBNA IgG antibodies (Screening) or measurement of the avidity of IgG antibodies (confirmation).
Finally, the results obtained here were compared with the recently published evaluation study of four commercial systems for the diagnostics of EBV primary infections (de Ory et al., 03/2011, Clinical and Vaccine Immunology, Evaluation of Four Commercial Systems for the Diagnosis of Epstein -Barr Virus Primary Infections).

Figure 1

Antibody type	Sensitivity %	Specificity %
Anti –EBV IgM antibodies		
recomWell EBV IgM (Mikrogen)	91.7	100
IF (ALLDiag, Immunoquick)	74,0*	100*
CLIA-L (DiaSorin, Liaison)	92,2*	93,3*
CLIA-I (Siemens, Immulite 2000)	77,9*	95,8*
ELISA (DiaSorin, ETI-EBV-M reverse)	67,1*	95,8*
Anti –EBV EA IgG antibodies		
recomWell EBV EA IgG	94.0	90.0
Anti -EBV VCA IgG antibodies		
recomWell EBV VCA IgG (Mikrogen)	98.6	99.4
IF (ALLDiag, Immunoquick)	79,4*	94,4*
CLIA-L (DiaSorin, Liaison)	86,9*	94,4*
CLIA-I (Siemens, Immulite 2000)	94,4*	100*
ELISA (DiaSorin, ETI-VCA-G)	89,1*	100*
Anti -EBV EBNA IgG		
recomWell EBV EBNA IgG (Mikrogen)	88.8	100
IF (ALLDiag, Immunoquick)	78,1*	91,4*
CLIA-L (DiaSorin, Liaison)	90,6*	82,3*
CLIA-I (Siemens, Immulite 2000)	93,8*	79,6*
ELISA (DiaSorin, ETI-EBNA-G)	87,5*	89,9*

*de Ory *et al.*, 03/2011, Clinical and Vaccine Immunology, Evaluation of Four Commercial Systems for the Diagnosis of Epstein – Barr Virus Primary Infections

DVP18

Beacon-Bbased FISH rapidly identifies Gram-negative bacteria causing ventilator-associated pneumonia

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Background: Hospital acquired pneumonia (HAP) is a leading cause of mortality, morbidity and increased hospital costs. We have tested a rapid method of identifying pathogens using a beacon-based fluorescent in situ hybridisation (bbFISH) method to identify the major Gram-negative pathogens causing HAP.
Methods: Respiratory specimens obtained from an surgical intensive care unit were examined and specimens of suitable quality, as judged by the Gram stain, were included in the study and subjected to additional bbFISH analysis using the lucesco HCAP kit (miacom diagnostics, Düsseldorf). The results of bbFISH were compared to culture. The investigator performing the bbFISH was blinded to the culture results of the specimens. Specimens were examined routinely according to standard microbiological methods and in addition at the time of performing the bbFISH were cultured on standard agar plates in order to analyse possible discrepancies.
Results: A total of 303 specimens were investigated. The sensitivity for the different species of Gram-negative pathogens varied between 20 % (*Klebsiella pneumoniae*) and 100 % (*Serratia marcescens*, *Proteus* spp. and *P. aeruginosa*). In all cases where there was a discrepancy between bbFISH and culture the results were compared to cultures performed at the same time as the bbFISH (generally 24 h after routine cultures). In 131 cases the results of both culture and bbFISH were identical and in 24 cases the bbFISH was negative for a pathogen found in culture. Cultures were negative in 148 cases of which in 3 cases the bbFISH was positive for a pathogen resulting in a sensitivity and specificity of 84,5 % and 98 % respectively.
Conclusions: The bbFISH is a very useful method to rapidly identify the major Gram-negative pathogens in respiratory specimens from patients with suspected pneumonia especially „problem pathogens“ such as *Pseudomonas aeruginosa* possibly leading to a more tailored empirical therapy. Furthermore bbFISH detects polymicrobial infections not easily detected by culture.

DVP19

Development of a mass spectrometry-based assay for rapid detection of ampicillin resistance in *Escherichia coli* strains

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Background: Sepsis is the most common cause of death in hospitalized patients worldwide. Promptness of pathogen detection and early-targeted antibiotic therapy are the cornerstones for successful management of systemic bacterial infections. Specifically, resistance to antibiotics is an increasing problem and rapid diagnostic tests for targeted therapy are urgently needed. To improve standard antibiotic susceptibility testing, we have developed a mass spectrometry-based assay capable of detecting ampicillin resistance in *Escherichia coli* strains within 60 minutes.
Materials and Methods: Suspensions of *Escherichia coli* strains were incubated with ampicillin under standardized conditions. Concentrations of ampicillin and ampicillin metabolites were measured in bacterial supernatants by mass spectrometry and ratios were calculated for

classification. The assay was validated with subcultures of 60 clinical isolates of *Escherichia coli* comprising 40 beta-lactamase positive strains including 20 strains with enhanced spectrum beta-lactamase activity and 20 beta-lactamase negative strains (training set). Independently, we tested our assay with subcultures of additional 50 randomized isolates of *Escherichia coli* strains (validation set). Classifications were subsequently done using the ratios of ampicillin and ampicillin metabolites. Finally, the assay was adapted for the analysis of *Escherichia coli* bacterial suspensions isolated from blood cultures, immediately after these turned positive.
Results: *Escherichia coli* strains that were beta-lactamase positive had ratios of ampicillin/ampicillin metabolites significantly different from those that were beta-lactamase negative. Classification accuracy was 100%. The total assay time was about 60 minutes. This may allow a fast adaptation of antibiotic therapy directly after identification of *Escherichia coli* with MALDI-TOF MS biotyping (Bruker Dalonics).
Conclusion: The accelerated metabolism of ampicillin can act as a surrogate marker for increased beta-lactamase activity in *Escherichia coli* strains. This might lead to the accelerated detection of antibiotic resistance combined with an early and targeted therapy of bacterial strains in septic patients.

DVP20

Differentiation of hospital-associated and colonizing Enterococcus faecium isolates using MALDI-TOF mass spectrometry

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Objective: Infections with hospital-associated strains of Enterococcus faecium increased in intensively cared patients. Hospital strains differ from colonizing strains by a specific core genome and an additional accessory genome of several 100 kb including a pathogenicity island, genomic islands, prophages and plasmids. Hospital strains are more often multi-resistant and express an enhanced spreading potential among the nosocomial setting. Our hypothesis suggests that the mentioned genomic differences may be reflected by specific protein patterns. We used a sophisticated extraction protocol and subsequent MALDI-TOF MS analysis to evaluate the discriminatory power of this method for differentiating isolates of E. faecium of various origins.
Materials: We included 86 pre-characterized E. faecium strains from animal/food (pig, poultry/chicken, pork, poultry/chicken meat) and human sources and from single infections and outbreaks isolated between 1995 and 2008. All isolates were MLST typed and strains were allocated to specific clonal complexes as based on eBURST/goeBURST. Strains had a varying resistance pattern and included vancomycin-resistant enterococci (VRE: vanA/B). Microbial sample preparation was carried out according to a trifluoroacetic acid (TFA)-based acid extraction protocol. Mass spectra were acquired in the linear mode from three independent microbial cultures of each microbial strain by an AutoFlex I MALDI-TOF mass spectrometer (Bruker Daltonik, Bremen). The strategy of MS data evaluation included unsupervised hierarchical cluster analysis and supervised artificial neural network analysis.
Results: Outbreak strains possessed MLST types allocating them to the clonal complex of hospital-associated strains (CC17). By using the supervised classification approach which included training, internal validation and external testing, we were able to differentiate in the independent test data between hospital-associated (MALDI pattern 1) and colonizing strain types (MALDI pattern 2). Systematic analyses for specifically identifying mass peaks linked to a corresponding origin of a strain, clonal type or complex did not reveal statistically relevant biomarkers. Currently, another 32 strains of various origins are being tested for assigning them to the respective MALDI pattern types.
Discussion: MALDI-TOF MS is a simple, rapid, and cost-effective method to differentiate between hospital (CC17) and colonizing strains of animals and humans of E. faecium allowing thus a timely and effective outbreak recognition and response. However, MALDI-TOF has its limitations; for instance, its discriminatory power is unable to sub-differentiate strains to the level of distinct clones.
Conclusion: MALDI-TOF MS analysis allows differentiating hospital-associated E. faecium strain types (CC17) from colonizing strains of animals and humans.

DVP21

A chromogenic culture medium for the reliable and facilitated detection of mycoplasmas

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The cultivation dependent method is considered the “gold standard” for testing cell cultures, biologicals and vaccines for mycoplasma contamination. This method requires complex nutritionally enriched culture media, which are well defined in Ph. Eur. chapter 2.6.7 and USP chapter <63>. Still mycoplasma detection remains a challenge because of their small colony size on agar media, limited turbidity in liquid culture and limited pH shifts produced in both, liquid and solid culture media. To enhance the sensitivity and reliability of the cultivation dependent method, we developed a chromogenic mycoplasma agar medium with improved formulation. Recovery rates of all Mycoplasma and Acholeplasma reference strains recommended by Ph. Eur. and EDQM were equal or even better (e.g. *M. orale* ATCC 23714: 281%, *M. pneumoniae* ATCC 15531: 175%, *M. synoviae* ATCC 25204: 312%) in comparison to culture media acc. to Ph. Eur. (USP). In addition, mycoplasmas grow within 3-7 days to dark violet colonies in most cases visible to the naked eye. Optimal results were obtained with a single medium, but two different incubation conditions (anaerobic and microaerophilic). The new mycoplasma medium greatly facilitates the detection of colonies and differentiation from any kind of artifacts.

DVV01

Comparison of three multiplex PCR assays for the detection of respiratory tract infections

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Background: Acute respiratory tract infections are the most widespread types of infections in adults and children and are responsible for a considerable morbidity and mortality worldwide. A high rate of respiratory tract infections is caused by viruses (approximately 80%), but symptoms are mostly similar. Therefore, the identification of the causative viruses is only feasible using multiplex PCR or several monoplex PCR tests in parallel.
Methods: The sensitivity of three multiplex PCR assays, RespiFinder-19, RespiFinder-SMART-22 and xTAG-Respiratory-Virus-Panel-Fast-Assay (RVP), were compared to monoplex real-time PCR with quantified standardized control material. All assays include the most common viruses (adenovirus, coronavirus, human metapneumovirus (hMPV), influenzavirus, parainfluenzavirus (PIV), respiratory-syncytial-virus (RSV), rhinovirus).
Results: To compare the sensitivity of the multiplex assays, samples were inoculated with 14 different quantified viruses in the range of 10^1 to 10^5 copies/ml. Concordant results were received for rhinovirus, whereas the RVP detected influenzavirus, RSV and hMPV more frequently in low concentrations. In contrast, the RespiFinder-19 and the RespiFinder-SMART-22 showed a higher sensitivity for adenoviruses and coronaviruses, whereas the RVP was incapable to detect adenovirus and coronavirus in concentrations of 10^4 copies/ml. A detection of influenzaviruses (10^4 copies/ml) and RSV (10^3 copies/ml) was not possible using the RespiFinder-19 and RespiFinder-SMART-22. However, the detection of all 14 viruses in one sample was only achieved using monoplex PCR. In order to analyze possible competitive amplification reactions between the different viruses, samples were further inoculated with only 4 different viruses in one sample. Compared to the detection of 14 viruses in parallel, only a few differences were found.
To assess the clinical usability, the incidence of respiratory viruses was compared in tracheal secretion (TS) samples (n = 100) of mechanically ventilated patients in winter (n = 50) and summer (n = 50). In winter, respiratory viruses were detected in 32 TS samples (64%) by RespiFinder-19, whereas the detection rate with RVP and RespiFinder-SMART-22 was only 22%. The most frequent viruses were adenovirus (32%) and PIV-2 (20%). Multiple infections were detected in 16 TS samples (32%) by RespiFinder-19. Fewer infections were found in summer (RespiFinder-19: 20%; RVP: 6%; RespiFinder-SMART-22: 4%). All positive results were verified using monoplex PCR.
Conclusions: Multiplex PCR tests have a broad spectrum of pathogens to test at a time, but a lack of sensitivity in comparison to monoplex tests. Our study shows that the application of a multiplex PCR method in the routine diagnostics has to balance the assay sensitivity the time-to-result, the hands-on-time and the clinical relevance of the detected pathogens.

DVV02

Occurrence of a newly described divergent *mecA* homologue with diagnostic impact in German clinical *Staphylococcus aureus* isolates

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates represent a major cause of hospital- and community-acquired infections that are increasingly difficult to manage. In addition, their detection and identification by culture- and nucleic acid-based methods is challenging by heterogeneous PBP2a expression and variability of the SCC*mec* elements. Recently, a new SCC*mec* element (XI) leading to phenotypic ß-lactam resistance was described (1, 2). It contains a novel *mecA* homologue, designated *mec*A_{LGA251}, that is not detectable by usual *mecA*-specific PCR approaches and PBP2a agglutination tests. The objectives of the study were to investigate i) the occurrence of this novel MRSA type in German isolates and ii) the resistance pattern of these strains against current available antibiotic classes with focus on ß-lactam antibiotics.
Method: Institutional collections comprising *S. aureus* isolates collected within the courses of German mono- and multi-center studies were screened for oxacillin-resistant, but *mecA* negative isolates. These strains were *spa*-typed and tested for *mec*A_{LGA251} and type XI SCC*mec* by PCR. Antimicrobial susceptibility testing was performed by Etest.
Results: Here, for the first time, MRSA isolates carrying *mec*A_{LGA251} were reported for German isolates. The earliest isolate was recovered from a clinical specimen already in 2004. All identified isolates were found to belong to *spa* type t843, which is described to be associated with CC130. The novel SCC*mec* type XI was confirmed in all respective isolates. Within the group of ß-lactams, MIC values of all isolates revealed resistance to benzyl-penicillin, ampicillin, oxacillin and cefoxitin and susceptibility to cefalothin cefuroxime cefepime *ceftobiprole* and imipinem. For other non ß-lactam antibiotics, isolates were tested susceptible.
Conclusion: At present, the detection of the *mecA* gene and/or its gene product PBP2a represents the cornerstone of the verification of the MRSA nature of a given isolate. Thus, the occurrence of *mec*A_{LGA251} poses a challenge for identification and confirmation of MRSA. Till the implementation of these novel diagnostic targets into MRSA detection approaches, methicillin/cefoxitin-resistant isolates determined by traditional susceptibility testing methods should not be disregarded as methicillin-susceptible even if classical *mecA*/PBP2a tests fail to detect their targets. As soon as possible, respective methods should be adapted to identify both *mecA* and *mec*A_{LGA251}. Furthermore, detailed epidemiological studies are warranted to investigate the prevalence and the clinical significance of MRSAs bearing the modified *mecA* gene.

Reference
1: García-Álvarez et al., Lancet Infect Dis. (in press); 2: Shore et al., Antimicrob. Agents Chemother. (in press)

DVV03

Clinical Value of the SeptiFast Multiplex PCR test in hematologic patients with neutropenic fever or sepsis: Interim study results

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Introduction: Sepsis continues to be a major cause of morbidity and mortality in neutropenic hematologic patients. Rapid identification of the causative pathogen and prompt initiation of appropriate antimicrobial treatment have a substantial impact on mortality. The LightCycler® Septi*Fast* (SF) multiplex PCR test for the direct detection and identification of microbial DNA in blood identifies major pathogens of hospital-associated bacteremia. This randomized clinical study aims to assess the clinical value of SF as an adjunct to the culture-based approach and clinical assessments (NCT01114165).
Methods: Hematologic patients with neutropenic fever and/or sepsis were randomly allocated into two groups. In the study group, the results of SF were made available to the clinicians and applicable to clinical decisions. In the control group, clinicians were blinded to all SF results. Concurrently with routine blood culture (BC) specimens, blood samples for SF were drawn from a peripheral vein and/or a central venous catheter. In patients presenting with persistent fever up to 72 hours, SF samples were collected once again. The study was performed under real-life conditions, i.e. routine transportation of samples and PCR availability only during the day.
Results: Until interim analysis, 101 blood samples of 61 patients (study group, n = 31; control group, n = 30) were collected. While SF was positive in 39.3% of patients (35.6% of samples, 37 microorganisms identified), blood culture was positive in 37.7% of patients (29.8% of samples, 31 microorganisms identified). Concordant results of SF and BC were obtained for 68.3% of all samples and 72.4% after exclusion of non-detectable microorganisms by SF. 33.3% of positive SF samples had the same microorganism(s) in BCs. 32.2% of positive BCs were confirmed by SF. The average duration between the blood draw and communication of SF results to the clinician was 22.2 h, while the mean time for performing SF itself was 5.7 h. The time between blood draw and communication of the Gram stain result from BCs was 27.9 h and time between blood draw and preliminary identification and susceptibility result from BC isolates was 57.1 h. In 22.6% of SF group patients, therapy was changed (55.5% of them due to the SF result, in 20.8% of patients with positive SF results therapy was changed). In the control group, 13.3% of patients had therapy changes. In total, there were nine therapy changes in the SF group and four therapy changes in the control group. Time to change to the targeted antimicrobial therapy was 24.9 h in SF group and 35 h in control group.
Discussion: The molecular approach for detection of pathogens in blood might serve as a valuable addition to the microbiological diagnostics in neutropenic patients. Final study results should enable conclusions concerning the effectiveness of SF to guide clinical decisions and to shorten the time to result.

DVV04

Culture-dependent and independent analysis of cardiac biopsies

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Objective: Endocarditis is a severe disease affecting cardiac valve functions. As a consequence a number of patients require cardiac surgery. In many cases the etiologic bacteria can be isolated in blood cultures and cardiac biopsies; however, following initial antibiotic treatment identification of the relevant bacteria is generally no more possible. Additionally, endocarditis can be also caused by fastidious or non-cultivable bacteria.
Methods: The Cardiac surgery department at the University of Saarland Hospital, a 1300-bed tertiary care facility, is highly experienced in cardiac valve replacement and reconstruction. Since August 2008 regular intra-operative cardiac valve biopsies are routinely analyzed also by culture-independent 16S RNA pan-bacterial PCR following sequence typing in addition to the classical culture-based analysis. Culture-dependent and culture-independent results were compared in the present retrospective single centre study.
Results: A total number of 761 cardiac valve tissues were analyzed between August 2008 and March 2011. 95 samples (12.5%) were positive by 16S PCR and 156 (20.5%) by culture-based methods. 54 samples (7.1%) were positive by PCR only, 115 (15.1%) by universal cultures only and 41 (5.4%) were independently confirmed by both methods. In both groups with positive PCR the predominant pathogens were streptococci, S. aureus and enterococci whereas samples with positive culture only, were dominated by the detection of potential contaminants of the skin flora (coagulase negative staphylococci [CoNS], Micrococci, Neisseria). The samples with positive PCR only were characterized by detection of typical pathogens for endocarditis (streptococci, *S. aureus*, enterococci) including fastidious organisms (Abiotrophia, Hemophilus, Paracoccus) and uncultivable bacteria as Tropheryma whipplei.
Analysing culture positive / 16S PCR negative samples bacterial growth was mostly restricted to broth culture without colonies on solid media whereas the 16S RNA PCR positive / culture positive samples were characterized by a significant growth on both, broth and solid media.
Conclusion: Culture-independent analysis of bacteria by 16S RNA PCR and sequence typing in cardiac biopsies is an important diagnostic tool for diagnosis of bacterial endocarditis even years after antibiotic treatment. Thereby, DNA of viable and also of dead bacteria can be detected in a very sensitive way at the site of infection. Culture-independent analysis may also help to discriminate bacterial contaminations from true infections.

DVV05

Using MALDI-TOF mass spectrometry to detect Carbapenem resistance within one to three hours

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Background: Resistance to carbapenems is the latest obstacle in the fight against bacterial infections. Fast and reliable detection of resistance is indispensable. However, time to result for agar diffusion or MIC determination is about 18 hours and sometimes resistance is not properly expressed. PCR is reliable but costly and can only detect known resistance mechanisms. We wanted to develop a method which is fast, easy, low in cost and reliable.
Methods: We used MALDI-TOF MS (matrix assisted laser desorption ionization time of flight mass spectrometry) to determine carbapenem resistance by monitoring the degradation of ertapenem through strains carrying the following carbapenemases: IMP-1, IMP-2, KPC-2, VIM-1, VIM-2 and NDM-1.
Results: Degradation of ertapenem was clearly visible within one hour for NDM-1 and IMP-1. IMP-2, KPC-2 and VIM-1 needed about 1.5 hours for degradation and VIM-2 needed 2.5 hours. All controls were negative (e.g. ESBL+ strains, K1+ strains).
Conclusions: We recommend this method as a fast confirmation test of carbapenem resistance in bacterial strains. This method would be especially helpful in an outbreak situation. Expanding this method to other classes of antibiotics (penicillins, cephalosporins or aminoglycosides) may clear the way for a routine use in detecting antibiotic resistance.

DVV06

Is it possible to identify bacteria directly from positive charcoal containing blood culture broths using MALDI-TOF MS?

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Introduction: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOS MS) is a fast and accurate diagnostic tool for identification of cultured bacteria in medical microbiology. To speed up the diagnostic process of bacteraemia several protocols have been established for direct identification of bacteria from positive charcoal-free blood culture bottles by MALDI-TOF MS. Here, we report the first results of our protocol for charcoal containing blood culture bottles.
Methods: 79 positive BacT/ALERT FA and FN bottles (bioMérieux, Marcy l´Etoile, France) were tested using the following protocol. Gram stain and subculture were performed from all positive blood culture bottles. 5 ml of blood were used for sample preparation for MS. In a first centrifugation step (10 min/400g) charcoal and blood cells were removed. After a lysis step with 5 % saponin the remainder of charcoal and blood cells were removed using a spin column. Before the standard Bruker extraction protocol for bacterial profiling was applied, a washing step with ultra pure water was performed. Zero point five µl and 1µl of the extract were spotted onto the MALDI target and overlaid with 1µl HCCA matrix. A Microflex II instrument with Biotyper 2.0 and 3.0 software was used to detect the protein profiles (Bruker Daltonics, Bremen, Germany). Criteria for correct species identification were a score value ≥ 1.4 and 4 best matches within the same species. Cultured bacteria were identified in parallel using biochemical methods (e.g. Vitek 2) or MALDI-TOF MS. Mixed cultures were excluded from this analysis.
Results: 73.4% of the isolates (n=58) were correctly identified on species level with our protocol. In 26.6% of our samples (n=21) no identification was possible. Incorrect identification did not occur. The following groups of bacteria were included: enterobacteriaceae (n=10), nonfermenters (n=5), staphylococci (n=50), streptococci/enterococci (n=8) and 'others' (n=6). Of these, all enterobacteriaceae were identified correctly on species level, whereas 1 nonfermenter (20%), 11 staphylococci (22%), 6 streptococci/enterococci (75%) and 3 'others' (50%, 2 x *P. acnes*, 1 x *Corynebacterium spp*.) could not be identified with our protocol.
Discussion: Our results indicate that direct MALDI-TOF MS is possible even from charcoal containing blood culture bottles. Where a score value ≥ 1.4 together with 4 best matches within the same species was obtained, the identification was correct on species level. All identifications were confirmed by a reference methods. The high rate of samples with no identification within the group of streptococci/enterococci corresponds to the observations reported in the literature.

DVV07

Impact of species specific score values using Biotyper 2.0 for *staphylococci*

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Objectives: Recent publications proved the high accuracy of bacteria identification by the use of Matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS). However many preceding studies differed in their conditions concerning sample preparation, quantity of shots and the chosen score cut-off value.In this study we measured over 600 isolates including 21 species of a local strain collection to establish if there should be a useful score cutoff value for different staphylococcal species.
Methods: With the exception of slight modifications in culturing samples were prepared according to the protocol of the "Ethanol/formic acid extraction" procedure (Bruker). All strains were prepared in duplicate and examined by MALDI-TOF MS using a Microflex LT instrument (Bruker Daltonics), Flexcontrol 3.0 software and the Biotyper 2.0 database (Bruker Daltonics).The cutoff value of 2.0, given by the manufacturer for probable species identification, was compared to species specific score cutoff values.Identification score values were grouped according to their score outcome of a given species in higher than 75% of the cases as excellent score cutoff value, 50 % of the cases as good, 25 % as poor and less than 25 % of the cases as insufficient score cutoff value.
Results: Twelve out of 21 species specific score cutoff values were evaluated as excellent, three as good, three as poor and three as insufficient for an identification approach. Interestingly these cutoff values were considerably lower for *Staphylococcus arlettae*, *Staphylococcus carnosus* and *Staphylococcus cohnii* compared to that of *S. aureus and S. epidermidis.*
Conclusion: According to this study we recommend to use species specific score cutoff values for the identification of staphylococci.

DVV08

In contrast to previous reports a detection of oxacillin resistance of *S. aureus* by whole cell matrix assisted laser desorption time of flight mass spectrometry in the mass-to-charge-ratio of 2000 to 15000 Da is not possible

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Several studies have been published that claimed to identify oxacillin resistance of *Staphylococcus aureus* by whole cell matrix assisted laser desorption time of flight (MALDI-TOF) mass spectrometry. In these studies selected methicillin susceptible *S. aureus* isolates were compared to selected methicillin resistant *S. aureus* (MRSA) isolates. Nevertheless, the study design in all of those studies was not suitable to achieve this goal, because only *mecA*/SCCmec isogenic strains could discriminate between strain specific- and *mecA* / SCCmec-dependent peak variations.
It have been also described that strains specific peaks exist within the species of *S. aureus.*. We identified in our study *mecA*/SCCmec isogenic strains of *S. aureus* and analysed their peak profiles by MALDI-TOF MS.
A large MRSA strain collection containing 120 strains with molecular divergent backgrounds was used. The *mecA* gene was found in only 104 out of 120 of these isolates after culturing the freezing culture. The loss of the *mecA* gene during freezing culture has been previously described. Single colonies of those isolates were identified by the cefoxitin resistance phenotype and *mecA* PCR results. The bacteria from freezing culture were submitted to LB media containing increasing oxacillin concentrations in order to select the corresponding *mecA* containing strain. In two out of 16 samples we could select a *mecA* containing strain and a corresponding isogenic strain lacking the *mecA* gene. These two pairs of molecularly characterised strains were submitted for whole cell MALDI-TOF MS. In order to increase the expression of the PBP2a, the oxacillin resistant strain was also cultured in LB medium containing NaCl and oxacillin. The corresponding oxacillin susceptible strain was cultured in LB medium only.
We do not found evidence for a difference in the peak profiles in a mass-to-charge-ratio (Da) of 2000 to 15000 Da in two *mecA*-isogenic pairs of *S. aureus* strains cultured on blood agar plates. The protein peak profiles of the *mecA* positive strains, cultured on oxacillin containing media, were also virtually identical compared to the *mecA* negative corresponding isolates.
In contrast to several reports in literature, we could show for the first time that whole cell MALDI-TOF MS-derived peak profiles are not suitable for the discrimination of *mecA* positive *S. aureus* strains in the mass-to-charge-ratio of 2000 to 15000 Da.

DVV09

Rapid identification of mixed- and single bacterial and fungal infections with PCR coupled to Electrospray-Ionization Time-of-Flight Mass Spectrometry (PCR-ESI/TOF MS)

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The identification of bacteria and yeast from blood culture is a time consuming step, shortening the benefit of the patient. Several technologies were developed to replace the second incubation step on agar plates and the biochemical characterization, most prominent analysis by mass spectrometry. However, the resolution of complex mixtures, detection of antibiotic resistance markers are still unmet needs.
In order to overcome these limitations, a new molecular technology, PCR coupled to Mass Spectrometry, was developed, allowing detection and identification of multiple pathogens and selected antibiotic resistance markers or virulence factors in a single test. After PCR with 18 different broad range and specific primers covering bacteria, Candida and key antibiotic resistance markers, the base composition of the amplicons was determined by mass spectrometry with the Abbott PLEX-ID analyzer, followed by bioinformatical identification and genotyping.
Clinical samples (positive and negative blood cultures) as well as spiked blood cultures were analyzed with the PLEX-ID instrument and the BAC Spectrum BC assay. The results were compared to traditional microbiology techniques, discordant results were additionally confirmed by 16s rRNA gene sequencing. The PLEX-ID technology displayed a high specificity, superior to traditional molecular techniques. We were also able to demonstrate the resolution of mixtures in several clinical samples and the resolution of up to 5 different bacteria and yeasts with a single primer pair.

DVV10

Evaluation of *S. pseudintermedius* differentiation using molecular marker analysis and MALDI TOF MS

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Introduction: The staphylococcus intermedius group (SIG) is comprised of three distinct coagulase-positive staphylococcal species *S. intermedius*, *S. pseudintermedius* and *S. delphini*. *S. pseudintermedius* is a common colonizer which usually inhabits the skin and mucosa of dogs. Like other opportunistic pathogens, *S. pseudintermedius* is able to cause a broad range of infectious diseases, for instance, wound infections, dermatitis and sepsis. In recent years, a rise in Methicillin- resistant *S. pseudintermedius* (MRSP) among diagnostic samples from dogs and cats was observed. In addition, MRSP isolates found in Europe were frequently associated with multi-drug resistance. Furthermore, *S. pseudintermedius* is potentially transferable to human hosts. Consequently, severe *S. pseudintermedius*- infections (including MRSP) in humans have also been recently described.
However, molecular methods currently used for the differentiation of members of the SIG are time consuming and do not allow rapid identification and distinction among this group. As the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)-based identification method is emerging as a fast and reliable method for microbial identification, the use of this method may provide significant advantages in the routine diagnosis of *S. pseudintermedius*.
Methods: We have developed a reference database of MALDI-TOF MS profiles for *S. pseudintermedius* (n=41). The present study utilises *S. pseudintermedius* (including MRSP) isolated from canine and human origin which were earlier characterised as *S. pseudintermedius* by sequencing of internal fragments of the house-keeping genes *cpn*60 and *pta*. A main spectra library (MSP) for each isolate was created from 27 spectra acquired from their biological and technical replicates. These MSPs were successfully added to the Bruker database by using BioTyper software.
Results: Analysis with Flex analysis software including representative spectra for *S. intermedius* and *S. delphini* provided by the Bruker database revealed that spectra obtained for strains which belong to the same staphylococcal species appeared to be comparable. A score oriented MSP-dendrogram displayed three clusters, each representing a distinct member of the SIG. The practical relatedness' between the spectra sets of the constructed database was verified by calculating the computing composite correlation index (CCI).
Conclusion: MALDI-TOF-MS can be used for rapid identification and differentiation of *S. pseudintermedius* in routine microbial diagnostic procedures.

Keywords
MALDI ToF MS, main spectra library, *Staphylococcus intermedius* group, Methicillin- resistant *Staphylococcus pseudintermedius*, microbial identification, composite correlation index

EKP01

Functional analysis of the two type II NADH dehydrogenase isoforms in *Toxoplasma gondii*

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NADH dehydrogenases play an important role in the energy metabolism by transferring electrons from NADH to the mobile electron carrier ubiquinone. Several bacteria, fungi and protozoa possess so called type II NADH dehydrogenases (NDH2s), which are structurally and functionally different from the common complex I of mammalian cells and are thus considered as putative drug targets. NDH2s are composed of a single subunit and are not involved in proton translocation. The apicomplexan parasite *Toxoplasma gondii* possesses two NDH2 isoforms, TgNDH2-I and TgNDH2-II, which are both targeted to the mitochondrion. Type II NADH dehydrogenases can occur either as internal enzymes, which utilize mitochondrial NADH, or as external enzymes, which utilize cytosolic NADH. We determined the orientation of both isoforms by applying a split GFP complementation approach and could demonstrate that both isoforms are internal enzymes, which face with their active site to the mitochondrial matrix. This internal orientation was confirmed by a protease protection assay after selective membrane permeabilization with digitonin. To obtain functional data for the contribution of the individual isoforms in the *T. gondii* energy metabolism, we generated single knock-out mutants for each of the isoforms. Both mutants were viable, but displayed a decreased replication rate, which was more severe in the *Tgndh2-II*-deleted than in the *Tgndh2-I*-deleted mutant. A functional complementation was possible by ectopic expression of the deleted gene, but not by expression of the heterologous isoform, suggesting that the two enzymes possess non-redundant, isotype-specific functions.

EKP02

*C. albicans eed1*Δ primary and secondary yeast cells display different filamentation phenotypes

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Introduction: The pathogenic fungus *Candida albicans* is able to colonize many different body sites and infections range from harmless superficial lesions up to severe and life threatening disseminated candidiasis. The transition from the spherical yeast to the filamentous hyphal growth form is involved in adhesion, invasion, tissue penetration and escape from host cells such as endothelial cells and macrophages after internalization. More recently it was shown that, despite identical morphology, yeast cells dispersed from a mature biofilm show major differences compared to their planctonic counterparts with respect to their adherence-, filamentation- and virulence properties. The Eed1 protein is a regulatory factor of filamentation in *C. albicans* and especially important for the maintenance of hyphal development and growth. Mutants lacking the *EED1* gene retain the ability to initialize hyphal growth by the formation of germ tubes, but do not form elongated true hyphae. In contrast, cells switch back into an elongated yeast cell-like growth form.
Aim and Methodology: In this work we address two major subjects: We first characterized the filamentation process and filament morphology of the *eed1*Δ mutant strain in comparison to the parental strain in more detail, with special attention to cell density effects. Secondly, we analyzed whether the *eed1*Δ mutant cells, which switched back into yeast cell-like growth form (referred to as secondary yeast cells) show the same properties as their primary counterparts. Therefore, we performed hyphae-induction experiments on plastic surfaces and determined filament induction, length and branching over a time course of 24 h.
Results: Surprisingly, within an inoculum range which does not affect wild type filamentation, filamentation of the *eed1*Δ mutant strain strongly depends on the inoculum size: Cells seeded at moderate cell densities formed shorter filaments than cells seeded at low cell densities. Furthermore, the *eed1*Δ mutant displayed increased branching, delayed filament induction and lower adhesion properties than the parental strain. The secondary *eed1*Δ yeast displayed an even more pronounced defect, characterized by a strong reduction in filament induction and adhesion properties and an even more pronounced effect of inoculum size to the filamentation process.
Discussion and Outlook: The increased influence of cell density on *eed1*Δ filamentation suggests a putative link of Eed1 to quorum sensing mechanisms. This hypothesis is currently under investigation. The observed differences between *eed1*Δ primary and secondary yeast cells might have implications for virulence, as it has been demonstrated for biofilm dispenser cells. Further experiments shall elucidate the pathogenic potential of *eed1*Δ secondary yeast cells and reveal possible molecular mechanism underlying these differences.

EKP03

Development of *Toxoplasma gondii* in skeletal muscle cells depends on differentiation of the host cell

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Toxoplasma gondii, an apicomplexan parasitic protozoan, infects almost every nucleated mammalian and avian cell including skeletal muscle cells (SkMCs). Replication of the parasite and its differentiation to the dormant bradyzoite stage in SkMCs are considered as crucial for the food-borne transmission to humans via undercooked or cured meat. Previous results from our laboratory indicated that SkMCs promote spontaneous differentiation from tachyzoites to bradyzoites *in vitro* more readily than fibroblasts. Therefore, the identification of cell-type specific factors which regulate the parasite development in SkMCs is of major interest.
In this study, cell type-specific interactions were investigated using permanent mouse-derived skeletal muscle cells (C2C12) and fibroblasts as controls (NIH3T3). C2C12 myoblasts were differentiated to polynucleated, contractile myotubes *in vitro*. Immunoblotting, immunofluorescence microscopy and quantitative RT-PCR validated the differentiation efficiency of C2C12 cells by measuring the expression of myosin heavy chain. Intriguingly, after infection with *T. gondii*, parasite replication was clearly lower in differentiated C2C12 myotubes than in undifferentiated C2C12 myoblasts or NIH3T3 control fibroblasts. After 2 days of infection, parasitophorous vacuoles (PV) contained an average of 6 and 7 parasites in C2C12 myoblasts or NIH3T3, respectively, whereas only 3 parasites were found in the PVs in differentiated C2C12. Additionally, no parasite-induced cell lysis could be observed in differentiated C2C12 in contrast to non-differentiated C2C12 or NIH3T3. Quantification of *Toxoplasma* by real-time PCR confirmed a reduced replication of the parasite in differentiated C2C12 myotubes as compared to undifferentiated C2C12 myoblasts. Mitogen Activated Protein Kinases (MAPK) such as p38 or ERK as well as Akt/PKB regulate the differentiation of SkMCs. Therefore, we also investigated whether MAPK might control *T. gondii* development in differentiated and undifferentiated C2C12 SkMCs. First results using immunoblotting showed a considerable increase of p38, ERK and Akt/PKB phoshorylation within differentiated C2C12. Furthermore, the phosphorylation of p38 and Akt/PKB proteins was enhanced after 24h post-infection with *T. gondii* in differentiated C2C12 as compared to undifferentiated C2C12. Further experiments on the impact of MAP Kinases on C2C12 differentiation as well as the infection process are in progress.
Together, these results suggest that the host cell type and the differentiation of SkMCs regulate *T. gondii* replication *in vitro*. This might also explain the different efficiencies of *T. gondii* to persist in differentiated tissues of the host.

EKP04

The *Candida albicans* factor H binding molecule Hgt1p also functions as complement receptor 3 (CR3) and HIV binding molecule

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Background: The complement system is tightly controlled by several regulators. Two of these, Factor H (FH) and C4b-binding protein (C4BP), can be acquired by pathogens conveying resistance to complement attack.
Objectives: The aim of the study was to characterise the FH binding molecule of *Candida albicans*, a potentially life-threatening yeast.
Methods: The gene coding for this molecule was initially identified by probing an expression library and homozygous deletion mutants of the respective gene have been constructed previously. Binding and functional assays were now undertaken to compare wild type and knock-out strains.
Results: The „high affinity glucose transporter 1" (CaHgt1p) was confirmed as FH binding molecule. Homozygous *hgt1Δ/Δ* deletion mutants, but not the restored strain in which *HGT1* was reintegrated, showed a decreased binding of FH and even of C4BP, demonstrating its function as a FH- and C4BP-binding protein. This led to an enhanced terminal complement complex deposition after incubation with human serum; CaHgt1p thus functions as complement inhibitor. *hgt1Δ/Δ* mutants failed to form rosettes with complement-coated sheep erythrocytes, and show reduced binding to HIV-gp160, implying that a complement receptor 3 (CR3) moiety, known as fungal HIV binding molecule is lacking.
Conclusions: CaHgt1p is a multifunctional evasion molecule, as complement inhibitor, CR3 analogue and HIV receptor.

EKP05

Ecm33 and Pst1 of *Candida glabrata* contribute to cell wall organization

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Background: *Candida glabrata* is the yeast second most frequently causing candidiasis after *Candida albicans*. Infections with *C. glabrata* are difficult to treat, as this fungus is often resistant to azoles, especially fluconazole. Ecm33 and its homologs are glycosylphosphatidylinositol (GPI)-anchored cell wall proteins of *C. glabrata*. A previous study found that Ecm33 and Pst1 are very abundant proteins in the cell wall of *C. glabrata*, but their functions remain totally unexplored. We therefore constructed *pst1*Δ, *ecm33*Δ and *pst1*Δ*/ecm33*Δ mutants in *C. glabrata* and aimed to functionally characterize both proteins.
Results: All mutants demonstrated (i) an increased sensitivity towards cell wall-perturbing chemical compounds such as calcofluor white and congo red, (ii) a decreased surface hydrophobicity and (iii) a loss of adherence towards polystyrene. In addition, *ecm33*Δ and *pst1*Δ*/ecm33*Δ showed an increased negative cell surface charge. Most interestingly, we found that these mutants release 1,6-β-glucan containing material from the cells to the environment.
Conclusions: Our phenotypic analyses show that Ecm33 and Pst1 in *C. glabrata* significantly contribute to cell wall organization. One possible explanation of our results is that the Ecm33 protein family is involved in cell wall assembly and/or cross linking of cell wall components.

EKP06

Impact of farnesol on human immune cells

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Introduction: Farnesol is an extracellular quorum sensing molecule produced by *Candida albicans* (Hornby *et al.*). It controls the morphological switch from yeast to hyphae which is essential for virulence of the fungus. Functions of this molecule in interaction with human immune cells have only partially been elucidated.
The purpose of this study is to determine the impact of farnesol on innate immune cells (monocytes, neutrophils, dendritic cells).
Methods: The monocytoid cell line THP-1, or primary monocytes and PMNs isolated from peripheral blood of healthy donors were used for the experiments. Monocyte-derived dendritic cells were generated over 6 days using GM-CSF and IL4. Using an *in vitro* apoptosis assay we examined the cytotoxicity of farnesol on THP-1 cells as well as on primary monocytes. The viability of the cells was monitored in time course analyses using Annexin V and PI staining. To define alterations in surface marker expression we performed FACS analysis. Reactive oxygen species (ROS) production was measured by quantification of H_2O_2 via oxidation of H_2DCF to DCF, a highly fluorescence component.
Results: To assess the effect of farnesol on the monocytic cell line THP-1, cells were treated with 50µM and 100µM farnesol (in RPMI + 10% Serum). After 4h and 6h incubation no significantly reduced viability was detectable. Previous work reported that in the presence of serum much higher levels of farnesol are needed to be effective on *C. albicans* than without serum (Mosel *et al.,* 2005). Accordingly we analyzed if serum modulates the impact of farnesol on immune cells. In the absence of serum, already after 2h incubation with 50µM farnesol the viability of the cells decreases to 60% and for 100µM less than 5% of the THP-1 cells were viable. Moreover we examined the influence of farnesol on primary immune cells. Until now we could not detect any significant effect on the viability of primary monocytes by farnesol in medium with 10% or 5% serum. However analysis of the surface phenotype of farnesol-treated monocytes showed a significant enhanced expression of CD86 and HLA-DR under low serum concentrations. In addition to monocytes we analyzed neutrophils and monocyte-derived dendritic cells. Neutrophils treated with high concentrations of farnesol showed significantly enhanced production of ROS. Current experiments focus on the cytokine expression pattern of neutrophils and dendritic cells in response to farnesol.
Conclusion: Our experimental results suggest that farnesol modulates the activivty of innate immune cells by altering surface of primary monocytes and enhancing ROS production in neutrophils.

EKP07

Caenorhabditis elegans as a host model system to screen novel antifungal benzimidazole derivatives

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Candida albicans, a commensal microorganism, is a causative agent of opportunistic oral and genital infections in humans. Infections with *C. albicans* account for 70 to 90% of all invasive mycoses. Treatment of invasive fungal infections is problematic due to several persisting therapeutic drawbacks, in particular development of resistance against the currently available drugs or toxic side effects for the patient. Various approaches have been developed to screen new antifungal drugs for their activity. Previously, the nematode *Caenorhabditis elegans* has been successfully used as a model host for human pathogens and to screen for novel antimicrobial drugs. It has been shown that *C. albicans* colonizes the intestinal tract of *C. elegans* and establishes a persistent lethal infection accompanied by deep tissue invasion of the entire organism. We screened a series of novel (S)-2-aminoalkyl benzimidazole derivatives for their antifungal activity against various *Candida* isolates using a standard EUCAST (European Committee on Antimicrobial Susceptibility Testing) protocol. One compound ((S)-2-(1-aminoisobutyl)-1-(3-chlorobenzyl) benzimidazole (EMC120B12)), showed very auspicious results and therefore was studied in more detail to analyze its antifungal activity against various *Candida* species, its ability to inhibit Biofilm formation in *C. albicans* and to determine the activity *in vivo* using *C. elegans* as an animal model. EMC120B12 showed the highest antifungal activity against a set of various clinical isolates that included *C. albicans, C. glabrata, C. parapsilosis* and *C. krusei*. Furthermore, EMC120B12 showed a significant reduction in *C. albicans* biofilm formation under *in vitro* conditions. Additionally, we found that EMC120B12 had no toxic effects to *C. elegans* at effective inhibitory concentration. During *in vivo* infection studies, we could demonstrate that EMC120B12 can inhibit filamentation of *C. albicans* and prolong survival of the *C. albicans* infected nematodes. EMC120B12 identified in this screen can potentially be used as an effective antifungal agent and will be further investigated for its activity against other fungal species and also in a murine model to study its activity against systemic candidiasis.

EKP08

The HexA protein of *Aspergillus fumigatus* and its role in stress resistance and virulence.

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The mycelium of filamentous fungi consists of an interwoven network of multi-nucleated hyphae. Individual cells within these hyphae are separated by septae. However, these internal walls contain central pores allowing the exchange of molecules, nutrients and even organelles enabling the mycelium to respond as a functional entity to changes in the environment. The draw-back of this organization is that a local damage of the cell wall is a potential threat for the whole syncytium. To prevent uncontrolled bleeding of cytoplasm, filamentous fungi developed a unique organelle, the Woronin body, which is able to seal the septal pore. In several fungi the Hex1 or HexA protein has been identified a major and essential component of the Woronin body and mutants generated in *Neurospora crassa* or *Aspergillus oryzae* show uncontrolled loss of cytoplasm after local damage. Loss of Hex1 in *Magnaporthe grisea* impairs the virulence of this plant pathogen, but no data are yet available on the role of Hex proteins in filamentous fungi causing infections in humans. In this study, we have analyzed the importance of Woronin bodies and in particular of the HexA protein for the stress resistance and virulence of the human pathogenic mold *Aspergillus fumigatus*. The phenotype of the *A. fumigatus* delta *hexA* mutant is less dramatic compared to the *N. crassa hex1* mutant. Under standard conditions growth of the *A. fumigatus* delta *hexA* mutant is comparable to that of the wild type, but in the presence of stressors, like Congo red or SDS, growth of the mutant is clearly impaired. We have also analyzed the localization of HexA using fluorescent fusion proteins. Whereas a GFP-HexA fusion is targeted to organelles showing the typical characteristics of Woronin bodies, a HexA-GFP fusion shows a striking localization at the septal wall. To further investigate the consequences of a loss of HexA, we analyzed the virulence of the delta *hexA* mutant in a murine model of infection and studied its phenotype in confrontation assays using human and murine immune cells.

EKP09

Functional analysis of *C. albicans* infection-associated genes with unknown function during interaction with host cells and in complex infection models

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Introduction: *Candida albicans* is a common fungal pathogen in humans and able to cause a variety of clinical infections. We hypothesized that *C. albicans* unknown function genes, which are transcriptionally upregulated during infection ("infection associated genes") might contribute to the infection process.
Aim and Methodology: We aimed to determine whether *C. albicans* infection associated genes contribute to virulence. Therefore, based on transcriptional profiling from oral infections, liver invasion and incubation in human blood, we selected a subset of infection-associated genes with unknown function for further analysis. Isogenic deletion mutants were constructed and subsequently tested in extensive *in vitro* screens, interaction with epithelial and endothelial cells and in complex infection models.
Results: Twentyfour isogenic deletion mutants were constructed and subsequently analyzed both *in vitro* and in infection models of increasing complexity. Sixteen mutants were impaired in their ability to damage monolayers of human endothelial and/or epithelial cells. Within this subset, seven mutants additionally displayed decreased stress resistance *in vitro*. Only two mutants showed filamentation defects. To determine whether the deleted genes influenced virulence in more complex infections models, all 24 mutants were tested *in ovo* for their ability to kill chicken embryos infected on the chorio-allantoic membrane. We recently showed that mortality in this model depends on the fungal ability to invade the membrane and that the pro-inflammatory host response likely contributes to pathogenesis. Surprisingly, only seven mutants were attenuated in this model, of which five were also attenuated in damaging epithelial cells *in vitro*. Three of the mutants, which were attenuated *in ovo*, and one mutant, which was attenuated in damaging endothelial and epithelial cells but fully virulent in chicken embryos, were subsequently analyzed in a systemic mouse model. Virulence in mice mirrored the results obtained *in ovo*.
Discussion and Outlook: Our results demonstrate that genes essential for causing full damage of epithelial or endothelial cells *in vitro* are not necessarily crucial for full virulence in complex infection models. We conclude that either such damage potential is not critical for full virulence in the complex host setting or that the greater complexity of the environment in animal models stimulates additional fungal regulatory networks leading to compensation of the defect.

EKP10

Thiol-dependent redox networks: analysing the effects of the anti-malarial agent Methylene Blue

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Introduction: The development and spread of resistance against standard antimalarial drugs such as chloroquine (CQ) and pyrimethamine/sulfadoxin (PSD) has been a challenge for 25 years. Thus standard drugs – standard implies that they are affordable for and available to those who need them the most - are continuously required. A number of drugs and drug candidates, for instance methylene blue (MB), are directed against the antioxidative redox-protein network of Plasmodium falciparum which contains a total of 25 redoxins and disulfide reductases [1]. Application of YANAsquare [2] allowed dynamic modelling of enzyme inhibition by different drugs including methylene blue. We analysed affected pathway modes, regulatory effects on affected enzymes, and combination effects of different drugs.
Materials and Methods: The enzyme activity of key redox enzymes with and without the influence of methylene blue was measured (in vivo) by real time PCR. Enzyme activity of enzymes affected by CQ and PSD was collected from literature and databases (PlasmoDB). The software YANAsquare was used for extreme pathway (EP) calculation in order to evaluate the strain-specific redox-network, folate synthesis, haemoglobin degradation as well as porphyrine and carbon-pool networks with all contained pathways.
Results: Modelling shows that metabolic fluxes through gluthation reductase as well as a number of redox-specific enzymes change in their activity (partial inhibition, MB was applied in sublethal concentrations).

Monitoring changes of enzyme mRNA expression, these predictions were validated by RT-PCR data. The strongest reductions (more than 10%) caused by MB are calculated for protein protection modes using thioredoxin and/or glutaredoxin. Furthermore, some enzymes of carbohydrate and nucleotide metabolism reacted to this inhibition by specific up or down regulation. These pleiotropic effects were considered next in a flux model where different resistance mutations were modelled. Resistance mutations (MDR, plasmodial transporter, CQ/PSD resistance) were considered by their mode of resistance on the enzymes targeted by drugs. The effects of the latter drugs are complementary to the fluxes affected by MB and there is favourable synergism, for instance against CQ resistance.

Conclusions: Taken together, our data suggest that MB could become a key factor in drug combination therapy efforts. This should be tested more as a promising local eradication strategy [3].

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EKP11

A C- terminal hyphal activating motif (CHAM) of the TEA transcription factor Tec1p is essential for the activation of morphological development of *C. Albicans*

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Candida albicans is a diploid fungal commensal of humans and mammals. The transition between yeast and hyphal forms is one of the virulence traits for *C. albicans*. *C. albicans* has developed numerous signaling pathways to activate the expression of the stage specific genes in response to environmental cues. The transcription factor Tec1p, a member of the TEA/ATTS transcription factor family, harbors an evolutionarily conserved N-terminal DNA-binding domain. The deletion of *TEC1* leads to defects in hyphal formation *in vitro* and attenuates virulence in a systemic model of murine candidiasis. In order to elucidate the putative DNA binding and gene activating domains spanned over the *C. albicans* Tec1p protein, we integrated modified open reading frames (ORFs) of the wild type *TEC1* under the control of the *C. albicans* -adapted reverse Tet-dependent transactivator (rtTA) into the *tec1/tec1* mutant CaAS12 and used a simple test for Tet-induced hyphal formation as a read-out for Tec1p-activity. Transformation of CaAS12 with wild type *TEC1* ORF was not only able to restore the hyphal growth ability in liquid medium but also it showed invasive growth on solid agar after adding Tet to the medium. The ORF constructs either with a deletion of the C-terminus from 637 to the stop codon at 744 aa or a deletion of the TEA domain from 216 to 244 aa showed abrogation in restoring the hyphal growth, invasive growth and to grow under different stress condition in the *tec1/tec1* mutants. Interestingly, the *tec1/tec1* phenotype was rescued in a mutant containing an ORF with an additional 72 aa that spanned the region from 1 to 709 aa. After expression of the modified ORFs of *TEC1* in *E. coli*, we tested for binding of recombinant rTec1p to the promoter consensus sequence TCS. The deletion of the proposed C- terminal CHAM of Tec1p did not affect DNA binding to the TCS probe. In conclusion, we show that CHAM of Tec1p is not required for DNA binding but it is essential for the biological activation of morphological development.

EKP12

The RNA-binding protein Khd4 of the pathogen *Ustilago maydis:* Identification of target mRNAs using *in vivo* UV-crosslink immunoprecipitation (iCLIP)

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Co- and post-transcriptional control is an essential component of the regulation of gene expression. RNA-binding proteins are the key mediators determining the spatio-temporal expression of proteins within a cell. Accumulating evidence indicates an involvement of RNA-binding proteins in regulating expression programmes underlying pathogenic development of the phytopathogenic fungi *Ustilago maydis*. We are currently investigating the role of the RNA-binding protein Khd4. Khd4 is the founding member of a novel class of fungal multi hnRNP K-homology domain proteins with homologues in human pathogens such as *Cryptococcus neoformans* and *Aspergillus fumigatus*. The deletion of *khd4* results in a disturbed cell morphology and severe reduction in virulence, implying an important role for Khd4 during morphogenesis. Khd4 recognizes a distinct hexanucleotide motif which is present in almost 25% of all annotated transcripts, suggesting a large regulatory potential of Khd4. Failure to bind this RNA element results in an aberrant phenotype similar to the deletion phenotype. The precise role and function of Khd4 however is still unclear. To address this question, we are currently applying *in vivo* UV-crosslinking and immunoprecipitation experiments to identify target mRNAs of Khd4. The iCLIP-technique enables the identification of target mRNAs by coupling *in vivo* UV-crosslinking to high throughput sequencing. Furthermore, it offers the advantage of resolving crosslink sites at individual nucleotide resolution, enabling detailed analysis of binding sites. Presented here are initial steps of establishing iCLIP in *U. maydis* using a control RNA-binding protein.

EKP13

Characterization of the two component histidine kinase TcsC of the pathogenic mold Aspergillus fumigatus

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Aspergillus fumigatus is a mold causing severe and systemic infections in immunocompromised patients. The high mortality of these infections is largely due to the limited therapeutic options. Since A. fumigatus seems to lack sophisticated virulence traits, alternative therapeutic targets have to be identified. The ability to respond to environmental changes is a vital requirement for all microbes. Interference with signaling pathways helping the fungus to adapt to situations of stress is therefore a promising option. However, this approach is hampered by the fact that many signaling pathways and molecules are conserved in fungi and men. Two-component systems (TCS) comprising a sensor histidine kinase and a response regulator are an exception. They are found in plants and microorganisms, but not in mammals.

In this study, we have analyzed the putative group III histidine kinase TcsC of A. fumigatus. TcsC is the only A. fumigatus protein that comprises several HAMP domains. A similar protein architecture has recently been proposed to constitute an osmo-sensing module. Deletion of tcsC has no severe impact on growth under standard conditions, but growth and sporulation of the delta tcsC mutant is clearly impaired in the presence of various stressors, including high osmolarity. This demonstrates that TcsC signaling is required to cope with different forms of stress.

In vitro, the delta tcsC mutant turned out to be highly resistant to several fungicides, including fludioxonil, which are supposed to activate the HOG pathway. Consistently, we observed a dramatic swelling of fludioxonil-treated A. fumigatus wild type hyphae. Mutants defective in genes of the cell wall integrity (CWI) pathway are significantly more sensitive to fludioxonil suggesting either a cross-talk between the HOG and the CWI pathway and/or a requirement for cell wall dynamics to bear fludioxonil-induced stress. The role of TcsC during infection is currently under study.

EKP14

Comprehensive gene deletion study to identify virulence factors in *Candida glabrata*

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Although *Candida glabrata* has become the second most important pathogenic *Candida* species, only few of its virulence mechanisms have been identified so far. Within the ERA-Net consortium FunPath the genes of about 800 potential virulence factors coding for predicted proteins of the cell wall, known signalling pathways, membrane-bound receptors, transporters and transcription factors were identified by comparative genome analysis and subsequently deleted. This library is screened with biological assays, e.g. for strains with altered cell wall stability, stress tolerance, or adhesion behaviour to get a more comprehensive idea of the virulence mechanisms of *C. glabrata*. Furthermore, fitness tests in mice with pools of different mutants are performed. A microarray based tool to read out the amount of cells from different deletion mutant strains after the tests was established which utilises the amount of genetic bar-codes (specific for the different strains) in a sample.

Up to now, several strains were found in survival assays on plates. According to their homologues in *S. cerevisiae*, some of the genes deleted in mutants are presumably involved in cell wall integrity. Since cell wall

integrity is crucial for stress resistance and virulence, further analysis of deletion mutant strains which are predicted to be part of one signalling network was performed.
Based on research done in the homologue *S. cerevisiae*, *SLG1* and *MID2* are presumed to be putative cell wall sensors, whereas *SLT2* is supposed to be a transcription factors involved in the activation of cell wall biogenesis genes. Regarding adhesion on different surfaces, Δ*mid2* and Δ*slt2* showed comparative behaviour to the wild type, whereas the mutant strain Δ*slg1* was stronger adherent. Similar behaviour could be observed while assaying cell wall stability and composition, with Δ*slg1* having considerably changed properties in comparison to the mutant strains Δ*mid2* and Δ*slt2* and the wild type. Comparing the trancriptomes of the deletion strains with the wild type in the presence or absence of congo red showed only very slight changes in the expression pattern of Δ*mid2* and Δ*slt2*, whereas in Δ*slg1* a significantly changed transcriptional profile could be detected.
The results obtained suggest that *SLG1* has a different role in *C. glabrata* than in *S. cerevisiae* compared to *MID2*. The function of the inspected genes of the cell wall integrity pathway in *S. cerevisiae* might therefore be different in *C. glabrata*.

EKV01

African trypanosomes transmigrate across an *in vitro* blood brain barrier model in both directions

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Introduction: The African Sleeping Sicknesss is a vector borne parasitic disease caused by human pathogenic *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. The disease threatens over 60 million people of 36 sub-Saharan nations. Without treatment the lethality is 100 % and 10.000 deaths occur each year [1]. The parasites are transmitted during a blood meal of tsetse flies from *Glossina* genus and multiply within the blood stream. Depending on the subspecies, the trypanosomes invade the central nervous system (CNS) within weeks, several month or even years, which leads to the fatal outcome of the disease [2].
Material & Methods: This project aims on the cellular mechanisms of Blood Brain Barrier (BBB) traversal. Therefore a transwell based *in vitro* BBB model was established and optimized using endothelial like ECV304 [3], glial C6 cells and a semi-synthetical basement membrane. The cell layers were analysed by electron microscopy (EM) and barrier integrity was evaluated by bacterial transmigration comparing a non invasive *E. coli* HB101 strain with meningitis causing *E. coli* K1 strain. J774 macrophages and blood stream cultures of different trypanosome strains, namely *T. b. brucei* TC221, *T. b. gambiense* STIB930 and *T. b. rhodesiense* STIB900, were used to analyse the transmigration across the *in vitro* BBB model.
Results: EM microscopy revealed a confluent layer of ECV304 cells with partly overlapping neighbour cells inside the transwell inserts and a loose meshwork of C6 cells underneath the insert membrane. The optimized ECV304-C6 model was permeable for pathogenic *E. coli* K1 transmigration whereas apathogenic *E. coli* HB101 did not transmigrate even at 100fold higher inoculums. African trypanosomes transmigrated efficiently and *T. b. gambiense* STIB930 strain showed the highest transmigration rate. The analyses of macrophage transmigration across the optimized ECV304-C6 BBB model indicated that paracellular extravasation of J774 macrophages does not enhance trypanosomal transmigration. An upside-down array of the BBB cells, representing an inverse BBB model, revealed brain to blood traversal of trypanosomes with similar amounts.
Discussion: The optimized ECV304-C6 BBB is a reproducible *in vitro* BBB model and selectively permeable for meningitis causing *E. coli* K1. Trypanosomal transmigration across the optimized ECV304-C6 model occurs in both directions underlining the possibility of a relapsing parasitemia after treatment with drugs that have no access to the CNS. This model promotes the analysis of the cellular and molecular mechanisms underlying the CNS invasion, which is urgently needed for therapy improvement and drug development.

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EKV02

Candida albicans affects barrier function and expression of tight and adherens junction proteins of intestinal epithelial cells

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Introduction: Intestinal epithelial cells (IEC) establish a tight monolayer, forming a barrier for microorganisms in the gut lumen. Tight junctional transmembrane proteins, notably claudins, regulate paracellular permeability of the intestinal barrier, constricting diffusion of small solutes and macromolecules. Barrier function can be affected by pathogenic microorganisms, toxins and other stimuli.
The polymorphic fungus and common commensal of the human gastrointestinal tract, *Candida albicans* is capable of establishing systemic disease within the host after traversing the barrier. Here we investigated the role of active barrier disruption for invasion of IEC by *C. albicans*.
Methods: Caco-2 derived C2BBe1 IEC, grown on permeable cell culture inserts served as *in vitro* model of the intestinal mucosal epithelium. Barrier function of C2BBe1 monolayer was examined by measuring transepithelial electric resistance (TEER). Cytotoxicity was determined by LDH release assay on culture supernatants. Fixed inserts with *Candida*-infected IEC were imaged with a confocal microscope.
Results: IEC were grown on membrane inserts for 12 days to establish stable monolayers. Addition of *C. albicans* with a MOI of ≈ 0.3 led to an increase of TEER of approx. 50 %. TEER peaked around 8 h after inoculation and then started to decrease until reaching background levels (i.e. insert without IEC) after ≈ 21 h. Decrease of electric resistance came along with an increasing LDH release. 21 h after infection cytotoxicity levelled at 30-40 %, while microscopic imaging revealed a profound disruption of the monolayers.
To examine the role of filamentation and active penetration, we infected the C2BBe1 monolayer with the non-filamentous *C. albicans* mutant Δ*efg1*/Δ*cph1*.
In contrast to wild-type inoculated IEC monolayer, TEER of mutant inoculated monolayer increased until reaching a two-fold level, which continued until the end of the experiment (22 h). Cytotoxicity of the latter remained at the level of non-inoculated control.
We examined the expression levels of junctional proteins to define more precisely the impact of *C. albicans* inoculation on IEC. Adherens junction (E-cadherin) and tight junction (JAM-A, occludin, and claudin -1, -2 and -3) protein levels decreased markedly in infected monolayers. Confocal imaging of *Candida*-infected C2BBe1 cells showed some localized E-cadherin accumulation at fungal filaments penetrating through cell-cell boundaries, whereas overall E-cadherin staining around the invasion site was lost.
Outlook: The results indicate an active participation of *C. albicans* in intestinal barrier breakdown. However, the relative impacts of host and fungus on barrier disturbance will have to be clarified in ongoing work.
Future analyses are intended to clarify localized vs. general effects of junctional protein distribution and/or degradation in *C. albicans* invasion.

EKV03

In vivo imaging of inflammation during invasive aspergillosis in mice

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Introduction: Invasive aspergillosis (IA) is an important disease in immunocompromised patients. Immunosuppressed mice are the gold standard to investigate IA *in vivo*. In mice immunosuppressed with cortisone acetate, infection is accompanied by a strong inflammatory response. Immigrating immune cells are characterized by an increased glucose uptake, which can be used for non-invasive imaging: Fluorine labeled glucose (FDG - 2-[^{18}F]fluoro-2-deoxy-D-glucose) is used as tracer for positron emission tomography/computer tomography (PET/CT). Alternatively, a fluorescence labeled glucose derivative (RediJect 2-DG-750 Probe, Caliper) is detectable by the In-Vivo-Imaging-System (IVIS). Since non invasive imaging tools to monitor inflammation have the potential to reduce the number of laboratory animals needed for the investigation of IA, we aimed to determine whether the above mentioned *in vivo* imaging systems are suitable for the quantification of the host's inflammatory response to IA in mice.
Materials and methods: After immunosuppression with cortisone acetate (25 mg/mouse intraperitonialy on day -3 and day 0) mice were infected intranasally with a dose of 1 x 10^5 conidia of a bioluminescent *Aspergillus fumigatus* strain (Brock *et al*. 2008). PBS mock-infected animals served as control. PET/CT imaging was performed on days -5/-4 and +3/+4 under isoflurane anaesthesia immediately after application of 100-150µl with 7-12

MBq FDG into the lateral tail vein. IVIS measurements were done with isoflurane anaesthesia immediately after PET/CT, which was 3 hours after injection either of a control dye or 2-DG-probe. The distribution of tracers was quantified in the right and left lung. After the last measurement, mice were euthanized, the lung was removed during necropsy and measured alone in the IVIS. The left and right lungs were homogenized independently. Myeloperoxidase and cytokine levels were quantified in lung homogenates as molecular markers of inflammation.
Results: The FDG and 2-DG-probe distribution in immunocompetent mice and immunocompromised PBS mock-infected mice were indistinguishable. In contrast, both the FDG and 2-DG-probe accumulated in the lungs of mice infected with *A. fumigatus*. Measurements of mice injected with the control dye revealed no accumulation, neither before nor after infection.
Discussion: We demonstrate that inflammation during IA can be monitored non-invasively by PET/CT and IVIS using labelled glucose as tracer. Both methods allow quantification of immune cell accumulation in the lung.

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EKV04

Malaria parasites hijack human factor H to protect from complement-mediated lysis in the mosquito midgut

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As part of the innate immune system, the human complement is a first line of defence against microbial infections. A number of blood-borne pathogens have evolved strategies for complement evasion, including mimicking host surfaces. While the intraerythrocytic blood stages of the malaria parasites are protected from any attack by the human complement, the parasite gametes have to exit the enveloping erythrocyte prior to fertilization in the mosquito midgut and are then vulnerable to factors of the blood meal, including human immune cells and complement factors. We here show that the human malaria parasite *Plasmodium falciparum* binds the regulatory protein factor H (FH) during sexual reproduction in the mosquito midgut, which protects the extracellular gametes from complement-mediated lysis in the bloodmeal. The alternative pathway of the human complement is active for approximately 1 h post feeding, while FH becomes degraded in the mosquito midgut after 8 h. Macrogametes and young zygotes bind FH, resulting in C3b inactivation. FH comprises 20 complement control protein modules, and binding of FH to malaria gametes appears to be primarily mediated by modules 1-7. Loss of FH-mediated protection results in impaired gametogenesis und reduced zygote numbers, and transmission blocking assays with antibodies against factor H significantly reduce transmission to the mosquito. This is the first time that a protozoan parasite is demonstrated to camouflage using FH in order to avoid complement-mediated lysis. Our findings might lead to novel approaches in the design of transmission blocking vaccines.

EKV05

Human natural killer cells mediate cytotoxic effects against *Candida albicans*

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Objectives: *Candida albicans* is the most common cause of invasive candidiasis. Natural Killer cells (NKC) represent an important population of cytotoxic immune cells with well known antitumor and antiviral properties. Up to now there is only little data on the interaction of human NKC with the pathogenic fungus. However, after NKC depletion mice have been shown to be more susceptible to systemic *Candida* infections. On the basis of these observations we investigated if there are direct or indirect effects of human NK Cells against *Candida albicans*.
Methods: Primary human PBMCs were isolated from buffy coat of healthy donors by standard ficoll gradient (Biochrom AG). Untouched NKC were separated by MACS using the NK cell isolation kit (Miltenyi Biotec), according to manufactorer's instructions. The isolated cells were primed with a cytokine cocktail (IL-2, IL-15, IFN-alpha, IFN-beta). Viable or heat-inactivated *Candida albicans* SC5314 was used for confrontation and receptor surface expression was quantified by FACS-analysis. Reduction of fungal viability was determined by an XTT assay. To analyze the mechanism of NKC mediated fungal killing, cells were treated with blocking antibodies (anti-FasL, anti-TRAIL), strontium chloride (inducing granula release) or concanamycin A (perforin inhibitor). Bioplex or ELISAs were used for quantification of secreted markers.
Results: Co-incubation of NKC and *C. albicans* resulted in down-regulation of Toll-like receptors (TLR2, TLR4) , cellular adhesins (CD11a, CD54) and activation markers/ ITAM-Bearing Receptors (CD25, CD314, CD335) on NKC but in parallel a marked increase of the degranulation marker CD107a as well as secretion of NK cell specific cytokines was detected. Activation of NKC was most prominent for *C. albicans* filaments. Degranulation was significantly reduced for inactivated yeast forms, whereas heat-inactivated filaments resulted in levels comparable to viable wild-type *C. albicans*.
The cytokine mediated priming of primary human NKC led to an enrichment of cytotoxic granules Perforin and Granzyme B. Using these activated NKC, XTT-based killing assays showed a significant decrease of fungal viability after a coincubation for four hours. Blocking experiments suggest that the cytotoxic effect is perforin dependent while FasL/TRAIL receptor pathways are not involved. In parallel to these direct effects, NKC may interact with other immune cells since cytokine release shows an upregulation of neutrophil activating cytokines.
Conclusions: Our observations suggest that NKC may play an important role in the first line defense of *C. albicans* infections. In this context, direct and indirect antifungal effects of NKC are currently further investigated.

EKV06

Characterisation of the innate immune activation in response to *Candida albicans* in a human whole blood model of infection

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Introduction: Fungal sepsis is an increasing clinical problem with high mortality rates. Although the incidence of fungal sepsis has increased recently, diagnosis is still difficult and mainly relies on the detection of the pathogen (*Candida spp.*) in blood cultures. Using a human whole blood model of infection we are investigating the innate immune activation in response to *Candida albicans* in a situation similar to *in vivo*. These analyses will allow identifying immune markers which might be potentially useful in clinical diagnostics.
Methods: Pathogen is added to human whole blood in different concentrations and patterns of immune activation are monitored in time course analyses. Initial assays included differential FACS staining to define activation patterns on immune cell populations. In parallel we quantified humoral markers of immune activation. Further investigation included the characterisation of the whole blood transcriptome in response to *C. albicans*. The obtained data will be integrated using bioinformatic methods to generate a network of innate immune activation during early steps of fungal sepsis.
Results: Fungal infection resulted in a strong and predominant activation of neutrophils detectable by an increased surface expression of markers for early activation (CD69, HLA-DR) and degranulation (CD11b, CD63, CD66b). As neutrophil granules contain important antifungal effector molecules, the release of these granules in response to *C. albicans* was studied. As expected, myeloperoxidase from primary and lactoferrin from secondary granules could be detected in plasma. NK cells also showed increased surface levels of activation marker CD69 whereas several receptors (FcγRIII, NKG2D, NKp46) decreased markedly over time during fungal infection. Furthermore, *C. albicans* blood infection induced a predominant increase in total number of platelets aggregated with granulocytes as well as PAC1 and CD62P surface expression. Multiplex assays revealed a secretion of proinflammatory and regulatory cytokines (IFN-γ, TNF-α, IL-1β, IL-6) as well as chemokines (MCP-1, MIP-1α/β, Rantes, IL-8, IP-10) corresponding to cellular activation as detected by FACS analyses. We also measured a strong increase in plasma concentration of anaphylatoxins (C3a, C4a, C5a) that are produced as a part of the activation of the complement system.
Conclusion: The human whole blood model of infection enables the analysis of innate immune activation in response to *C. albicans* in a situation similar to *in vivo*. Advantages over isolated primary cells are the physiological conditions and the possible communication between immune cell populations. Furthermore we are currently performing comparative analyses with Gram-positive and Gram-negative bacteria to identify pathogen-specific activation patterns and get a global view on innate immunity of microbial blood infections.

EKV07

Exploring molecular mechanisms of apoptosis in the protozoan parasite *Leishmania major*

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Protozoans like Leishmania can undergo apoptosis. In this process phosphatidylserine (PS) is externalized and DNA fragmentation occurs. During leishmanial apoptosis an endonuclease (Lm Endo G) is activated, involved in DNA degradation. In addition, an anti apoptotic peroxidase (Lm APX) has been found that inactivates reactive oxygen species (ROS). However, the protein machinery mediating apoptosis in mammalian cells like caspases or members of the bcl-2 families are completely absent in Leishmania. In this study we want to identify the mechanism and the proteins that are involved in the protozoan apoptotic program. We used axenic amastigote cultures and promastigote cultures and induced apoptosis using staurosporine treatment. First we performed quantative FACS and microscopical analyses, subsequently we used mass-spectrometry and characterized the most abundant proteins that were either up or down regulated upon apoptosis induction.
Using timelapse imaging we observed that induction of apoptosis in eGFP expressing promastigotes resulted first in rounding of the promastigotes, then in loss of eGFP and subsequently in externalization of PS. A more detailed investigation of apoptosis revealed that reactive oxygen species (ROS) are generated in a parasite stage and apoptosis induction dependent manner. Focusing on Lm Endo G we found an upregulation upon apoptosis induction. In the same context, we observed a downregulation of anti apoptotic Lm APX.
Mass spectrometry quantified a total of 707 leishmanial proteins: In promastigotes apoptosis induced the upregulation of 3 proteins and the downregulation of 7 proteins, for amastigotes this was 6 and 22 respectively. We have selected 3 proteins for which specific inhibitory compounds exist. Currently we are investigating these proteins for a role in leishmanial apoptosis.

EKV08

Identification of apoptosis-like markers and regulators in *Toxoplasma gondii* Programmed Cell Death (PCD)

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Programmed cell death (PCD) is a common feature of multicellular organisms and has been well characterized. In recent years, there has been increasing evidence for the occurrence of PCD in distinct unicellular organisms as well, however, there is only little information about putative PCD in *Toxoplasma*. Therefore, identification of apoptosis-like markers and molecules that might play a role in *T. gondii* PCD need to be elucidated.
TUNEL assays followed by flow cytometry were used to determine DNA strand breaks after treatment of the parasite with several proapoptotic inducers (staurosporine and miltefosine). The results showed that staurosporine and miltefosine increased the number of TUNEL-positive cells to various extents and time-dependently from 24 to 72 hours post treatment. Treated parasites showed a slight reduction in cell size as compared to untreated parasites. Such reduction in cell size was most obvious in miltefosine-treated parasites which also displayed the highest percentage of TUNEL-positive parasites. Furthermore, treatment with staurosporine and miltefosine led to a higher percentage of Annexin V-positive parasites exposing phosphatidylserine on the cell surface as compared to untreated controls. Some proteins that are involved in mammalian apoptosis pathways have been identified by in silico analysis of the *T. gondii* genome database. In this project, we have been focusing on *T. gondii* Bax inhibitors. In silico analysis of the *T. gondii* genome demonstrated the presence of putative Bax inhibitor genes, named *T. gondii* Bax inhibitor-1, -2, and -3 (TgBI-1, TgBI-2 and TgBI-3). Amino acid sequence analysis of TgBI-1, TgBI-2 and TgBI-3 showed 33-38% identity with human BI-1. Putative TgBI-1, TgBI-2 and TgBI-3 contain 7-9 transmembrane domains and have a molecular weight of 37,974 Da, 29,322 Da and 37,345 Da, respectively. In order to unravel possible functions of *T. gondii* BI proteins in regulation of apoptosis, we heterologously expressed TgBIs in HeLa cells. Stable transfection of the genes encoding TgBI-2 and TgBI-3 into HeLa cells was confirmed by immunofluorescence microscopy as well as Western Blot analysis. Hoechst staining showed lower levels of chromatin condensation in TgBI-2-expressing HeLa cells than in HeLa wild type cells after treatment with brefeldin A and staurosporine. Measurement of caspase 3/7 activity demonstrated an inhibitory effect of TgBI-2 in HeLa cells after treatment with either staurosporine, brefeldin A, tunicamycin or thapsigargin. Moreover, TgBI-2- and TgBI-3-expressing HeLa cells showed lower induction of hypoploid DNA (subpeak G0/G1) after treatment with inducers as above. These findings suggest the existence of apoptotic-like cell death in *T. gondii* and protective effects of TgBI proteins in regulation of apoptosis.

EKV09

Histone deacetylase inhibitors rescue macrophages from the *Toxoplasma gondii*-mediated inhibition of IFN-γ responsiveness

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Toxoplasma gondii is an eukaryotic parasite that is highly prevalent in warm-blooded hosts including 25 -30% of humans worldwide. Infection of individuals with premature or suppressed immunity can lead to life-threatening toxoplasmosis whereas in immune competent hosts, infection is mostly asymptomatic but leads to parasite persistence for the host's life. Cell-mediated immunity including the production of IFN-γ is critical for control of the obligatory intracellular parasite. However, by using genome-wide transcriptome analysis, we have shown that *T. gondii* leads to global unresponsiveness of murine macrophages to IFN-γ and this may be essential for establishing a long-term infection. Analysis of the underlying mechanisms revealed normal activation and nuclear translocation of STAT1 in *Toxoplasma*-infected macrophages though a defect in the activation of STAT1-responsive promoters in response to IFN-γ. Isolation and characterization of proteins that bound to oligonucleotides containing a STAT1-responsive consensus sequence indicated a defective recruitment of non-muscle actin and Brg-1 in *Toxoplasma*-infected macrophages, i.e. components of chromatin remodelling complexes. In addition, chromatin immunoprecipitation showed that *T. gondii* inhibited the acetylation of histone H4 *in vivo*, i.e. a major prerequisite for chromatin remodelling prior to transcription. Histone acetyl transferase (HAT) activity towards histone H4 and H3 was not generally decreased in nuclear extracts of *T. gondii*-infected macrophages. Furthermore, histone deacetylase (HDAC) activity was not increased after infection. Nonetheless, treatment of macrophages with HDAC inhibitors MS-275 or sodium butyrate prior to stimulation with IFN-γ largely abrogated the inhibition of IFN-γ-induced MHC class II and CIITA gene expression exerted by *T. gondii*. Together, these data unravel novel insights into the mechanisms by which *T. gondii* evades IFN-γ-regulated immunity and open up novel strategies for therapeutic intervention against toxoplasmosis.

EKV10

A C-terminal hyphal activation motif (CHAM) of the transcription factor Tec1p is essential for biofilm formation and nematode infection in *Candida albicans*

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Candida albicans is the most frequent human fungal pathogen and infections range from superficial mucosal lesions to life-threatening invasive mycoses with a high mortality rate. Hyphal growth, biofilm formation and the transcriptional regulation of putative virulence traits for adaptation to the host environment are key issues during *C. albicans* pathogenesis. Hyphal formation is a prerequisite for penetration of host tissues causing systemic candidiasis, and the formation of biofilms allows this pathogen to persist in the host and to increase resistance against anti-fungal compounds. The transcription factor Tec1p, a member of the TEA transcription factor family, plays an important role in morphogenetic development in *C. albicans.* In order to define a structure function relationship for the Tec1p protein, we integrated modified open reading frames (ORFs) of the wild type *TEC1* under the control of the *C. albicans* adapted reverse Tet-dependent transactivator (rtTA) into the *tec1/tec1* mutant. We screened the series of such *C. albicans* mutants with modifications of the Tec1p for their ability of *in vitro* biofilm formation and also examined the hyphal formation and survival of a nematode host using C. *elegans* as an infection model. In biofilm assays, the full length *TEC1* ORF was able to restore the ability for biofilm formation ability in the *tec1/tec1* mutant background after Tet induced gene expression, and the biofilm mass was similar to the biofilm formed by the wild type SC5314 strain, whereas the TEA domain mutant and mutants lacking the proposed C- terminal motif were impaired in biofilm formation under similar growth conditions. Previously, *C. elegans has* been successfully used as an

infection model host to understand the virulence mechanisms of various human pathogenic microorganisms. Further *in vivo* investigations with *C. elegans* infection model revealed that the *C. albicans TEC1* mutant lacking this C-terminal motif shows less virulence against nematodes in terms of hyphal invasion and killing of the nematodes. This *TEC1* mutant library screening revealed that besides the already known essential regions as the TEA domain and the important N-terminal end of Tec1p, also a C-terminal motif of the transcription factor plays an important role in morphogenetic development. It can be demonstrated that this C-terminal motif of Tec1p is essential for yeast-hyphae transition, infection and killing of *C. elegans* nematodes and biofilm formation.

EKV11

Candida albicans hypha form fosters binding of Fibronectin and Platelets

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Introduction: *Candida albicans* is a dimorphic fungus that can switch between the yeast and hypha form. The ability to germinate and grow in hypha is an important virulence factor *in vivo*. Platelets can bridge the innate with the adaptive immunity. To attack microorganisms, platelets store microbicidal and immune stimulatory proteins in their α-granules. Platelet integrins and their plasma ligands can govern both binding to the pathogen and activation of platelets to release their granules. Here we tested the hypothesis that fibronectin (which is also stored in platelet α-granular and bound by platelet integrins) supports the host defense against *C. albicans* infections.
Material and methods: Platelet-rich plasma from citrated anticoagulated blood samples was washed with phosphate buffered salt solution (PBS) pH 6.5 containing 2 U/ml apyrase. Platelet pellet was resuspended in Tyrode buffer pH 7.3 containing $MgCl_2$, $CaCl_2$ and 10 µM of the cell tracker dye CMFDA (Molecular Probes). Isolated plasma fibronectin was labeled with alexa fluor 488. *C. albicans* (SC5314) cultured over night in yeast extract pepton glucose medium at 30°C was washed in PBS pH 7.3 and starved for 1 h at room temperature. Germination was induced at 37°C by adding 10 % serum. Samples were taken over the germination time. For flow cytometry *C. albicans* yeast and hypha forms with an optical density (OD) of 0.4 were labeled for 1h with 40 µg/ml Fn-alexa fluor 488 and $1x10^7$ fluorescently stained platelets under static conditions.
Results: Only *C. albicans* hypha bound fibronectin. The mean fluorescence intensity (arbitrary units, AU) increased from 140 AU to 380 AU after 60 min and 120 min time of germination. Furthermore, germination of *C. albicans* increased binding of platelets up to 40 % and 90 %, after 90 min and 180 min, respectively. Moreover, pretreatment of *C. albicans* hypha with fibronectin enhanced their binding to platelets.
Conclusion: Hypha of *C. albicans*, an important virulence factor, is attacked by platelets. Fibronectin, a ligand of the platelet integrins ($\alpha_5\beta_1$, $\alpha_5\beta_1$, $\alpha_{IIb}\beta_3$) opsonizes the hypha form of *C. albicans* and fosters platelet binding. Hence, platelets and fibronectin, with its intrinsic multivalent binding site can support the platelet mediated host defense and immune response against *C. albicans*.

EKV12

Molecular characterization of components implicated in cell wall integrity signaling in *Aspergillus fumigatus*

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The organization and biosynthesis of the fungal cell wall, an essential structure that defines the organism's shape and shields it from environmental stress, is regulated by the cell wall integrity (CWI) signaling pathway. Its central MAP kinase module was subject of diverse studies and is conserved from *Saccharomyces cerevisiae* to filamentous fungi, such as *Aspergillus spp*.. On the contrary, components upstream of this MAP kinase module are well characterized in *S. cerevisiae* but largely unknown in *Aspergillus spp*.. We have identified, localized and functionally characterized homologues of the *S. cerevisiae* cell surface sensors ScWsc1 - ScWsc3 and ScMid2/ScMtl1 in *A. fumigatus*. Moreover, we have analyzed the roles of three Rho-GTPases that have been implicated in CWI signaling in other fungi. We show that AfRho1 is essential and conditional downregulation of *rho1* or deletion of *rho2* or *rho4* result in a severely impaired CWI. In addition, our data indicate fundamental functional differences on the sensor level of the CWI pathways of *S. cerevisiae* and *A. fumigatus*.

ERP01

Trends in multidrug resistance among *Escherichia coli* and klebsiellae in Germany

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Introduction: Escherichia coli (ECO) and klebsiellae are among the most common pathogens causing infections in in- and outpatients. Antimicrobial agents such as 3rd generation cephalosporins (3GC), carbapenems (carba) and fluoroquinolones (FQ) are essential drugs to treat serious illness. Treatment outcome, however, has increasingly been threatened by the emergence and dissemination of resistant (R) strains (multi-drug R [MDR] strains in particular). The objective of this study was to document changes in the R patterns of ECO, Klebsiella pneumoniae (KPN) and Klebsiella oxytoca (KOX) in Germany between 1995 and 2007.
Methods: Susceptibility data of clinical isolates collected in 21, 20, 21, 22 and 21 laboratories that participated in five surveillance studies conducted by the Paul-Ehrlich-Society in 1995, 1998, 2001, 2004 and 2007, respectively, were analysed. MICs of antimicrobial agents were determined by the broth microdilution procedure according to the standard DIN ISO and interpreted by EUCAST criteria. The CLSI MIC method using ceftazidime (CAZ) +/- clavulanic acid (CLA) and cefotaxime (CTX) +/- CLA was employed as screening test for ESBL-producing isolates. MDR was defined as antimicrobial R to more than two of the following four drug classes: 3-cef, carba, FQ, aminoglycosides.
Results: A total of 3,964 isolates primarily recovered from urine (33%), wounds (20%), respiratory specimens (19%), and blood (11%) were tested. There were 890 (24%) ICU isolates and 3,074 (76%) non-ICU isolates. Single R to ciprofloxacin (CIP) in ECO, KPN, and KOX increased from 6% (26/449), 4% (9/224) and 2% (2/83), respectively, in 1995 to 28% (115/418), 13% (25/190) and 17% (17/101) in 2007. R to CTX in all three species rose from 1-4% in 1995 to 11-15% in 2007. Concurrently, an increase in the percentage of strains showing the ESBL phenotype was observed, while R to carba (ertapenem [ERT], imipenem [IMP], meropenem [MEM]) remained uncommonly. MDR rates for each of the species and study years are shown in the table. Of the 36 MDR isolates recovered in 2007, none were susceptible (S) to CTX and CIP, 8% to gentamicin and 39% to piperacillin-tazobactam. In contrast, carba were active against MDR isolates, except one KOX (MICs of ERT, IMP, MEM 16-64 mg/l).
Conclusions: Single R to CIP and CTX, representing FQ and 3GC, respectively, as well as MDR in ECO and klebsiellae increased between 1995 and 2007. In contrast, activity of carba did not change over the last 10-15 years.

Figure 1

	phenotype	1995	1998	2001	2005	2007
ECO	R to >1 drug class	3.8%	4.2%	6.6%	10.8%	15.6%
	MDR	0%	0,2%	1.2%	3.4%	5.3%
KPN	R to >1 drug class	1.8%	3.7%	5.8%	5.2%	10.5%
	MDR	0%	0%	2.6%	1.6%	6.3%
KOX	R to >1 drug class	1.2%	7.7%	0.9%	10.9%	8.9%
	MDR	0%	0%	0%	0.8%	2.0%

ERP02

No evidence of an overall increase in vancomycin MICs of German MRSA isolates between 2001 and 2009

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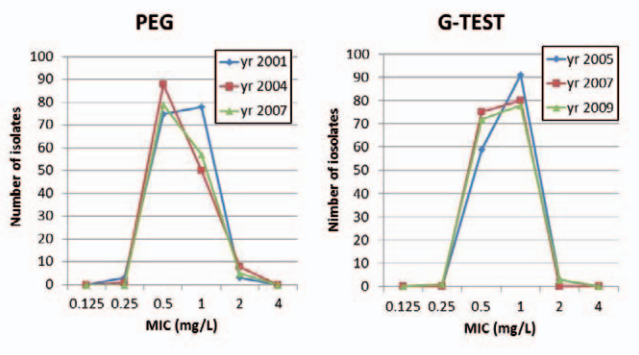
Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of infections in hospitals (healthcare-acquired MRSA; HA-MRSA). Moreover, isolates of community-associated MRSA (CA-MRSA) have been identified in recent years. Vancomycin (VAN), a glycopeptide, has been in clinical use for more than 50 years to treat MRSA infections. Evidence in the literature indicates that treatment success with VAN decreases as the VAN minimum inhibitory concentration (MIC) of the MRSA strain increases. A target area under the serum drug concentration–time curve (AUC)/MIC ratio of ≥400 was predictive of *S. aureus* eradication. However, using a conventional dosages of VAN (e.g., 1 g every 12 hours [15 mg/kg every 12 hours]), it is unlikely to achieve this ratio if the VAN MIC is ≥1 mg/L. The objective of this study was to evaluate two collections of MRSA isolates recovered between 2001 and 2009 for a possible increase of VAN MICs (MIC creep).
Method: MIC data of VAN for a total of 909 MRSA isolates collected in six surveillance studies were analysed. Three studies each were conducted by the Paul-Ehrlich-Society (PEG: years 2001, 2004 and 2007) and the German Tigecycline Study Group (G-TEST: years 2005, 2007 and 2009). The network of PEG and G-TEST comprised ca. 25 and 15 laboratories, respectively. In all studies, MICs were determined by the broth microdilution procedure in accordance with the standard DIN ISO and interpreted by EUCAST criteria. Microdilution trays containing VAN in a dried form were purchased from two manufacturers, MERLIN Diagnostika (PEG) and TREK Diagnostics (G-TEST). A VAN MIC ≤2 mg/L was defined as susceptible. Data of PEG and G-TEST were separately analyzed for MIC creep. Comparison of the log2 MIC population distributions achieved in the three study years was made using ANOVA, that provides a statistical test of whether or not the means of the MIC distributions are all equal. A P value of
Results: All isolates were susceptible to VAN. In either analysis, the comparison of the three MIC distributions did not reveal a shift towards higher VAN MICs over time (PEG, P=0.6021; G-TEST, P=0.1144) (see Figure). The proportion of isolates with a MIC ≥1 mg/L varied between 39.5% and 50.5% in PEG and between 51.6% and 61.4% in G-TEST.
Conclusions: This work provides no evidence of an overall increase in VAN MIC of MRSA isolates between 2001 and 2009. However, about 50% of the MRSA isolates had a MIC ≥1 mg/L, which may lead to treatment failure using standard dosages of VAN.

Figure 1



ERP03

Susceptibility of major bacterial pathogens to tigecycline in Germany, 2004-2009

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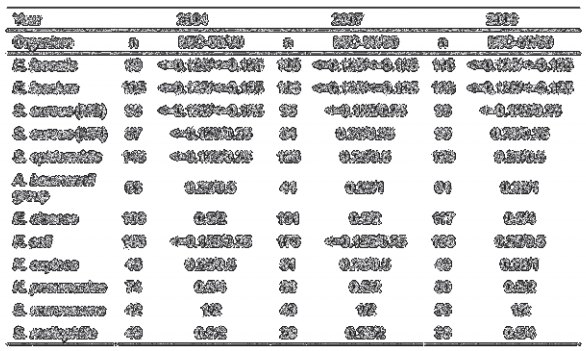
Introduction: Following regulatory approval for use in complicated skin and skin structure infections and complicated intraabdominal infections, tigecycline (TGC) was introduced in Germany in 2006. Data of the German Tigecycline Evaluation Surveillance Trial (G-TEST) conducted between 2005 and 2009 were analyzed for the susceptibility of most important aerobic Gram-pos. and Gram-neg. pathogens to TGC and comparators.
Methods: The susceptibility of isolates of four Gram-pos. and seven Gram-neg. species consecutively collected in three surveillance studies conducted in cooperation with the same 13 laboratories in 2005, 2007, and 2009 (G-TEST I-III) were tested. Each laboratory collected appr. 250 pathogens from hospitalized patients. MICs were determined in a central laboratory using the microdilution method according to the standard ISO 20776-1:2006. EUCAST breakpoints were applied to all antibiotics for interpretation. ESBL-producing organisms were identified by CLSI criteria.
Results: A total of 3,248 isolates recovered from wounds (n=1,573; 48.4%), the peritoneal cavity (n=567; 17.5%), and blood (n=1,108; 34.1%) were tested. Based on MIC-50/90 values, TGC demonstrated unchanged (within +/- one dilution) in vitro activity against all species tested (see Table). In contrast, a considerable increase of resistance (R) to beta-lactams and fluoroquinolones (FQ) was noted for members of the Enterobacteriaceae family. Between 2004 and 2009, R to FQ in *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae* increased from 20% to 27%, 6% to 11%, and 7% to 23%, respectively. The increase of R to cefotaxime in *E. coli* and *K. pneumoniae* went along with a rise of ESBL-producing strains, from 6% to 11% and from 5% to 14%, respectively. R to imipenem in *Acinetobacter baumannii* group isolates was not observed in 2005, but was 18% and 8% in 2007 and 2009, respectively. The rate of vancomycin-resistant strains among *Enterococcus faecium* isolates varied between 9% and 19%.
Conclusion: TGC retained its very good in vitro activity against all Gram-pos. and Gram-neg. organisms tested after the introduction in Germany. Against a background of pathogens that are frequently resistant to various antibiotic classes, TGC remains an important treatment option.

Figure 1



ERP04

Pharmacodynamics of three carbapenems considering the current susceptibility patterns of drug-resistant Gram-negative bacteria in Germany

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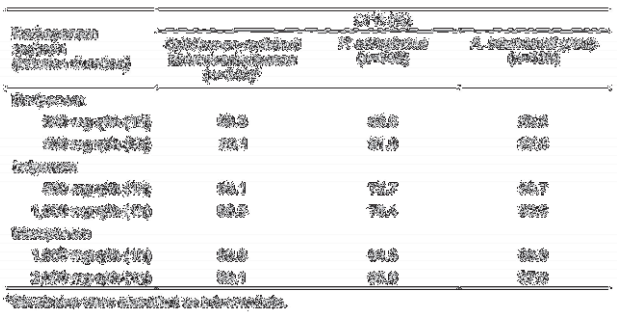
Introduction: The carbapenems (CARBA) are widely used for empiric therapy of serious infections involving drug-resistant Gram-neg. pathogens like *P. aeruginosa*, *A. baumannii* or Enterobacteriaceae expressing an ESBL phenotype or a stably de-repressed AmpC ß-lactamase. The objective of this study was to predict the probabilities of attaining targeted pharmacodynamic exposure for clinically used dosing regimens of doripenem (DOR), imipenem/cilastatin (IPM), and meropenem (MEM) against a German collection of contemporary drug-resistant Gram-neg. pathogens using Monte Carlo simulation (MCS).
Methods: Isolates from hospitalised patients were collected in 15 medical microbiology laboratories during two resistance surveillance studies conducted in 2007 and 2009. Bacterial groups investigated were *P. aeruginosa* (n=149), *A. baumannii* group (n=139), and ceftriaxone (CRO)-resistant Enterobacteriaceae (n=349). MCS modelled 5,000 patients receiving the following dosing regimens: DOR 500 mg every 8 hours (1-h infusions), DOR 500 mg every 8 hours (4-h infusions), IPM 1,000 mg every 8 hours (1-h-infusions), IPM 500 mg every 6 hours (1-h-infusions), MEM 1,000 mg every 8 hours (1-h-infusions), and MEM 2,000 mg every 8 hours (1-h-infusions). Pharmacodynamic targets to predict efficacy were the free drug concentrations above the MIC for 40% of the dosing interval. Simulations were based upon a normal distribution of volume of distribution and total body clearance. Mean pharmacokinetic parameters and their distribution were taken from published studies. The cumulative fraction of response (CFR) was calculated considering the MIC distribution data for each of the three groups of organisms.
Results: All CARBA regimens achieved CFR rates of >98% against CRO-resistant Enterobacteriaceae, but varied between 80% and 88% against *A. baumannii* group isolates (see Table). Against *P. aeruginosa*, the highest CFR (91.9%) was obtained with DOR 500 mg every 8 hours (4-h infusions) and the lowest (72.7%) with IPM 500 mg every 6 hours.
Conclusion: Considering the current susceptibility patterns of drug-resistant Gram-neg. pathogens in Germany, all clinically used dosing regimens of CARBA are sufficient to achieve a probability of 95% or greater success against CRO-resistant Enterobacteriaceae. In contrast, only the DOR prolonged-infusion and MEM 2,000 mg dosing regimens achieved at least 90% CFR against *P. aeruginosa*. None of the CARBA regimens achieved 90% CFR against *A. baumannii* group.

Figure 1



ERP05

Trends of antibiotic resistance in outpatients and hospitalized patients in Thuringia/Germany 2007-2010

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Background: Current regional antibiotic susceptibility data from hospitalized patients and outpatients are needed to support local networks for the defence against the spreading of multiresistant bacteria.
Material and methods: Results of routine suseptibility data for twelve antibiotic agents of a total of 90.043 isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* from four Thuringian microbiology laboratories were analysed retrospectively for the years 2007 to 2010. Susceptibility testing was performed by agar diffusion test or breakpoint-testing according to the German standard DIN 58940. The results were compared to nationwide German data published online by the Robert Koch-Institute on the Antibiotic Resistance Surveillance website (ARS, https://ars.rki.de).
Results: Resistance rates of *S. aureus* from outpatients against penicillin, oxacillin, and ciprofloxacine were significantly lower than those of hospitalized patients ($p<0.05$). The rate of methicillin resistant *S. aureus* (MRSA) in outpatients rose from 6.9% in 2007 to 8.2% in 2010, whereas in both large (>500 beds) and small (<500 beds) hospitals, the MRSA rate remained constant at around 15.0%. Resistance rates of *E. coli* against ampicillin, cefotaxime, gentamicin, and ciprofloxacin were significantly lower in outpatients ($p<0.05$). The resistance rate of *E. coli* against cefotaxime as a marker for extended spectrum betalactamase (ESBL) production was constant at about 9.0% in hospitals but quadrupled in outpatients from 1.3% in 2007 to 5.9% in 2010. Resistance rates of *P. aeruginosa* against piperacillin, ciprofloxacine, and ceftazidime were significantly lower in outpatients ($p<0.05$), whereas gentamicin was ineffective against *P. aeruginosa* in 6.0% of both in- and outpatients. *E. faecalis* isolates remained highly susceptible to ampicillin.
Conclusions: As of December 2010, in Thuringia/Germany, MRSA rates in both outpatients (8.2%) and hospitals (15.0%) remained well below nationwide levels as determined by the ARS (12.7% and 24.6%, respectively). However, there was an alarming increase of ESBL rates in outpatients from 1.3% in 2007 to 5.9% in 2010.

ERP06

The German EHEC outbreak strain ST678 harbours the CTX-M-15 extended-spectrum β-lactamase (ESBL)

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Background: In May 2011 a nationwide outbreak with Enterohemorrhagic *E. coli* (EHEC) was observed in Germany with the main focus in northern Germany. Probably the outbreak was caused by contaminated vegetables.
Methods: An EHEC outbreak isolate was sent to the National Reference Laboratory for multidrug-resistant gramnegative bacteria for further characterization. Susceptibility testing was performed by disk diffusion and Etest. Virulence genes of *E. coli* pathovars were tested by PCR. MLST typing and PCR based determination of the phylogenetic group were performed. The presence of several β-lactamase genes was tested by PCR and the genetic environment characterized. Transconjugation and transformation of a resistance plasmid was attempted. S1 restriction followed by pulsed-field gel electrophoresis and subsequent Southern Blot hybridization was done to determine a plasmidic localization of resistance genes.
Results: The EHEC outbreak isolate was isolated from a female patient from Northern Germany and tested positive for *stx2* and *aggR*, but negative for *stx1* or *eae*. Typing revealed the MLST type ST678 and *E. coli* phylogenetic group B1. The isolated displayed an ESBL phenotype and $bla_{\text{TEM-1}}$ as well as $bla_{\text{CTX-M-15}}$ could be identified. The genetic environment of $bla_{\text{CTX-M-15}}$ showed IS*Ecp1* upstream and ORF477 downstream of the gene. No carbapenemase gene like KPC, VIM, OXA-48 or NDM was found. Furthermore, resistance to tetracycline and sulfamethoxazole-trimethoprim was observed. S1 restriction revealed a plasmid of ca. 90 kb. Transconjugants and transformants with a plasmid harbouring $bla_{\text{CTX-M-15}}$ where obtained. No co-resistance to tetracycline or sulfamethoxazole-trimethoprim could be demonstrated.
Conclusion: Within two days after receipt of the EHEC outbreak isolate the molecular basis of its unusual ESBL phenotype could be clarified and was immediately reported to the German health authorities. EHEC strains harbouring CTX-M-15 have never been reported before. The presence of resistance genes in diarrheagenic bacterial strains is worrisome since it might contribute to an enhanced spread of resistance determinants, e. g. by horizontal gene transfer.

ERP07

Antimicrobial susceptibility of ocular isolates in Germany, 2004-2009

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Introduction: Antimicrobial resistance has increased over the past two decades in Germany, as in many other countries. Data on the in vitro susceptibility of pathogens isolated from ocular infections, however, are scarce. Two multi-centre studies were carried out in order to assess the occurrence of resistance amongst ocular isolates against antibacterial agents used topically in the treatment of superficial eye infections.
Methods: Bacterial isolates were prospectively collected in two surveillance studies conducted in cooperation with the same 23 medical

laboratories in 2004 and 2009. Minimal inhibitory concentrations were determined in a central laboratory using the broth microdilution method according to the German DIN standard (now DIN-EN-ISO 20776-1:2006). Interpretive criteria applied were EUCAST clinical breakpoints for systemic use of antibacterial agents as well as EUCAST epidemiological cut-off values (ECOFFs), where available. Susceptibility data of nine antibacterial agents were analysed: levofloxacin (LVX), moxifloxacin (MXF), ofloxacin (OFX), gentamicin (GEN), kanamycin (KAN), azithromycin (AZI), erythromycin (ERY), chloramphenicol (CHL) and oxytetracycline (OTE). Additionally, the susceptibility of staphylococci to oxacillin (OXA) was determined.

Results: A total of 2,006 ocular isolates (2004, n=933; 2009, n=1,073) primarily recovered from conjunctival swabs were tested. *S. aureus* was the predominant pathogen in either study (28-34%), followed by *H. influenzae* (13-18%) and *S. pneumoniae* (15-16%). Patient related data were comparable in both studies, with two third of the isolates derived from out-patients and more than half of the patients being *S. aureus* isolates collected in 2004 and 2009, 10.3% and 9.8%, respectively, were OXA-resistant (i.e. MRSA). Resistance to CHL, GEN and KAN among *S. aureus* decreased between 2004 and 2009, while resistance to ERY and the fluoroquinolones (FQ) remained unchanged (see Table). No significant changes in the rates of resistance were observed for *S. pneumoniae* and *H. influenzae*. In contrast, isolates with reduced susceptibility to LVX and OFX among Enterobacteriaceae increased from 0% and 1% in 2004 (n=103) to 1.9% and 5.8% in 2009 (n=104), respectively.

Conclusion: Overall, studied aminoglycosides and CHL displayed lower resistance rates in 2009 as compared to 2004, while resistance rates of FQ remained either unchanged or slightly increased.

Figure 1

Table: Proportion of antibiotic resistance (%)

	S. aureus		*S. pneumoniae*		*H. influenzae*	
Year	2004	2009	2004	2009	2004	2009
No. tested	310	325	155	175	117	125
Levofloxacin	13.5	15.4	0.7	0	0	0
Moxifloxacin	n.t.	13.1	n.t.	0	n.t.	0
Ofloxacin	14.1	15.4	0.7	0	0	1.0
Kanamycin	34.5*	6.5*	n.a.	n.a.	n.a.	n.a.
Gentamicin	15.4*	4.3*	n.a.	n.a.	0	0
Azithromycin	n.t.	15.0	n.t.	11.5	n.t.	1.0
Erythromycin	27.0	28.0	16.4	16.0	0	1.0
Chloramphenicol	6.5*	1.0*	0	0	0	2.1
Oxytetracycline	n.t.	9.2	n.t.	7.5	n.t.	1.0

Resistance rates determined by ECOFFs are given in italic.
n.t., not tested; n.a., no clinical or epidemiological breakpoint available;
*Significant difference ($p < 0.05$)

ERP08

Description of a novel IMP carbapenemase in two *Pseudomonas aeruginosa* isolates from Germany

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Introduction: The worldwide increase of multidrug-resistance in gramnegative bacteria has become an important clinical challenge. Carbapenem resistance in *P. aeruginosa* can be caused by a variety of mechanisms, however the worldwide spread of carbapenemases is especially important. Most carbapenemases in *P. aeruginosa* belong to Ambler class B metallo-β-lactamases (MBL). Here we describe a novel IMP-type MBL that was found in two isolates from patients in Germany.

Methods: Susceptibility to antibiotics was determined by disk diffusion and Etest. The presence of a MBL was determined by EDTA combined-disc-tests, MBL Etest and by a bioassay based on cell-free extracts. A modified Hodge-Test was performed. PCRs and subsequent sequencing were performed for VIM, IMP, NDM, GIM, SIM, SPM, AIM, DIM and KHM. Clonal relatedness of the isolates was determined by RAPD-PCR. Integron structures were analysed by PCR and sequencing.

Results: Two *P. aeruginosa* isolates from single patients hospitalized in different geographic regions in Germany were sent to the National Reference Laboratory for Multidrug-resistant Bacteria for further characterization. The isolates were resistant to piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem (MICs 32 mg/L and 128 mg/L, respectively), meropenem and doripenem (MICs >32 mg/L) as well as to gentamicin, tobramycin, amikacin, ciprofloxacin and levofloxacin. Both isolates were susceptible for colistin with a MIC of 0,38 mg/L. The modified Hodge-test was positive for imipenem, meropenem and ertapenem. The isolates showed synergy with EDTA in the combined disk test and the MBL Etest. An unspecific result due to toxicity of EDTA could be excluded by a positive bioassay based on cell-free extracts. With consensus primers for IMP a 587 bp fragment was amplified and sequenced. The sequence showed only 88 % similarity to IMP-8 and IMP-24 and revealed 18 amino acid substitutions relative to IMP-8 (23 compared to IMP-1). The isolates also harbour at least one class I integron containing the gene for an aminoglycoside adenylyltransferase (aadA6).

Conclusion: The two clinical isolates harbour a novel IMP-type carbapenemase. It clearly shows a MBL phenotype, while the MIC differences for imipenem are likely to be the result of additional mechanism for imipenem resistence. With 23 amino acid substitutions compared to IMP-8, the identified IMP-type carbapenemase shows significant differences to any known IMP-type carbapenemase.

ERP09

Carbapenemases arrived in Germany: Report of the National Reference Laboratory for multidrug-resistant gramnegative bacteria

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Introduction: Multidrug-resistance in *Enterobacteriacea*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since no innovative antimicrobial drugs against gramnegative bacteria will be introduced into clinical practice within the next five years. Among all resistance mechanisms the worldwide spread of carbapenemases is the most worrisome development. However, the correct identification of carbapenemases is challenging for the microbiological laboratory.

Methods: The National Reference Laboratory for Multidrug-Resistant Gramnegative Bacteria offers the free service of carbapenemase detection in bacterial isolates with elevated carbapenem MICs. All isolates are tested by a wide array of phenotypic and molecular methods. A bioassay based on cell-free extracts allows the detection of still unknown β-lactamases.

Results: A total of 808 isolates were sent to the National Reference Laboratory in 2010 mainly for investigation for carbapenemases, but also for clarification of the resistance mechanism to 3rd generation cephalosporins or molecular strain typing. Several different carbapenemases could be detected, including OXA-48 (n = 59), KPC-2 (n = 31), KPC-3 (n = 26), VIM-1 (n = 37), VIM-2 (n = 20), VIM-4 (n = 4), VIM-5 (n = 1), IMP-7 (n = 2), IMP-8 (n = 12), IMP-16 (n = 1), NDM-1 (n = 5), NDM-2 (n = 1), GIM-1 (n = 4), OXA-23 (n = 69), OXA-72 (n = 2) and OXA-58 (n = 6). In *Enterobacteriacea* most Carbapenemases were found in *K. pneumoniae*, especially OXA-48, KPC-2 and KPC-3. VIM-2 was the most frequent carbapenemase in *P. aeruginosa* and OXA-23 in *A. baumannii*.

Discussion: Almost all carbapenemases found worldwide have arrived in Germany. However, the molecular epidemiology in Germany with a predominance of OXA-48 differs significantly from observations made in other countries like Greece, Israel, USA or the United Kingdom. An ongoing surveillance of resistance determinants is necessary, especially for infection control and diagnostics.

ERP10

Dissemination of multidrug-resistant Enterobacteriaceae producing NDM-1 and OXA-48 carbapenemases - examples from Oman

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Objectives: The successful worldwide dissemination of carbapenemase producing Enterobacteriaceae is of special concern because of limited therapeutic options and higher mortality. While the dissemination of carbapenemase KPC is predominantly bound to distinct *Klebsiella pneumoniae* strains, spread of OXA-48 and NDM-1 enzymes is mainly due to conjugative transfer of different plasmids carrying these carbapenemase genes and facilitating the transmission among many enterobacterial species. Here we report on molecular analysis of multidrug-resistant Enterobacteriaceae isolates from Oman.

Methods: Carbapenem resistant isolates of *Klebsiella* spp. (n=13), *E. coli* (n=4) and *Enterobacter cloacae* (n=1) were collected from three hospitals in Oman between September 2010 and February 2011. Relevant resistance genes were identified by PCR and sequencing. Conjugation experiments and PFGE-typing were performed.

Results and conclusion: The carbapenemase OXA-48 was identified in five isolates. Ten Isolates produced NDM-1 and OXA-181 was identified in two isolates. Three patients recently travelled to India and Pakistan before their admission to the hospital. XbaI-macrorestriction analysis revealed seven closely related NDM-1 isolates and three isolates with OXA-48 indicating several events of clonal transmission of carbapenemase

producing strains in two hospitals. Furthermore additional ESBL CTX-M-15 (n=14) and CTX-M-24 (n=3) as well as other beta-lactamases (TEM-1, SHV-11, OXA-1, OXA-9) were detected in the majority of isolates. The bla_{OXA-48} genes were successfully transferred into recipients and plasmids of ca. 60kb size were isolated from transconjugants. The bla_{NDM-1} genes were located on conjugative plasmids of different size. The reduced carbapenem MIC values of transconjugants indicate the occurrence of further carbapenem resistance mechanisms like increased production of efflux pumps or loss of porins (outer membrane proteins, OMPs) in the clinical isolates.
In Germany the NDM-1 carbapenemase was identiefied in only 13 isolates in the last three years. Although carbapenemase producing Enterobacteriaceae are still rare in many European countries there is an urgent need of surveillance to prevent further spread of these multidrug-resistant pathogens.

ERP11

How should we treat double - and triple - resistant *Helicobacter pylori* in Germany?

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Introduction: When the first line eradication therapies for *H. pylori* containing amoxicillin, clarithromycin and metronidazole fail, further second or third line schemes containing amoxicillin, levofloxacin and rifabutin are used. To find out which rescue eradication therapy is most successful in a given pattern of antimicrobial resistance, the National Reference Centre (NRC) for *H. pylori* conducts this treatment advice study.
Material and Methods: Since 2006 the NRC for *H. pylori* offers individual treatment recommendations for eradication therapy based on the isolates' resistance pattern and patient's risk factors (e.g. pre-treatment, smoking, diabetes and allergy). Data on the chosen treatment scheme and eradication control are collected three months later, to evaluate the outcome.
Results: Written treatment advices were made for 1539 patients. Outcome data were available for 767 patients. The resistance rates before treatment recommendation were 8% for metronidazole (MZ), 13% for clarithromycin (CLA), 41% for double- (MZ & CLA) and 23% for triple-resistant strains (MZ & CLA & fluoroquinolones). Patients harbouring double-resistant strains were best treated with a therapy containing proton pump inhibitor (PPI) + levofloxacin + rifabutin (70% success/9% failure). In patients with triple-resistant *H. pylori* isolates PPI + amoxicillin + rifabutin was most successful (54% success/15% failure).
Discussion: We have effective but not optimal treatment schemes even for double- and triple-resistant *H. pylori* strains. The use of the second and third line drugs levofloxacin and rifabutin should be based always on the results of antimicrobial susceptibility testing. A strong indication for eradication therapy is needed to use these drugs with a severe side-effect profile.

ERP12

Prevalence of CTX-M producing strains among *Escherichia coli* isolates from hospitalized patients in Germany, 2005-2009

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Introduction: CTX-M type ESBLs have emerged since the detection of the first *bla*CTX-M in 1990. They are now the most widespread ESBL enzymes in Enterobacteriaceae isolates worldwide. So far, 115 CTX-M type enzymes have been described. Dominance and frequency of the various types, however, have been shown to be different between geographical regions. The objective of this study was to evaluate the prevalence of CTX-M type ESBLs among German *Escherichia coli* (ECO) isolates between 2005 and 2009.
Methods: 300, 292 and 297 ECO recovered from 15 laboratories during three multicentre studies conducted in 2005, 2007 and 2009, respectively, were studied. Susceptibility testing was performed by the broth microdilution procedure according to the ISO standard. Antibacterial agents tested were various cephalosporins as well as piperacillin-tazobactam (P/T), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IPM) and tigecycline (TGC). ESBL-producing organisms were identified according to CLSI criteria. Isolates expressing an ESBL phenotype were further characterized by isoelectric focusing (IEF), amplification of *bla*CTX-M genes using specific primers for CTX-M groups, and sequencing.
Results: The percentage of CTX-M producing ECO was 4.7% (14/300) in 2005, 11.6% (34/292) in 2007 and 11.4% (34/297) in 2009. In 2005, 50% (7/14) of the CTX-M producing ECO harboured CTX-M-1, 35.7% (5/14) CTX-M-15 and 14.3% (2/14) CTX-M-14, while 26.5% (9/34), 58.8% (20/34), 11.8% (4/34), 2.9% (1/34) and 2.9% (1/34) of the CTX-M positive isolates produced CTX-M-1, -15, -14, -9 and -2, respectively, in 2007. In 2009, 44.1% (15/34), 50% (17/34) and 5.9% (2/34) of isolates expressed CTX-M-1, CTX-M-15 and CTX-M-14, respectively. Resistance to P/T, CIP and GEN was detected in 3, 9 and 4 strains, respectively, in 2005, in 4, 24 and 14 strains, respectively, in 2007, and in 1, 26 and 10 strains, respectively, in 2009. All isolates were susceptible to IPM and TGC.
Conclusions: Our data suggest that the rate of CTX-M producing strains among ECO isolates doubled between 2005 and 2007, but remained unchanged between 2007 and 2009. The most frequent CTX-M type in 2005 was CTX-M-1, while in 2007 and 2009 CTX-M-15 predominated. Resistance of CTX-M producing ECO to carbapenems or TGC was not detected.

ERP13

Emergence and Dissemination of ESBL and Fluoroquinolone Resistance in Enterobacteriaceae - the RESET Network

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Background and Objective: *Enterobacteriaceae* have been shown to play a crucial role in the transmission of antimicrobial resistances. The most prominent enterobacterial species in humans and animals are *Escherichia* (*E.*) *coli* and *Salmonella* (*S.*) *enterica.* Dissemination of antibiotic resistance can be due to the transfer of resistant bacteria between different host organisms or by horizontal transfer of resistance properties between isolates of the same or different species and genera within and beyond the family *Enterobacteriaceae*. Especially, resistance to β-lactams mediated by production of Extended-spectrum beta-lactamases (ESBL) and resistance to (fluoro)quinolones influenced by different plasmid-mediated quinolone resistance determinants are of concern since they limit dramatically the therapeutic options in both, veterinary and human medicine. Therefore the RESET research consortium aims at assessing the impact of different origins, transmission routes and pathogen attributes on the risk for humans being exposed to ESBL-producing and (fluoro)quinolone-resistant *Enterobacteriaceae*.
Studies and Methods: The RESET consortium consists of 10 network partners and 5 associated partners from human and veterinary medicine, fundamental and application-oriented research and epidemiology. The RESET network will perform different studies to provide indispensable, reliable information on
- the pathogen distribution in specific human and animal populations as well as in food, feed and the environment,
- the occurrence of ESBL and PMQR (plasmid-mediated quinolone resistance) genes within the pathogen populations,
- the transmission pathways between the different populations (animal-, environment-, human- or pathogen-related), and
- the impact of the use of antibiotics in animals and humans in respect to the resistance situation.

Conclusion: In 2008, the Federal Ministry of Health (BMG) started the DART (Deutsche Antibiotika-Resistenzstrategie) initiative for prevention and control of further spread of antimicrobial resistances in human and veterinary medicine. The RESET network includes different interacting and complementary studies on factors associated with the dissemination of emerging resistance properties in *Enterobacteriaceae* from humans, animals and the environment. As such, the results expected from RESET will be a substantial contribution to DART.

ERP14

ContFiltSE: 8020 Resistance frequencies to 18 antibiotics in *vanA*- and *vanB*-positive *E. faecium* isolates from German hospitals between 2008 and 2010

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ContFiltSE:8020

Introduction: We report resistance frequencies (based on minimal inhibitory concentrations, MICs) to 18 antibiotics of total 490 *vanA*- and 404 *vanB*-positive clinical isolates of *Enterococcus* (*E.*) *faecium* and 13 *vanA*- and 16 *vanB*-positive *E. faecalis* isolates from 2008, 2009 and 2010 .

Materials and Methods: The MICs of the bacteria to antibiotics tested were determined by broth microdilution using cation-adjusted Mueller-Hinton broth (for some antibiotics special requirements are necessary). The MICs of few strains were additionally determined by E-test. The evaluation of MICs was done according to the MIC breakpoints of EUCAST published in January 2011.

Results: The resistance frequencies to the antibiotics of about 150 - 200 VanA-type *E. faecium* and 100 - 160 VanB-type *E. faecium* isolates per year are presented. Vancomycin resistance in *E. faecalis* is very seldom: we received 3 - 6 VanA and 3 - 9 VanB isolates per year between 2008 and 2010.

Nearly all clinical Vancomycin-resistant *E. faecium* strains are resistant to ampicillin (MICs >8 mg/L) and possess high level resistance to ciprofloxacin (>16 mg/L); typical features of hospital-associated clonal types. High level resistance (HLR) to gentamicin (MICs >256 mg/L) occurs in 40 - 57% of the strains (with increasing frequencies between 2008 and 2010); HLR to streptomycin (>512 mg/L) in 36 - 58% of the strains. Resistances to antibiotics of last resort such as linezolid (0 - 4%), tigecycline (0 - 2%), daptomycin (0%) or quinupristin/dalfopristin (1 - 18%) are very seldom or of moderate frequency.

Discussion/Conclusions: The presented frequencies of antibiotic resistances among Vancomycin-resistant *E. faecium* add important data to existing resistance surveillance systems such as ARS or GERMAP; the latter collect resistance data of all enterococci not especially focusing on VRE. Our data show frequent co-resistances to therapeutically relevant antibiotics (ampicillin, aminoglycosides) for enterococci; resistances to antibiotics of last resort are still rare. Comparably high rates of *vanB* VRE suggest a changing epidemiology of *van* genotypes in recent years.

ERP15

Fosfomycin susceptibility in multidrug-resistant *Enterobacteriaceae*

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Background: The prevalence of multidrug-resistant gramnegative pathogens is increasing worldwide. Especially the spread of carbapenemase producing *Enterobacteriaceae* is worrisome since those strains are usually also resistant to most other antibiotics. No new antibiotics with activity against gramnegative bacteria will be introduced within the next five years. Therefore, testing the activity of older and rarely used antibiotics is of high clinical impact.

Methods: *Enterobacteriaceae* (n = 107) with elevated carbapenem MICs were sent to the National Reference Laboratory for Multidrug-Resistant Gramnegative Bacteria from several laboratories all over Germany and investigated for the presence of carbapenemases by the modified Hodge test, synergy testing using EDTA and boronic acid, a microbiological assay using cell-free extracts and PCR followed by sequencing. Resistance testing of fosfomycin was performed by Etest, disk diffusion and agar dilution on Glucose-6-phosphate containing Mueller-Hinton agar.

Results: In a collection of multidrug-resistant *K. pneumoniae* (n = 57), *E. coli* (n = 18), *E. cloacae* (n = 12), *K. oxytoca* (n = 7) and further species various β-lactamases like OXA-48 (n = 24), KPC-2 (n = 12), KPC-3 (n =11), VIM-1 (n = 15), CTX-M-15 (n = 9) were found.

Regarding agar dilution as the reference method the MIC_{50} was 8 mg/L and the MIC_{90} was 512 mg/L. Overall 77 isolates (72%) were susceptible according to EUCAST with an MIC ≤ 32 mg/L. Susceptibility rates were 66.7% for *K. pneumoniae*, 88.9% for *E. coli* and 75% for *E. cloacae*, respectively. Etest gave concordant results in 92.5% and showed seven very major errors (6.5%) and one major error (0.9%). Applying a threshold of ≥ 16 mm as susceptible in disk diffusion (fosfomycin 200 µg) as recommended by CLSI for *E. coli* eleven very major errors (10.3%) and no major errors were found.

Conclusions: Using EUCAST criteria and agar dilution a diverse collection of multidrug-resistant *Enterobacteriaceae* strains showed fosfomycin susceptibility of 72%. With less than 10% very major or major errors Etest is an acceptable method for fosfomycin susceptibility testing.

ERV01

Molecular analysis of daptomycin resistance in *Staphylococcus aureus*

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Objectives: After European approval of Daptomycin in 2006 selection of daptomycin-non-susceptible *S. aureus* isolates had been demonstrated in vitro and in vivo. To date, we have collected a total of 99 clinical daptomycin-resistant *S.aureus* strains (MIC ≥ 2,0 mg/l) sent to the German Reference Centre for Staphylococci. In this study we report on increasing frequency of resistance and molecular characterization of resistant isolates.

Methods: All isolates sent to the German Reference Centre for Staphylococci were tested for antibiotic susceptibility by broth microdilution and were assigned to clonal complexes by *spa*-typing. For a subset of isolates antibiotic susceptibility was tested by automatic susceptibility testing (VITEK) and E-test in comparison.

To elucidate the molecular basis of resistance 20 representative isolates from different geographic origin were subjected to Solexa whole genome sequencing of pooled DNA-samples. Additionally, the DNA of two clinical isolates obtained before and after unsuccessful Daptomycin treatment was sequenced by Solexa whole genome sequencing. All sequence data were mapped to appropriate *S. aureus* reference strains. Based on the whole genome data the *mpr*F locus, which was previously shown to be associated with daptomycin resistance was re-sequenced in 64 daptomycin resistant and susceptible isolates.

Results: Among isolates received at the National Reference Centre frequency of daptomycin resistance increased from 0.7% in 2008 to 1.8% in 2011. Most isolates were MRSA, predominantly belonging to clonal complexes CC5/ST225 and CC22. Automatic susceptibility testing (VITEK) generally indicated slightly elevated MIC values as compared to broth microdilution and E-test. Sequence analysis of whole genome data revealed a small number of SNPs putatively associated with resistance. However, after re-sequencing the corresponding loci in a larger number of resistant and susceptible isolates strong association of resistance to mutations in a certain locus could be confirmed only for *mpr*F. Re-sequencing of *mpr*F revealed point mutations in 44 out of 46 resistant isolates (MIC ≥ 2µg/ml).

Conclusion: Results corroborate the role of *mpr*F-mutations in resistance development towards daptomycin; however further studies must elucidate the complete mechanism leading to reduced susceptibility.

ERV02

Spread of KPC-3 producing *K. pneumoniae* belonging to the international clone ST258 in Germany

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Background: The worldwide spread of carbapenemase producing *Enterobacteriaceae* is worrisome since therapeutic options for such strains are severely limited and no new antibiotics will be licensed in the near future. Data regarding carbapenemases in Germany are scarce. This study focuses on the spread of KPC-3 carbapenemases from different geographic regions in Germany.

Methods: Strains with elevated carbapenem MICs were sent to the National Reference Laboratory for multidrug-resistant gramnegative bacteria for further characterization. Carbapenemases were detected by phenotypic tests, PCR and subsequent sequencing. MICs for carbapenems were determined by Etest. The genetic environment of bla_{KPC} was characterized by a set of specific PCRs. Plasmid characterization was performed by S1 nuclease restriction and conjungative transfer of the plasmid was tried.

Results: KPC-3 was detected in *K. pneumoniae* (n=27) and *E. coli* (n=1) single patient strains from ten different geographic regions all over Germany. The MICs for ertapenem fell in a range from 0.5 to >32 mg/L, for imipenem from 0.38 to >32 mg/L and for meropenem from 0.25 to >32 mg/L. The modified Hodge-Test and the combined disk test with boronic acid was positive in all strains. PFGE demonstrated relatedness of 26 *K. pneumoniae* strains and MLST typing of five exemplary strains revealed ST258. PCR detected bla_{OXA-1} in 14 and bla_{TEM} in 25 strains. Transconjugation experiments were successful only in one strain, however the presence of bla_{KPC} on plasmids could be demonstrated by restriction with S1 endonuclease and Southern Blot. PCRs demonstrated that bla_{KPC-3} was surrounded by Tn4401a showing a 100-bp-deletion in all cases.

Conclusions: KPC-3 producing *K. pneumoniae* belonging to the ST258 clone are spreading also in Germany. Similar to reports from the USA and Israel *bla*KPC-3 is surrounded by the Tn4401a isoform. The low MIC for

carbapenems in some strains emphasizes that detection of carbapenemases can be challenging.

ERV03
Resistance to Zink in MRSA

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Objective: The finding of a gene coding for a Zn^{2+} porter (*czr*C) contained by an SCC*mec*V element in livestock associated MRSA CC398 and the previous use of zinkoxide together with organic acids in pig feeding raised the more general question of coselection of MRSA by use of zinkoxide. In this context it is of interest whether *czr*C and the already known resistance gene *cad*A (both code for an ATPase based efflux) are disseminated among different clonal lineages of MRSA prevalent in humans and livestock.
Methods: Defined collection of MRSA from humans (HA-MRSA, CA-MRSA) and from other animals (LA-MRSA) attributed to different clonal lineages. Phenotypic susceptibility testing for $ZnCL_2$ by means of microbroth MIC in cation adjusted MH broth. PCR demonstration of Zn^{2+} resistance genes *cad*A and *czr*C.
Results: *czr*C was exclusively associated with MRSA ST398 (20/27 from pigs; 21/23 from humans exposed to pigs; 10/20 infections; 10/10 infections in humans) and contained by a defined SCC*mec*-element with tandem *ccr*C. It was not found in HA-MRSA and CA-MRSA from central Europe clonal lineages. *cad*A is typical for CA-MRSA ST30 (as to be expected!) and ST80, it was also detected in 2/5 CA-MRSA ST22 but not in CA-MRSA ST1, ST5, ST8, and ST152. Among HA-MRSA CC5 *cad*A was only found in isolate of ST228, not in isolates ST225 and ST5. Among HA-MRSA CC8 only the two isolates attributed to ST239 contained *cad*A, and one among 5 isolates attributed to CC45.
Discussion: It can not be excluded that the previous use of zinkoxide ions in conjunction with organic acids as feed additives has selected for *czr*C and possibility coselected MRSA ST398. In human medicine zinkoxid is widely used in hospitals as constituent of ointments for care of skin. As the most widely disseminated MRSA (MRSA ST22, IV, ST225, II) remain susceptible to Zn^{2+} there is obviously no selection in favour of MRSA. With respect to multiresistant but methicillin susceptible *S. aureus* ST30 ("80/80 complex") in the past and use of zinkoxide ointments containing also boronic acid this situation might have been different.

ERV04
Susceptibility of Gram-negative pathogens to colistin and comparators in Germany

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Introduction: The emergence of healthcare associated infections caused by multidrug-resistant Gram-negative bacteria has become an increasing problem over the past 20 years. Colistin, a 'forgotten' antibiotic, has attracted attention, because of its activity against multidrug-resistant Gram-negative bacteria. It is, however, not active against Proteeae and *Serratia* spp. The objective of this study was to evaluate the susceptibilities of clinical isolates of *Enterobacter cloacae* (ECL), *Escherichia coli* (ECO), *Klebsiella pneumoniae* (KPN), *Klebsiella oxytoca* (KOX), *Pseudomonas aeruginosa* (PAE) and *Acinetobacter baumannii* group (ABA) to COL and comparators.
Methods: A total of 1,529 isolates were prospectively collected from 21 microbiology laboratories across Germany, which participated in the surveillance study conducted by the Paul-Ehrlich-Society in November 2007. MICs of COL, ciprofloxacin (CIP), ceftazidime (CAZ), gentamicin (GEN) and meropenem(MEM) were determined by the broth microdilution procedure according to the standard DIN ISO. MICs were interpreted by EUCAST criteria, if available. Breakpoints of COL were <=2 mg/L (susceptible) and >2 mg/L (resistant). The CLSI MIC method using CAZ +/- clavulanic acid (CLA) and cefotaxime +/- CLA was employed as screening test for ESBL-producing isolates.
Results: Isolates were primarily recovered from wounds (26%), respiratory specimens (26%) urine (20%) and blood (9%). There were 460 ICU isolates and 1,069 non-ICU isolates. Of the ECO, KPN and KOX isolates, 11.5%, 10.5% and >15.8% showed an ESBL-phenotype. MIC-50 and MIC-90 values (mg/L) are displayed in the table. Of the ECO isolates, 100% were susceptible (S) to COL and MEM, while 72% were S to CIP and 87% to GEN. Of the ECL isolates, 91% and 9% were S and resistant to COL, respectively. Susceptibilities of ECL to CAZ, CIP, GEN and MEM were 52%, 89%, 88%, and >99%, respectively. Among KPN and KOX isolates, susceptibilities to COL and MEM were 98-100%, while susceptibilities to CIP and GEN were 82-85% and 94-96%, respectively. COL was the most active drug against ABA and PAE, with susceptibility rates of 100% for either species. For ABA, susceptibilities of comparators varied from 78% (CIP) to 95% (MEM). Susceptibility rates of PAE for comparative agents were as follows: GEN (91%) followed by CAZ (87%), MEM (85%) and CIP (75%).
Conclusions: Overall, susceptibility to COL seems to be high among Gram-negative pathogens in Germany. COL may be considered as an important treatment option.

Figure 1

	ECO	ECL	KPN	KOX	ABA	PAE
n	418	191	190	101	113	516
COL	≤0.25/≤0.25	≤0.25/2	≤0.25/0.5	≤0.25/≤0.25	≤0.25/0.5	0.5/1
CAZ	≤0.25/2	1/≥64	≤0.25/4	≤0.25/1	4/32	2/16
CIP	≤0.06/≥16	≤0.06/1	≤0.06/4	≤0.06/4	0.25/≥16	0.25/4
GEN	1/≥32	0.5/4	0.5/1	0.5/1	1/16	2/4
MEM	≤0.25/≤0.25	≤0.25/≤0.25	≤0.25/≤0.25	≤0.25/≤0.25	≤0.25/1	0.5/8

ERV05
Antibiotic susceptibility of invasive bacterial pathogens *Neisseria meningitidis* and *Haemophilus influenzae*

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Bacterial pathogens *Neisseria meningitidis* and *Haemophilus influenzae* are infrequent causes for invasive disease manifesting as sepsis or meningitis; their respective incidence rates in Germany were 0.47 and 0.26 per 100,000 in 2010. We present antibiotic susceptibility data over a period of nine and three years for Meningococci and *H. influenzae*, respectively. Using gradient test systems (Etest), 3283 non-duplicate meningococcal strains were tested for susceptibility against benzylpenicillin, rifampicin, and ciprofloxacin between 2002 and 2010; 417 meningococcal strains were also assayed for cefotaxime susceptibility. Between 2008 and 2010, 298 non-duplicate *H. influenzae* strains were assayed for ampicillin susceptibility. In meningococci, no temporal trends in minimal inhibitory concentrations (MIC) or susceptibility categorization according to EUCAST were observed; 85.5%, 13.8%, and 0.7% of strains were categorized as susceptible, intermediate, and resistant to penicillin, respectively. Rate of rifampicin-resistant and -susceptible isolates was 0.4% and 99.6%, respectively. Only 0.1% of strains were not susceptible to ciprofloxacin and all tested strains were susceptible to cefotaxime. MIC to penicillin depended on serogroup: using serogroup B and rare serogroups (e.g. A, Z, X, 29E) as reference category with a mean MIC of 0.056 mg/L, serogroups C, W-135, and Y showed significant deviations with MICs of 0.074, 0.107, and 0.067 mg/L, respectively. Interestingly, 51.9% of serogroup W-135 strains were intermediate susceptible to penicillin. Most resistant isolates (82.6%) were of serogroup C, while serogroups W-135 and Y had no resistant isolates at all. Although no resistance was observed for cefotaxime, cefotaxime MICs showed a weak yet significant linear correlation with penicillin MICs (r^2 0.23). Accordingly, mean cefotaxime MICs were 0.003, 0.005, and 0.011 mg/L for penicillin susceptible, intermediate, and resistant isolates, respectively. Due to low rate of non-susceptibility, no meaningful analyses were possible for antimicrobials rifampicin and ciprofloxacin in meningococci. In *H. influenzae*, 10.4% were resistant to ampicillin according to EUCAST. A downard temporal trend was observed between 2008 (16.7%) to 2010 (8.4%). Resistance was significantly more common in non-typeable *H. influenzae* lacking a polysaccharide capsule (13.0%) compared to capsulate strains (3.7%). Rate of beta-lactamase production was 9.3%. Three ampicillin-resistant strains (1.1% of total) lacked beta-lactamase activity and were thus categorized as beta-lactamase-negative-ampicillin-resistant (BLNAR).

ERV06

Antimicrobial resistance in *K. pneumoniae* - Results from the German ARS System 2009

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Introduction: High prevalence of resistance in invasive *K. pneumoniae* isolates from hospitalised patients to third-generation cephalosporins (3GC), fluoroquinolones (FQ) and aminoglycosides (AG) and a high level of combined resistance to these classes in most European countries was a major result of the European Antimicrobial Resistance Surveillance Network (EARS-Net) Report 2009. In Germany, the ARS (Antimicrobial Resistance Surveillance)-System allows to study resistance and resistance patterns in further settings: urine and respiratory samples from inpatients as well as from outpatients.
Methods: Analysis is based on non-duplicate isolates of *K. pneumoniae* collected in 2009 by nine laboratories covering 160 hospitals and 865 practices. Species identification and antimicrobial susceptibility testing is performed by VITEK 2, results are evaluated according to CLSI guidelines. Isolates are classified as resistant to an antibiotic class if they show resistance to one of its agents: 3GC: ceftazidime or cefotaxime or ceftriaxone; FQ: ciprofloxacin or levofloxacin; AG: gentamicin or tobramicin or amikacin. The distinct class resistances are combined to resistance patterns.
Results: The sample is composed of 5,548 non-duplicate *K. pneumoniae* isolates from inpatients (blood: 299; respiratory samples: 1,118; urine: 4,131) and 2,666 isolates from outpatients (respiratory samples: 173; urine: 2,493). Results are displayed in table 1. Proportions of resistance are highest against FQ in all subsets ranging from 21.1% in blood cultures from inpatients to 4.6% in respiratory samples from outpatients followed by 3GC (16.4% to 3.5%) and AG (13.4% to 2.3%). Regarding resistance against the three antibiotic classes simultaneously reveals that triple resistance is the most frequent pattern in all inpatient subsets reaching 10% in blood cultures, 9.3% in respiratory samples and 6.8% in urines. In outpatient samples, single resistance against FQ is the most frequent pattern followed by triple resistance in second place accounting for 3.0% in urine samples and 1.2% in respiratory samples.
Discussion: While the EARS-Net-Surveillance limited to invasive isolates from hospitalised patients captures the sector with highest levels of antimicrobial resistance in *K. pneumoniae,* the extended approach of ARS reveals an emerging problem in outpatient care that physicians should be aware of, even if resistance proportions might be overestimated as samples are more likely to be taken from pre-treated patients.

Figure 1

	Hospitalised patients			Outpatients	
	Blood	Respiratory	Urine	Respiratory	Urine
	n = 299	*n = 1,118*	*n = 4,131*	*n = 173*	*n = 2,493*
Resistance to antibiotic class					
Fluorochinolones (FQ)	21.1	15.0	16.1	4.6	9.9
Third-gen. cephalosporines (3GC)	16.4	14.5	13.8	3.5	7.1
Aminoglycosides (AG)	13.4	10.9	8.9	2.3	4.3
Resistance patterns					
Fully susceptible	**76.3**	**82.4**	**80.5**	**93.6**	**88.0**
Single resistance	**6.7**	**4.1**	**7.0**	**3.5**	**5.7**
FQ	5.0	2.5	4.5	1.7	4.0
3GC	1.7	1.5	2.1	1.2	1.4
AG	0.0	0.1	0.4	0.6	0.3
Double resistance	**7.0**	**4.2**	**5.6**	**1.7**	**3.2**
FQ + 3GC	3.7	2.7	4.0	1.2	2.3
FQ + AG	2.3	0.5	0.8	0.6	0.6
AG + 3GC	1.0	1.0	0.9	0.0	0.4
Triple resistance FQ + AG + 3GC	**10.0**	**9.3**	**6.8**	**1.2**	**3.0**

Table 1: Resistance in *Klebsiella pneumoniae* isolates from hospitalised and outpatients in Germany 2009: proportions of resistance against fluoroquinolones, third-generation cephalosporines and aminoglycosides and frequencies of combined resistance to these antibiotic classes stratified by origin: blood culture, respiratory and urine samples

ERV07

10 Years *Resinet* study - An update

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The German multicentre study *ResiNet* was launched by the National Reference Centre (NRC) for *H. pylori* in 2001. The aims of this ongoing surveillance study are to monitor and to prospectively investigate risk factors for the development of antimicrobial resistance in *H. pylori*. 14 microbiological centres spread over the whole country each collaborating with 2 to 7 gastroenterologists participate in the study. At the end of December 2010, 1423 *H. pylori* strains were tested. Most of the patients suffered from gastritis (44%) followed by ulcus ventriculi or duodeni (19%). The overall resistance rates are 42% for metronidazole (MZ), 29% for clarithromycin (CLA) and 17% for fluoroquinolones (FQ). Double- (MZ & CLA) and triple-resistant strains (MZ & CLA & FQ) were found in 19% and 5%, respectively. Antimicrobial pre-treatment is the main risk factor for resistance development. After one course of *H. pylori* eradication therapy resistance rates reach 50% (MZ), 59% (CLA) and 31% (MZ & CLA). More than one eradication therapy led to resistance rates of 82% (MZ), 74% (CLA) and 64% (MZ & CLA). When we started the study in 2001, 32% of the strains were resistant against MZ. At the end of 2010, 42% of the strains were resistant against MZ. For CLA the resistance rates increased from 15% to 29%. In 2007, we started a follow up study to investigate the outcome of our *ResiNet* patients. 451 patients have been examined so far. The majority of the patients (87%) were treated with an eradication therapy. The most common scheme used was the French triple therapy. After treatment 70% of the patients reported an improvement of their complaints or no complaints anymore. Eradication control was performed in 80% of the patients using the urea-breath test predominantly. The eradication rate for all patients was 61%. 19% of the patients were still *H. pylori* positive after treatment, which might be explained by the high rate of clarithromycin resistance (29%) within this population. In conclusion, these results underline the hypothesis that development of resistance in *H. pylori* in contrast to other pathogens is dependent on treatment strategies in the individual patient. Further, the data clearly demonstrate that culture and susceptibility testing from gastric biopsy specimen is mandatory already after the first treatment failure.

ERV08

MRSA-Surveillance in Germany: data from the Antibiotic Resistance Surveillance System (ARS) and the mandatory surveillance of MRSA in blood and cerebrospinal fluid.

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Introduction: Surveillance is an indispensible component of most strategies for control and prevention of MRSA. Data from ARS, the German Antibiotic Resistance Surveillance system, and mandatory reporting of MRSA in blood cultures are presented.
Methods: ARS: Within a framework of a voluntary laboratory based surveillance system resistance data of all clinical pathogens and sample types from hospital and ambulatory care are transmitted electronically to the central data-base of the national public health institute (Robert Koch Institute, RKI).
Mandatory reporting: Since the 1st July 2009, all microbiological laboratories are obliged to report the detection of MRSA in blood and cerebrospinal fluid to the local public health authority from where it is reported to the RKI via the state health department. MRSA-incidences have been calculated by using denominators based on the German population 2009. The relationship between regional incidences and MRSA-rates of eight federal states has been assessed by application of spearman rank correlation.
Results: ARS: In 2009, 10 laboratories supplying 226 hospitals and 3222 medical practices provided resistance data from 37076 Staphylococcus aureus isolates. MRSA-rates stratified by hospital and outpatient care are presented in table 1.
Mandatory reporting: From 01.07.2009 to the 30.06.2010 3504 cases, accounting for an incidence of MRSA-bacteraemia of 4.2/100,000 inhabitants/year, were reported. Males >65 years present with the highest incidence (23.5/100,000 inhabitants/year). Stratification by federal states shows considerable regional differences (range:1.8-6.4/100,000 inhabitants/year), which were positively correlated to regional MRSA-rates (6.7%-27.3%) generated in ARS (spearman correlation coefficient: 0.74; p=0.04).
Conclusion: While mandatory reporting of MRSA-bacteraemia serves as an indicator of the situation in hospital care, ARS additionally provides a view on the outpatient setting. Expectedly, in ambulatory care MRSA-rates are considerably lower compared to hospital care. Nevertheless, the results indicate that in Germany MRSA plays a relevant role in the outpatient setting as well. Particularly, high resistance rates in urine samples suggest that further investigations about the epidemiological context in which infections occur and patient characteristics are necessary to assess possible consequences for antibiotic management.

Figure 1

Table1: MRSA-rates 2009 generated in ARS from hospital and outpatient care stratified by ward type, medical speciality, sample type, age and sex.

	Hospital care		Outpatient care	
		Number of		Number of
	MRSA-Rate	*S. aureus* isolates[a]	MRSA-Rate	*S. aureus* isolates
	%	n	%	n
total	**21.6**	**22,251**	**12.5**	14,825
Ward type				
General ward	20.7	19,916	n.a.[b]	n.a.
Intensive Care Unit	24.8	2,335	n.a.	n.a.
Medical department				
General ward				
Surgical	18.2	6,624	n.a.	n.a.
Medical	24.4	9,248	n.a.	n.a.
Other	19.2	4,044	n.a.	n.a.
Intensiv Care Unit				
Surgical	31.8	358	n.a.	n.a.
Medical	23.4	872	n.a.	n.a.
Other	23.7	1,105	n.a.	n.a.
Sample type[c]				
Urine	33.0	2,412	22.4	1,289
Other swaps	26.9	7,528	10.4	8,216
Respiratory samples	26.7	3,528	12.9	498
Wound swaps	23.8	7,623	16.1	4,880
Blood	22.1	1,610	21.4	70
Punctures	17.8	715	9.6	157
Other	25.3	3,304	24.7	178
Agegroup				
<=15	4.8	2,044	3.0	2,004
16-59	13.6	6,262	6.5	6,282
>=60	27.7	13,945	21.2	6,539
Gender				
Male	18.5	7,150	12.1	6,045
Female	16.5	5,257	10.2	5,395
Unknown	26.7	9,844	16.6	3,385

[a]Copy-strain-rule: 1 isolate per species/patient /quarter

[b]not applicable

[c]Copy-strain-rule: 1 isolate per species/patient/sample type/quarter

ERV09

Comparable high carriage rates of Extended-spectrum beta-Lactamases producing *Escherichia coli* in birds of prey from Germany and Mongolia.

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Introduction: Several studies give evidence for the carriage of Extended-spectrum beta-Lactamases (ESBL) producing *E. coli* in the feces of wild birds even in remote areas, where the presence of antibiotic selection pressure is assumed to be very rare. In the present study we compared wild avian fecal *E. coli* isolates from remote areas in Mongolia with those from an intensively agriculturally used area in Germany with respect to the rate of ESBL production and their clonal relatedness.
Methods and Results: 281 cloacal swabs, mostly taken from juvenile birds, were screened for *E. coli*, resulting in a number of 108 isolates. By means of a selective growth medium ESBL-production was confirmed in 13.8% (n=9) German and in 11.6 % (n=5) of the Mongolian avian *E. coli* isolates. In both groups we determined ESBL enzymes of the CTX-M family as the most prominent ones, while $bla_{\text{CTX-M-1}}$ dominated the German samples and $bla_{\text{CTX-M-9}}$ the Mongolian ones. Genotyping led to the frequent detection of virulence determinants associated with extraintestinal pathogenicity whereas genes associated with intestinal pathogenic pathovars were only rarely observed. Phylogenetic typing via MLST and structure analysis revealed high rates of ancestral group B2 - a group linked with extraintestinal virulence - particularly in the German ESBL strains - most of the Mongolian ESBL strains were assigned to phylogenetic groups D and A.
Among the Mongolian samples we identified several STs, such as ST167 (ancestral group A) or ST648 (hybrid group ABD) which have previously been reported from human clinical ESBL producing isolates. Macrorestriction patterns revealed a clonal relatedness of wild avian fecal isolates from Asia to animal and human clinical isolates from Europe, indicating the global and interspecies dissemination of multiresistant strains.
Conclusions: Our findings underline the urgent need to unravel the role of wild birds in the spread of antimicrobial resistant bacteria. Due to their migration behavior and with respect to the omnipresence of bird´s feces in wild and urban environments, wild birds might represent a non-point source of infection and could by that considerably contribute to the transmission of ESBL producing bacteria.

FTP01

An approach for enabling an automatic qRT-PCR – ANALYSIS

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Introduction: qRT-PCR has widely been used for over ten years and has entered into domains with medical needs. The detection of microorganisms in industrial environment takes place with the methods of microbiology. The issue of reducing the time of detection of microorganisms is not resolved with the usage of the qRT-PCR method , but improved stronglyAll developed PCR analyze methods need an additional pre-processing to reduce the wide range of noise. The currently used methods require the use of experts to evaluate the result of noise removal, because the methods need some parameters which correlate to the detected noise. The adaption/calibration can only be executed by a manual process. This fact prevents the usage of the qRT-PCR-analysis in an automated industrial environment. The focus of this article lies in the development of automated pre-processing method which can reduce the significantly influence of the noise.
Noise: Generally, in test results, two different types of noise can occur (Wilhelm, 2003). The first type of noise is the background noise and the second type of noise is called *signal trend*. The possible causes of the occurrence are not yet resolved. The effects of background noise and signal trend for the repeated execution of sample analysis may lead to varying results.
Noise removal: The noise removal cannot generally detect and mathematically formulate the *background noise* and the *signal trend* of a curve. Existing methods do not try to detect the noise but only try to remove it. Such methods use parameters to control the strength of the noise removal.
Pre-processing using the Bézier curve: The smoothing with Bézier curve generally changes the curve progression so that the local extramas will be removed and the four phases of an exponential curve will be pronounced.
Evaluation: The project ProDIAP (This project which this report was funded by the *Bundesministeriums für Bildung und Forschung* under support code 01RI0709A-C) used 640 references for the evaluation. The evaluation of the quality of the qualitative analysis with the Bézier smoothing estimated with the criteria: recall, precision and the specificity. The results of the recall is 100%, the average of the precision is 96% and the average of the specificity is 97%.

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FTP02

Susceptibility of the new fluorochinolone Ulifloxacin vs. Ciprofloxacin vs. Norfloxacin against bacteria from Urinary tract infections

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Introduction: Ulifloxacin is a new fluorochinolone approved for the treatment of lower urinary tract infections and exacerbations of chronic bronchitis. The prodrug Prulifloxacin is available for oral administration and is metabolized in the liver to its active form Ulifloxacin. Ulifloxacin demonstrates a broad spectrum of antimicrobial activity against gram-positive and -negative bacteria.
In this study the susceptibility of Ulifloxacin was compared to other fluorochinolones in 267 isolates of urinary tract infections.
Materials and Methods: During testing period in April 2011 in 267 clinical isolates of urinary tract infections Ulifloxacin was added to routine resistance testing. Used method was disc diffusion; interpretation was performed according to CLSI criteria. For Ulifloxacin (disc containing 5 µg) interpretative criteria were as followed: ≤ 15 and ≥ 19 mm for Enterobacteriaceae, ≤ 16 and ≥ 20 mm for Nonfermenters, and ≤ 14 and ≥ 18 mm for Gram-positive bacteria.
Tested urinary tract infection strains include mainly E. coli (n= 159), other Enterobacteriaceae (n= 57), Pseudomonas sp (n= 14), Enterococci and Staphylococci (n= 24).

Results: Comparing Ulifloxacin vs. Ciprofloxacin and Norfloxacin concordant results could be obtained in 250 of the 267 (93.6%) tested strains.
Differences in the interpretation between tested substances were found in some isolates of *E. coli* (7 of 159), *Pseudomonas* sp. (2 of 14) and Enterococci (8 of 21). In 2 of 17 cases Ciprofloxacin and Norfloxacin were resistant, whereas Ulifloxacin was interpreted as susceptible. The other 15 of these 17 cases showed minimal interpretation variations only.
Regarding tested isolates E. *coli* (n= 159) revealed a susceptibility of 79.2% for Ciprofloxacin and Norfloxacin, and 80.5% for Ulifloxacin, respectively. Pseudomonas sp. (n=14) showed a susceptibility of 71.4% for Ciprofloxacin and Norfloxacin respectively and 85.7% for Ulifloxacin. For Enterococci following results could be found: Susceptibility to Ciprofloxacin, Norfloxacin and Prulifloxacin was 38%, 42%, 28% respectively.
Discussion: Ulifloxacin is a new fluorochinolone approved for lower urinary tract infections. Comparing the results of Ciprofloxacin, Norfloxacin and Ulifloxacin concordant results in 250 of 267 strains (93.6%) could be obtained. Ulifloxacin was as active as Ciprofloxacin and Norfloxacin against gram negative isolates. Against gram positive strains Ulifloxacin was slightly less active as Ciprofloxacin and Norfloxacin, but this statement is limited by the low number of isolates.
Because of some minor differences in the interpretation of the susceptibility pattern testing of Ulifloxacin and/or other chinolones resistance testing seems to be noteworthy if therapy with this fluorochinolone in intended.

FTP03

Impact of thymidine on the antimicrobial activities of trimethoprim/sulfamethoxazole, 5-iodo-2'-deoxyuridine, and rifampicin against Staphylococcus aureus

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Objectives: A treatment option against S. aureus in general and MRSA in particular is trimethoprim/sulfamethoxazole (SXT) either alone or in combination with rifampicin (RIF). SXT inhibits different enzymatic steps of the folic acid pathway, leading to cessation of bacterial synthesis of thymidine monophosphate via thymidylate synthase. However, the antimicrobial activity of SXT can be antagonised by bacterial utilisation of thymidine, which can be present in infected and/or inflamed tissues such as the airways of cystic fibrosis (CF) patients. The aim of this study was to determine thymidine concentrations in the sputa of CF patients and to analyse the antimicrobial activity of SXT, 5-iodo-2´-deoxyuridine (IdUrd) and RIF alone and in combination against S. aureus in the presence of thymidine.
Material and Methods: Thymidine concentrations in CF sputa were determined by high performance liquid chromatography. The antimicrobial activities of SXT, IdUrd and RIF alone and in combination against S. aureus were analysed by time kill assays.
Results: Thymidine concentrations in the sputa of ten different CF patients varied from <100 µg/L to 38 845 µg/L. The abolished antimicrobial activity of SXT against 22 S. aureus strains in the presence of thymidine was restored by combination with IdUrd. In contrast, SXT combined with RIF in the presence of thymidine did not show a synergistic effect and, furthermore, allowed the emergence of RIF-resistant bacteria. Adding RIF to the combination of SXT and IdUrd did not improve antimicrobial activity against S. aureus.
Conclusions: Our data suggest that SXT in combination with rifampicin may fail to cure clinical S. aureus infections in tissues with elevated thymidine concentrations. SXT in combination with a thymidine kinase inhibitor, however, may become an interesting antimicrobial combination for this setting in the future. Further in vitro and in vivo studies are therefore required.

FTP04

Non-nucleoside analogs as bacterial thymidine kinase inhibitors identified by multi-step virtual screening

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Objectives: The antimicrobial activity of folic acid antagonists can be antagonized by thymidine as detected in tissues with necrotic cells such as pus and sputum from cystic fibrosis patients.
Trimethoprim/sulfamethoxazole (SXT) inhibits different enzymatic steps of the folic acid pathway leading to cessation of the bacterial synthesis of deoxythymidine monophosphate (dTMP) by thymidylate synthase. However, several bacterial species including S. aureus possess an alternative pathway for synthesis of intracellular dTMP by uptake of extracellular thymidine and subsequent intracellular phosphorylation to dTMP via thymidine kinase. This study was aimed at (i) screening for non-nucleoside analog inhibitors of S. aureus thymidine kinase by multi-step virtual screening, and (ii) determining the in vitro activity of these thymidine kinase inhibitors against S. aureus in combination with SXT in the presence of thymidine.
Material and Methods: An adaptive virtual screening protocol for novel antibiotics using a combination of ligand- and structure-based approaches was applied. Two consecutive rounds of virtual screening and in vitro susceptibility testing were performed that resulted in several non-nucleoside hits.
Results: The most potent compound exhibits substantial antimicrobial activity against both methicillin-resistant S. aureus strain ATCC 700699 and nonresistant strain ATCC 29213, when combined with SXT in the presence of thymidine.
Conclusions: Our study demonstrates that multi-step virtual screening can help identify bioactive substances from a large screening compound pool with limited experimental effort. Rapid focusing on promising candidate structures was possible, so that inhibitors of bacterial thymidine kinase with non-nucleoside scaffolds were identified. These inhibitory compounds exhibit moderate to high antimicrobial activity when combined with folic acid antagonists in the presence of thymidine, and provide rich opportunity for further optimization.

FTP05

Drugs from bugs: The bacterial protein YopM is an autonomous `self-delivering´ anti-inflammatory agent that reduces inflammation in rheumatoid arthritis

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Cell-permeable proteins such as 'cell-penetrating peptides (CPP)' have the ability to cross cellular membranes, either alone or in association with a bioactive cargo. We identified the *Yersinia* protein YopM as a novel bacterial cell-permeable protein and described the ability of isolated recombinant YopM to enter host cells without a requirement for additional factors. Furthermore, treatment of cells with recombinant YopM leads to the down-regulation of inflammatory cytokines such as TNFα. This might contribute to systemic effects on innate immunity and, furthermore, suggests potential therapeutic applications of YopM e.g. in chronic inflammatory diseases. Hence, we were interested to investigate whether YopM might have the potential to act as an immune modulating molecule by exhibiting beneficial effects on inflammation and the production of cytokines that maintain the inflammatory processes linked to autoimmune diseases such as rheumatoid arthritis (RA). First, we analyzed the penetration of recombinant YopM into cells that actively drive joint and bone destruction in RA (bone marrow macrophages, osteoclasts, and synovial fibroblasts) by confocal laser scanning microscopy. In addition, we studied the effects of YopM on osteoclastogenesis using an *in vitro* osteoclast formation assay. To unravel the signaling pathways involved in the effects of YopM on cellular activation and differentiation, we investigated the activation of MAP-kinases and NFκB signaling by Western Blot analysis. With respect to a potential *in vivo* application of YopM, we injected YopM intra-articularly (i.a.) and intra-venously (i.v.) in wild-type and hTNFtg mice and monitored its distribution by fluorescence reflection imaging (FRI). Furthermore, the effects of YopM on the disease manifestations were monitored by histopathology and established clinical scoring.
Our investigations revealed that once inside RA-driver cells, YopM is an effective reducer of the TNFα-induced production of pro-inflammatory cytokines and cartilage-degrading enzymes (e.g. IL-6, MMP-1, and MMP-3). Furthermore, YopM is also capable of preventing structural damage by inhibition of RANKL induced osteoclastogenesis of murine bone marrow cells via the NFκB-pathway. After i.a. injection of the protein into hTNFtg mice, we observed a drastically reduced onset of symptoms and inflammation of skeletal joints.
These encouraging results combined with the possibility for focused topical applications in skeletal joints underline and strengthen the potential of YopM as a novel pathogen-derived immune modulatory agent for the treatment of inflammatory RA.

FTP06

Comparison of genotypic, proteotypic and phenotypic methods for the identification of bacteria

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Accurate classification of unknown bacterial isolates is an essential first step in understanding the impact these organisms have on an environmental monitoring program. There are many methods, technologies, and strategies utilized to determine the identity of unknown microorganisms, however, the selection of these methods is often impacted by more than the performance of the technology. Cost, time and the amount of expertise required to perform an assay are major points to consider during the selection process. Current available methods of identification range from genotypic to phenotypic, with 16S sequencing being universally acknowledged as the standard for routine bacterial identifications. Still, there is even variability within this process as not all 16S sequencing methods are comparable. When identifications are based on phenotypic characteristics, the methods are more subjective and results can be impacted by many variables. The first practical proteotypic identification systems utilize matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF) spectroscopy for microbial identification. This technology is based on whole cell protein profiles that are subject to less expression variability than phenotypic systems. This study directly compared performance between several of these technologies, including metabolic profiling (bioMerieux VITEK® 2 Compact), MALDI-TOF (Bruker BioTyper™), automated DNA sequence analysis (ABI MicroSEQ® 2.1), and DNA sequencing with a reference-quality, customized data analysis process and curated libraries (Accugenix). These microbial identification methods were used to analyze 60 unknown environmental bacterial isolates. Accuracy, as well as assay cost, time, and ease of use for each method are discussed. While 16S rRNA gene sequencing remains the standard for microbial identification of environmental isolates, proteotypic MALDI-TOF technology outperformed more phenotypic methods without compromising assay cost and turnaround time.

FTP07

YopM as a bacteria-derived anti-inflammatory cell-penetrating peptide (CPP) in a murine Psoriasis model

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Psoriasis is one of the most common immune-mediated chronic inflammatory skin disorders affecting approximately 2-3% of the population. It is considered as an incurable, life-long skin condition that affects all races, age groups and both sexes. The pathogenesis of psoriasis is complex and not clearly understood. Ongoing research indicates that immune T-cells appear to play a dominant role for the development of this disease. These cells release TNF-a, IFN-α and other pro-inflammatory cytokines that lead to vasodilation, leukocyte migration, and activation of keratinocytes. This further activates dendritic cells which in turn leads to inflammation. A common disadvantage (besides their side effects) of all drugs currently available for treating this primarily cutaneous disease is that they are only effective when given systemically but are largely ineffective when applied topically. In our previous work, we identified the T3SS-dependent YopM of *Yersinia enterocolitica* as a bacterial CPP capable of penetrating eukaryotic cells without a need for additional factors. Moreover, we showed that cell-penetrating YopM efficiently down-regulates the transcription of several pro-inflammatory cytokines (e.g. TNFα). Therefore, YopM might be applicable as a topical therapeutic agent for the treatment of psoriasis.
To investigate whether YopM might be functional as an immune-modulator for the treatment of psoriasis, we utilized the murine imiquimod (IMQ)-induced psoriasis model. In this model, daily topical application of imiquimod (IMQ), a TLR7/8 ligand and potent immune activator, on mouse back skin induces and exacerbates psoriasis. Therefore, this model could serve for the analysis of pathogenic mechanisms in psoriasis-like dermatitis. Here, we applied YopM either topically, intraperitoneally, or subcutaneously (injected every 48 h) to groups of mice over a period of two weeks. Our current results confirmed the 'self-delivering' abilities of YopM across the cutaneous barrier for topically applied YopM, and already indicated a remarkable dampening of overt inflammatory reactions. Repression of disease symptoms was also found in the group of mice subcutaneously injected with YopM, whereas no significant phenotypic changes were observed in intraperitoneally injected mice.

FTP08

Genomic islands of *Legionella pneumophila* Corby

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Exchange of genetic information by horizontal gene transfer is an important mechanism for the evolution of bacterial genomes. Horizontal gene transfer was also observed in *Legionella pneumophila* and other *Legionella* species. In the genome of *Legionella pneumophila* Corby we identified and characterized a new *trb/tra* conjugation type IVA secretion system, localized on the genomic islands Trb-1. The genomic island is integrated within the $tRNA^{Pro}$ gene (*lpc2778*) and can exist in an integrated chromosomal or an episomal circular form. Additional to all essential *trb/tra* genes require for conjugation and an *oriT* region, a site-specific integrase (*int-1*; *lpc2818*) and a *Legionella vir* region (*lvr*) also had been identified. By deleting the *int-1* gene, we could demonstrate that the excision and forming of an episomal circle is integrase dependent. First experiments with a mutant lacking the *vir* region, also indicate a growth dependent regulation of the alteration between the integrated and the circular form of Trb-1. The whole genomic island can be transferred to other *Legionella* strains and integrated site-specifically into the genome of the transconjugants. Using the *int*-1 mutant as a donor in conjugation experiments, we obtain reduced conjugation frequencies, compared to the parental strain. Further analysis of the *L. pneumohila* Corby genome led to the identification of two additional genomic islands (Lpc-GI3 and Lpc-GI4). GI3 and GI4 are flanked by a tRNA gene and exhibit several putative integrases. Both islands exhibit an *lvr* region, the genes *traG*, *traD*, *virB4*, *pilT* and a *hel* gene locus. In addition, GI4 contains genes encoding an ATP synthase and various efflux systems for heavy metals and other toxic substances. Similar genomic islands are also present in the genomes of *L. pneumophila* Philadelphia, Paris, Lens and Alcoy. We could demonstrate that both genomic islands can exist in an integrated chromosomal and an exercised episomal form, similar to Trb-1.

FTP09

Production of L-asparaginase by a protease-deficient *Bacillus megaterium* strainITBHU01: Characterization and production optimization

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Abstract: Bacterial asparaginases are widely used in combined chemotherapy of acute lymphoblastic leukemia, Hodgkin' diseases, non Hodgkin's lymphoma, melanosarcoma and multiple myeloma (Verma et al., 2007) as well as and in food industries to prevent acrylamide formation in fried food at high temperature (Pedreschi et.al., 2008).
Methods: Present study reports production optimization of L-asparaginase in a strain of *Bacillus megaterium*. A strain designated ITBHU01, was isolated from soil near degrading hospital waste site characterized phenotypically as well as genotypically. Optimization of media component like pH, temperature, carbon source and nitrogen source was performed for maximum L-asparaginase production. Effect of different inducer compounds and salts on synthesis was optimized as well.
Results: The isolate ITBHU01 was renamed as *Bacillus megaterium* ITBHU01 based on its phenotypical features, biochemical characterstics and phylogenetic similarity of 16S rDNA sequences. The strain was found protease-deficient and its optimal growth occurred at 37°C and pH 7.5. ITBHU01 was capable to produce enzyme L-asparaginase with maximum specific activity of 3.62±0.3 U/mg protein, when grown in the medium containing glucose (3 g/L) and beef extract (0.2%w/v) as carbon and nitrogen sources, respectively. Addition of L-asparagine at the concentration of 3 g/L to the medium induced approx. three fold production of L-asparaginase, proved that it was an inducible enzyme. Among different salts tested, sodium chloride and magnesium sulphate were found to be maximum effective for the production of the enzyme.
Conclusion: The present study has been introduced a new member of *Bacillus* sp. for production of L-asparaginase. The report also helps to optimize enzyme production and its regulation pattern by combinatorial influence of different factors. Further, the information obtained in this study signifies its importance during scale-up studies.

Keywords
L-asparaginase production; *Bacillus megaterium* strain ITBHU01; 16S rRNA gene; protease-deficient; optimization

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FTP10

Unravelling glycosphingolipid receptors of enterohemorrhagic *Escherichia coli* by means of solid phase bacterial adhesion assay combined with IR-MALDI mass spectrometry

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Glycosphingolipids (GSLs) play pivotal roles in the manifestation of infectious diseases as attachment sites for pathogens. Many germs are known to exploit GSLs as targets on the surface of mammalian cells. This initial protein (pathogen) carbohydrate (host) interaction contributes to colonization and virulence of many pathogens. Enterohemorrhagic *E. coli* (EHEC) causes diarrhea, bloody diarrhea, and hemolytic-uremic syndrome in humans. Although a substantial amount of information about the GSL-receptors for Shiga toxins (Stxs), the major virulence factors of EHEC, is available, less is known about the receptors for EHEC bacterial cells. The thin-layer chromatography (TLC) overlay assay coupled to infrared matrix-assisted laser desorption/ionization orthogonal time-of-flight mass spectrometry (IR-MALDI-o-TOF-MS) represents a powerful tool for the detection and structural characterization of GSL receptors of microorganisms. Here we report on the employment of this strategy aimed at the identification and structural characterization of GSL-receptors of the most common EHEC, *E. coli* O157, which consists of two types: non-sorbitol-fermenting strains of serotype O157:H7, which are spread worldwide, and Sfp-fimbriated sorbitol-fermenting nonmotile strains (O157:NM), which are pathogens in Europe. In the present study the potential of GSL recognition by various EHEC O157 strains was investigated employing neutral GSLs and sialylated GSLs (=gangliosides) from different types of mammalian cells. We were able to identify certain neutral GSLs carrying oligosaccharides with β-configurated terminal galactose residues showing binding activity towards EHEC. This type of interaction contrasts the common binding structures of Stxs that specifically recognize neutral GSLs with α-linked galactose molecules. EHEC-binding GSLs were further characterized by IR-MALDI-o-TOF-MS in parallel to TLC overlay assays using specific anti-GSL antibodies. The ganglioside GM3 (=II^{3}Neu5Ac-Lc2Cer) and terminally sialylated gangliosides with neolacto-series core were negative indicating that sialylation abrogates EHEC interaction. In conclusion, this study may help to better understand host-pathogen interactions and in particular the adhesion process of EHEC toward target cells and, moreover, to develop strategies to prevent and treat EHEC infections.

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FTP11

Glycosphingolipid Shiga toxin receptors in lymphoid and myeloid cell lines: subcellular receptor distribution and expression profiles of glycosyltransferases related to their biosynthesis pathway

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Glycosphingolipids (GSLs) are amphipathic membrane constituents comprising a hydrophilic oligosaccharide chain and a hydrophobic ceramide moiety. The ceramide lipid anchor of GSLs is embedded in the outer leaflet of the plasma membrane and the carbohydrate chain protrudes into the extracellular environment. GSLs are preferentially organized in microdomains known as lipid rafts, which play crucial functional roles in GSL-mediated cell-surface interactions. GSLs organized in lipid rafts are capable for efficient interaction with pathogens such as viruses and bacteria or pathogen-released virulence factors like bacterial toxins. Microdomain association of GSLs is believed to be the linchpin in effective binding, internalization and subcellular routing of GSL-binding AB_5-toxins such as Shiga toxin (Stx) to intracellular targets. The pentameric B-subunit of Stxs specifically binds with high and low affinity to the neutral GSLs globotriaosylceramide (Gb3Cer/CD77) and globotetraosylceramide (Gb4Cer), respectively. Our investigations are focused on the interaction analysis of Stxs with these GSL receptors that are expressed on the surface of leukocyte derived cell lines.

The structural characterization of the globo-series neutral GSLs by means of electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-QTOF-MS) with collision-induced dissociation (CID) in conjunction with Stx as well as anti-Gb3Cer and anti-Gb4Cer antibody binding assays revealed Gb3Cer (d18:1, C24:1/C24:0) and Gb3Cer (d18:1, C16:0) as the prevalent Stx-receptors in cell lines of lymphoid (B cell) and myeloid (monocyte) origin. Studies on the subcellular distribution of Stx receptors in both cell types showed their predominant association with and enrichment in lipid rafts. Here, we also provide data on the high sensitivity of Stxs to GSL receptor positive B-cell and monocyte descendants. As expected from the GSL profiles granulocyte and T-cell derived cell lines lacking Stx-receptors were found to be resistant to the cytotoxic action of Stxs. Moreover, studies on the expression of the glycosyltransferases responsible for biosynthesis of GSL receptors showed high compliance with immunostaining of GSL isolates. Our findings provide the basis for further exploring the functional role of lipid raft-associated Stx-receptors in cells of leukocyte origin.

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FTP12

Biological activity of *Bacillus* extracts against *Legionella*

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Legionella pneumophila is an intracellular parasite of aquatic and soil protozoa. Due to man-made water systems like cooling towers or air conditioning systems, *L. pneumophila* is also able to accidentally infect humans causing a severe, sometimes fatal pneumonia termed Legionnaires´ disease. The bacterial-protozoa interaction and sheltering biofilms lead to a remarkable resistance to biocide compounds and chlorination, thus representing ecological niches for legionellae persistence in such man-made environments.

From air samples we isolated two different *Bacillus* species that showed strong growth-inhibitory activity against legionellae on agar plates. The observed inhibitory activity was *Legionella*-specific since the growth of a brought spectrum of Gram-positive and Gram-negative bacteria was not affected.

By 16S rDNA sequencing the two bacilli strains were identified as *Bacillus pumilus* and *Bacillus licheniformis*. The inhibitory compounds were purified from *Bacillus* cultures by solvent extraction or binding to XAD-16 resin. The compounds revealed resistance to high temperatures (121 °C for 20 min) and remained active in agar growth-inhibition assays after acid (pH 0) and alkali (pH 14) treatments, respectively. On human blood agar plates the compounds exhibited hemolytic activity. Minimal inhibitory concentrations (MIC) were determined in liquid assay with 10^7 bacteria/ml. The crude extracts showed specific bactericidal activity against *L. pneumophila* (MIC 6.25 µg/ml), whereas concentrations of 800 µg/ml were not able to inhibit other Gram-negative or Gram-positive bacteria. The cytotoxicity of the compounds against the human lung epithelial cell line NCI-H292 was analyzed by the MTT assay. Compared to the high bactericidal activity, the crude extracts showed only low cytotoxic effects on the eukaryotic cells at concentrations above 200 µg/ml. Phenotypic characterization by transmission electron microcopy (TEM) after co-incubation of *L. pneumophila* with *B. licheniformis* or *B. pumilus* extracts for 48 h revealed a dose-dependent effect on the *L. pneumophila* membrane integrity. The bacterial cell exhibited increased blebbing and plasmolysis.

The narrow spectrum of activity and the results of the TEM revealed a specific interaction of the bacilli extracts against the highly hydrophobic and structurally unique membrane of *Legionella*. These promising initial data suggest further characterization of the compounds and an evaluation of putative applications.

FTP13

Functional analysis of the translation elongation factor EF-Tu from *Chlamydophila pneumoniae*

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The bacterial translation elongation factor EF-Tu is well known to be involveld in prokaryotic protein biosynthesis. EF-Tu from *Escherichia coli* has been shown to polymerize *in vitro* and a recent study provided evidence that the protein serves besides its function in translation another vital role in *Bacillus subtilis*: it affects cell morphology, apparently via interaction with the cytoskeleton protein MreB. In rod-shaped bacteria the actin-ortholog MreB is thought to direct incorporation of cell wall material into the side wall. Surprisingly, chlamydiae harbor, despite their spherical shape and the absence of a cell wall, MreB and we recently proved *in vitro* activity for this protein.
Here, we show that chlamydial EF-Tu is functional *in vitro*. The purified, strep-tagged protein polymerized in a concentration, pH and ion strength dependent fashion in light scattering and sedimentation assays. Additionally, co-polymerization assays revealed interactions between the elongation factor and the cytoskeletal protein MreB.
A deeper insight into the functions of EF-Tu and its role in chlamydial cell biology on molecular level will provide valuable information for the design of new anti-chlamydial antibiotics.

FTP14

Ecological fitness investigations of antibiotic resistant mutants of Legionella pneumophila strain MTZ OLDA under natural conditions in artificial microcosm.

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Legionella pneumophila is a gram-negative, rod-shaped bacterium. This ubiquitous bacterium can cause two forms of legionellosis – Legionella-pneumonia and Pontiac fiver. Newer macrolides and fluoroquinolones are currently the drugs of choice for the treatment of legionellosis. Although the failure in the therapy occurs from time to time, there are no reports about the isolation of the macrolide or quinolon-resistant Legionella mutants from the patients and environment to date.
A number of macrolide resistant mutants of L. pneumophila strain MTZ OLDA were selected in vitro using two-step selection. To investigate the interaction of Legionella antibiotic resistant mutants and their protozoan host under the influence of environmental flora, we used a previously established natural microcosm with simulated natural conditions.
This microcosm was established based on the microbial community of the water supply of a large building. The diversity of this community was determinated using the 16S rRNA clone libraries. It includes multiple heterotrophic species such as Sphingomonas sp., Chromobacter sp., Flavimonas sp. In addition the microcosm contains L. pneumophila strain MTZ OLDA wilde type (SG1) and amoeba strain Hartmanella sp.
Results showed that the resistant mutants cannot survive within the microcosm and that they disappeared after approx. ten weeks of cultivation. Furthermore they are less fit in co-cultivation experiment. The reduced ecological fitness of the macrolide resistant mutants might be an explanation for the lack occurrence of resistant strains under natural conditions.

FTP15

Recombinant linked cytokines as potential enhancers of bacterial oncolysis

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For more than a hundred years, it has been known that patients with solid tumors who concomitantly suffer from a bacterial infection experience a regression of their tumor burden. Still, the phenomenon of bacterial tumor targeting and lysis remains only poorly understood. The vision behind the PROTumor research project is not only to explore the mechanisms of bacterial oncolysis, but also, among other things, to find ways to further augment this effect. Amid several theoretically interesting approaches, we focused our efforts on immunosuppressive and pro-inflammatory cytokines. The immunosuppressive cytokines are supposed to expand the inoculum in the tumor and should enable the bacteria to penetrate the host immune defense shielding the tumor from total lysis. On the other hand, the pro-inflammatory cytokines should attract inflammatory cells, boost NK cell and CD8+ T-cell activity and eventually lead to tumor vaccination.
Here, we are going to present first results with recombinant Interleukin-10 and Interleukin-12 fusion proteins expressed in *E. coli*. In both cytokines, the corresponding monomeric proteins were linked with a glycine-serine-linker in order to achieve an improved stability and an optimized yield of biologically active protein. The genes used were synthesized as codon-optimized DNA sequences by a commercial provider. The recombinant proteins are secreted into the periplasmatic fraction with help of the OmpF signaling sequence of *E. coli*.
The Interleukin-10 fusion protein was expressed in *E. coli* BL21 (DE3) and Origami B (DE3) under varying conditions. The quantity of expressed Interleukin-10 was determined with a quantitative ELISA technique and its biologic activity was assessed in a cell-based assay measuring phosphorylation of the STAT-3 transcription factor.

FTP16

5-nitro-8-hydroxyquinoline (nitroxoline): potential new application as carbapenemase inhibitor in the face of spreading "Superbugs".

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While the preceeding decade was marked by the efficient spread of enterobacteriaceae carrying the ESBL phenotype in Germany and other European countries, we are now facing an alarming distribution of carbapenemase harbouring isolates (referred to as superbugs in the yellow press). These isolates are resistant to all ß-lactams except monolactams and-like ESBL- typically carry further resistance markers. On basis of structural differences, carbapenemases are divided into several classes, with superbug enzymes like NDM-1 belonging to the class B metallo-enzymes. These require zinc in their catalytic centre and inhibition of the lactamase activity by the cation chelator EDTA is used during screening procedures. However, while EDTA has found widespread application as a conservative, its clinical use is hampered by toxic side effects. Looking for clinically administrable alternatives, we studied the cation chelator and broad-spectrum antibiotic nitroxoline (NIT).
Mono-disk assays were carried out with carbapenemase expressing strains, applying either 2 µM EDTA or NIT with 10 µg imipenem disks. Interaction of NIT with imipenem was further evaluated by checkerboard assays and hydrolytic activity in cell lysates or whole cell suspensions was followed spectrophotometrically at 298 nm in the presence or absence of NIT (150 µM imipenem in PBS, 37°C, 1 hour).
NIT had comparable effects to EDTA in the mono-disk assay. In checkerboard assays synergism was demonstrated for NIT and imipenem in strains harbouring class B enzymes, but additive or indifferent effects were observed with strains expressing class A or D lactamases. Hydrolysis of imipenem by VIM and IMP enzymes was inhibited by NIT in a concentration- dependent manner (complete inhibition reached at 100-150 µM NIT), whereas no effect on KPC enzyme activity was noted (class A). The inhibitory effect depended on the respective carbapenemase type, but was not influenced by the bacterial species carrying the enzyme or the integrity of the bacteria (no difference between lysate or cell suspension). The inhibition was completely resolved by addition of 100 µM Zn^{2+} in the incubation mixture, whereas Ni^{2+} had only a minimal effect.
In conclusion, we found NIT to be an effective inhibitor of class B carbapenemases. As this antibiotic has been in clinical use over the last 50 years without serious side effects having been reported, it constitutes a promising candidate for the development of new therapeutic strategies.

FTP17

Factors influencing the results of microbial surface water testing in South Africa.

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Problem Statement: Faecal coliforms, the hydrogen-sulphide test and *E. coli* are used in monitoring of microbial water quality. *Rhodococcus coprophilus* indicates faecal contamination from animal sources such as cattle. Their mutual agreement and factors influencing the results have not been studied in detail in South Africa. Thus a study was conducted at 15 sampling sites in the Eastern Cape Province of South Africa to address this knowledge gap.
Materials and Methods: Sampling sites were located in the Makana and Buffalo Municipalities. Concentrations of faecal coliforms and *E. coli* were measured either using the MPN procedure with the A1 medium, the membrane filtration technique on the m-FC agar and the indole test. Cultivations were always conducted at 44.5 ºC. Concentrations of *Rhodococcus coprophilus* was measured by spread-plating using the M3 medium and cultivation at 30 ºC for 21 days. The hydrogen-sulphide test medium contained 0.5 % of deoxycholate. Grab samples of the water column and sediment were transported to laboratory at 4 ºC. Sediment samples were extracted by vortexing and the one-quarter strength Ringer solution. Model waters from selected sampling sites were prepared and the survival of faecal coliforms and the hydrogen-sulphide test bacteria were measured.

Results: Concentrations of faecal coliforms and *E. coli* ranged from 2 to above 1.6 x 10^4 cells or CFUs/100 mL. Suspension of accumulated sediment led to concentration changes ranging from 0 to 40 %. Positive results in the hydrogen-sulphide test were observed after 14 to 60 hours; and these results were susceptible to influence by sediment re-suspension. Temperatures varied between 10 and 32 ºC during the sample collection and transport, but did not change the results of the hydrogen-sulphide test. Levels of *Rhodococcus coprophilus* ranged from 0 to 8.18 x 10^6 cells/100 mL. If a suspension of the sediment matter on-site took place, this would lead to a change in the bacterial concentrations between 0 and 24 %. Survival studies showed that faecal coliforms and the hydrogen-sulphide producing bacteria survived for 22 to 29 days after faecal contamination depending on the chemical composition of the water column.
Conclusions: Re-suspension of sediment particles can compromise the results of microbial water quality testing. Temperature fluctuations between 10 and 32 ºC are not expected to influence the applicability of the hydrogen-sulphide test in detection of faecal contamination of surface water.

FTP18

Adaption of multiplex PCR analytics for the detection of microorganism contaminants in the industrial production of emulsion paint

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Introduction: Classic microbiological colony counting on agar plates is the diagnostic gold standard for the detection of microbial contaminants (MC) in raw material, intermediate goods and final products in the industrial emulsion paint production. As time-to-result takes up to 3 days, corrective actions like separation and cleaning of contaminated goods or addition of preservatives are usually retrospective. A sustainable in-time management of emulsion paint production should rely on fast quantitative MC analysis of products and their effective separation, thereby minimizing preservative input and waste of contaminated emulsion paint. Aim of this study was an overall process optimization by introduction of a faster and more sensitive analysis method for tracing microbial contaminations within the production line of a leading German emulsion paint producer. A proposed fast and sensitive multiplex PCR assay for the detection of MC was developed and evaluated against the gold standard method.
Methods: QRTPCR is well established in the medical routine diagnostics of human microbial infections. This study focuses on method adaptations for effective DNA preparation from emulsion paint samples and the development of a multiplex qRT PCR assay for the detection of relevant MC in emulsion paint precursors and products. In addition a new PCR expert software for automated result analysis of qRT-PCR runs from the RotorGene instrument (RG) was developed in order to standardize the analysis of qRT PCR data.
Results: The functionality of multiplex qRT PCR assays as a central tool of quality control to trace spiked MC in emulsion paint samples was demonstrated and evaluated against the gold standard. While the PCR method was clearly faster and equally sensitive to classic microbiology plate counting regarding ready emulsion paint, it failed when applied to some raw materials containing pure acrylate and some other compounds. This was clearly due to the insufficient separation of highly PCR inhibitory acrylate compounds which simply passed the DNA preparation columns used in this study. All PCR runs were analysed twice, manually using the standard RG software, and automatically using the new PCR expert software. No discrepant results were found.
Conclusions: Further development effort would be needed to optimize sample preparation with special focus on removal of inhibitory emulsion paint compounds. All methods should run on (or must easily be adaptable to) integrated sample and analysis robotics applying qRT-PCR, automated analysis and interpretation of results. This requirement is crucial since a final qRT-PCR based QC strategy should be able to manage the emulsion paint production without interaction of human PCR experts in a model environment at JWO in Coesfeld.
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FTP19

Biofilm formation between bacterial genera isolated from pharmaceutical water system

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Introduction and Objectives: Pharmaceutical-water systems are known to harbor biofilms, even though these environments are oligotrophic and often contain a disinfectant. Control of these biofilms is important for aesthetic and regulatory reasons. In pharmaceutical water, bacteria are affected not only by the environment they live in but also by the variety of other species present. By performing studies on the interactions present in multispecies biofilms, basic knowledge on several aspects of sociomicrobiology can be gained. The aim of this work was to assess the role of interspecies interactions in species biofilm formation and characteristics.
Material and Methods: The microorganisms used throughout this study were isolated from a model laboratory pharmaceutical-water distribution system. Preliminary, presumptive bacteria identification was done using selective media Chromocult TBX agar, *Pseudomonas* isolation agar, and methanol minimum medium, Gram staining, and biochemical methods. In order to assess the biofilm formation abilities and specific respiratory activities of the several bacteria isolated from pharmaceutical water, the standard 96-well microtiter plates with Cristal Violet and XTT staining were used to characterize biofilms**.**
Results: A ranking of biofilm formation was produced according to the method of Stepanovic´ *et al.*, classifying test bacteria as non-biofilm producer, weak biofilm producer, moderate biofilm producer, or strong biofilm producer.
Conclusions: Biofilms are recognized as focal points where bacteria and other microorganisms interact. Recent investigations into the microbial ecology of pharmaceutical water systems have found that pathogen resistance to chlorination is affected by the community biodiversity and interspecies relationships. A range of interactions has been observed among microorganisms in biofilms, including competitive, mutualistic, antagonistic and commensal relationships. In this work, some of the bacterial isolates tested are recognized as problematic opportunistic bacteria. The selected bacterial species were also detected in pharmaceutical-water biofilms.

Keywords
Biofilm, Pharmaceutical-water

GIP01

Are *Campylobacter jejuni* secretory proteins suitable antigens for serodiagnostics?

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Campylobacter spp. are the worldwide leading cause for bacterial gastroenteritis. The main reservoir and therefore the most common cause of an infection is poultry. A primary Campylobacteriosis manifests as diarrhea and is mainly diagnosed by stool culture. Following an infection, there is a risk of suffering from a sequela, such as reactive arthritis, neuropathia, myositis or a Guillian-Barré-Syndrome. These post-infectious complications can only be traced by serological assays. The sensitivity and specificity of most of these tests suffer since they are based on crude whole cell lysates which entail cross reactivites with related species. Hence, the use of species specific antigens becomes more and more favorable. *C. jejuni* secretes a number of proteins which are amongst others required for cell invasion and therefore confer the pathogenicity of the germ. These proteins are presumably highly antigenic due to the exposure to the host's immune system and may be used as antigens in diagnostic purposes. In this study could be demonstrated that recombinantely produced Cj0069, one of the secreted proteins, has a similar sensitivity and specificity compared to the already and commercially established antigens OMP18 and P39.

GIP02

The H-NS protein silences the *pyp* regulatory network of *Yersinia enterocolitica* and is involved in controlling biofilm formation

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Background: Horizontal gene transfer plays an important role in bacterial evolution. DNA acquired by horizontal gene transfer has to be incorporated into existing regulatory networks. The histone-like nucleoid structuring protein H-NS acts as a silencer of horizontally acquired genes to avoid potential damage. Here we analyzed the influence of H-NS on the

transcription of the horizontally acquired *pyp* regulatory network controlling transcription of the *hreP* gene encoding a proteinase essential for full virulence of *Yersinia enterocolitica*.

Methods: Using β-galactosidase and biofilm assays the effect of a dominant-negative H-NS variant (H-NSΔ) on gene expression of *hreP* and its regulators *pypA*, *pypB* and *pypC* were analyzed. Utilizing electrophoretic mobility shift assays (EMSA) the interaction of H-NS with the promoter regions of *hreP* and the *pyp* genes was investigated.

Results: H-NS represses the transcription of *pypA*, *pypB*, and *pypC* in *E. coli*. Moreover, a dominant-negative version of H-NS relieves repression of *hreP* and the *pyp* genes in *Y. enterocolitica.* RovA*,* an antagonist of H-NS in *Yersinia,* only activates transcription of *pypC*, but not of *pypA, pypB,* or *hreP*. With EMSA we show that H-NS interacts directly with the promoter regions of *hreP* and the *pyp* genes. In addition, the analysis of biofilm formation clearly demonstrates that overproduction of H-NSΔ strongly increases the formation of biofilms.

Conclusion: This study reveals that *hreP* and the *pyp* genes are part of a complex regulatory network that is repressed by H-NS, further underlining our hypothesis that these genes have been acquired horizontally. These results also underscore that dominant-negative versions of H-NS as the H-NSΔ protein are an important and valuable tool to study H-NS-dependent phenotypes in strains where *hns* deletions are lethal such as in *Yersinia spp.*

GIP03

Host-specific flagellin recognition in *Salmonella enterica* - infected cells

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Bacterial flagellin is one of the major pathogen-associated molecular patterns and plays a pivotal role in recognition of *Salmonella* by Toll-like receptors such as TLR5 and intracellularly by NOD-like receptors Ipaf and Birc1e/NAIP5. Both pathways trigger inflammatory processes and though the host immune response to prevent the infection. *Salmonella* is an important zoonotic pathogen, able to infect and cause disease in humans and animals ranging from reptiles to mammals. Depending on the serovar, host species, age and immune status the course of disease can range from asymptomatic carriage or localized acute diarrhea to severe systemic infections with fatal outcome.

To evaluate possible host differences in flagellin recognition in human and animal cells and subsequent induction of immunostimulatory signalling cascades, we have used the activation of transcription factor nuclear factor - κB (NF-κB) in NF-κB reporter cell lines of different species origins that express a luciferase during NF-κB activation. Mutants of *Salmonella enterica* with different flagellar gene deletions (*fliC*, *fljB*, *fliD*, *flhDC*) or expression patterns were used to infect NF-κB reporter cell lines of human, porcine and avian origins (THP-1, LoVo, Caco-2, 3D4/31, IPEC-J2, HD-11).

The loss of flagellar-associated genes affected the invasiveness of *Salmonella* differently between cells of different host species. In addition, loss of flagella affected NF-κB activation in human macrophages and epithelial cells but not in porcine or chicken cells. The varying behaviour and sensing of *Salmonella* flagellin in different host species might contribute to the different outcome of infection and host adaptation of *Salmonella* serovars.

GIP04

Shiga toxin glycosphingolipid receptors of human glomerular microvascular endothelial cells

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Shiga toxins (Stxs) are secreted by Stx-producing *Escherichia coli* (STEC) that cause diarrhea, bloody diarrhea and potentially lethal hemolytic-uremic syndrome (HUS). Stxs are AB5 toxins composed of a single A-subunit and 5 identical B-subunits. The catalytic A-subunit has rRNA *N*-glycosidase activity and inhibits eukaryotic protein biosynthesis. The pentameric B-subunit is responsible for binding to the high- and less-effective cell surface glycosphingolipid (GSL) receptors globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer), respectively. Although preferential Stx-mediated damage of glomerular microvascular endothelial cells (GMVECs) is known as the major mechanism underlying HUS caused by STEC, the exact nature of the Stx receptor GSLs of GMVECs including the expression of different lipoforms remains to be defined. Here, we report for the first time the structures of Stx receptors of GMVECs employing a combination of thin-layer chromatography (TLC) immunostaining using anti-Gb3Cer and anti-Gb4Cer antibodies and biophysical mass spectrometry-based analysis. Full structural characterization of Stx receptors was achieved by electrospray ionization (ESI) mass spectrometry combined with collision-induced dissociation (CID) analysis. Gb3Cer and Gb4Cer lipoforms with ceramide moieties mainly composed of C24:0/C24:1 or C16:0 fatty acid and sphingosine (d18:1) were determined in GMVECs. These lipoforms were comparable to those previously detected in human brain microvascular endothelial cells (HBMECs). Thin-layer chromatography (TLC) immunostaining of isolated GSLs demonstrated a 2.0-fold higher content of Gb3Cer and a 1.4-fold higher content of Gb4Cer in GMVECs than in HBMECs. Comparative cytotoxicity assays revealed high cytotoxicity of Stx towards GMVECs in a dose-dependent manner. Importantly, Stx caused a significantly higher toxic effect on GMVECs than on HBMECs over the whole span of applied concentrations. This higher sensitivity of GMVECs correlated with the enhanced expression of Stx-receptors in GMVECs. However, as GSLs are believed to localize abundantly in lipid rafts tightly associated with cholesterol and raft-associated membrane proteins and since lipid raft association of Stx receptors is assumed as a functional prerequisite for Stx-mediated cytotoxicity, future investigations on the subcellular distribution of GSLs in GMVECs are required to explore the functional role of the molecular assembly of GSLs in microdomains of GMVECs.

GIP05

Influence of the Cag type IV secretion system of *Helicobacter pylori* on the migration of phagocytes

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The Cag type IV secretion system (T4SS) is known to be a major virulence factor of the Gram- negative bacteria *Helicobacter pylori*. Together with the CagA effector protein, it is encoded on the *cag* pathogenicity island (*cag*-PAI), the presence of which is associated with more severe disease. The T4SS is able to translocate CagA directly into host cells, such as epithelial cells. As a consequence, the infected host cell is subject to several changes, such as actin cytoskeleton rearrangements and loss of cell polarity. Moreover, epithelial cells are stimulated by the T4SS to produce and secrete chemokines such as interleukin-8. *H. pylori* induces a massive inflammatory response in the host, but cannot be effectively eliminated by the immune system. Therefore, it is likely that *H. pylori* is able to modulate the immune response to its advantage.

In this study, we have examined the interaction between phagocytes and the Cag T4SS. It has been shown before that macrophages aggregate under the influence of the Cag T4SS, and that CagA can be translocated into these cells as well. We infected phagocytic cell lines (THP-1, HL-60) and freshly isolated human phagocytes (granulocytes, dendritic cells) with *H. pylori* and examined their migration in chemotactic gradients. To do so, we used two methods to examine cell migration, time-lapse microscopy of three-dimensional collagen gels and migration in transwell (Boyden) chambers. We confirmed that CagA is translocated into the cells examined and subsequently proteolytically processed and phosphorylated. The migration assays suggest that *H. pylori* infection results in a chemotactic defect of phagocytic cells and in an inhibition of phagocyte migration. Infection with *H. pylori* mutants showed that the inhibitory effect on human granulocyte migration is likely to be caused by the Cag T4SS.

In conclusion, our results suggest that interaction of the Cag T4SS with phagocytic cells may contribute to immune evasion and persistence of *H. pylori*.

GIP06

withdrawn abstract

GIP07

Changing epidemiology of *C. difficile* infections in a tertiary care German University hospital

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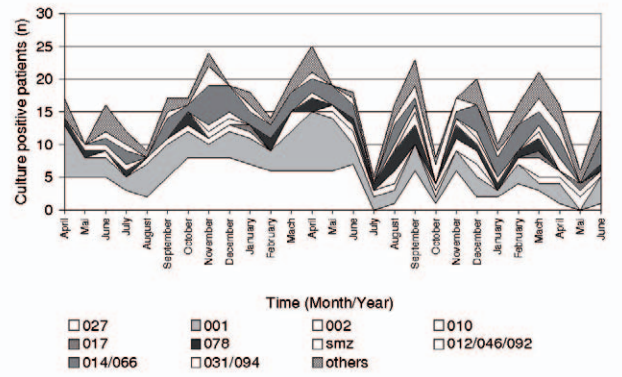
Objective: *Clostridium difficile* is a pathogen with emerging evidence for nosocomial and community acquired diarrhoea. New 027 outbreak strains with global spreading are characterised by toxin A, B and binary toxin expression, mutations of a potential toxin repressor gene (tcdC), and increased toxin production *in vitro.* Recently, high 027 rates have been detected in the region of Saarland, Germany. Single locus sequence typing of surface layer protein A (slpAST) is established in our reference laboratory for genotyping.

Methods: The University of Saarland Hospital is a 1300-bed tertiary care facility with150 ICU-beds. Between April 2008 and December 2010 *C. difficile* isolates (n = 792) of 524 consecutive adult and 36 pediatric patients with diarrhoea were propagated by standard anaerobic stool cultures using CCFA media (CLO-Agar). Genotypes were detected by slpAST as

previously described (Kato et al., JMM 2005) following sequence alignment with BLAST search and with a growing institutional database. In the present single centre retrospective study the appearance of different *C. difficile* genotypes was analyzed in the different age-groups.
Results: Since July 2009 the frequency of 027 significantly decreased from up to 50% to about 6% at the end of 2010. The proportion of other highly prevalent genotypes as e.g. 001, 014 and 017 remained stable. Interestingly, the rate of various *C. difficile* genotypes was different between children and adults with high percentage of non-toxigenic strains in the pediatric group (50%). Using an institutional database the genetic relationship between the various slpAST was analysed showing clustering in at least three major genetic groups subdivided in different subgroups with close association to known PCR ribotypes.
Discussion and conclusion: 027 is a pandemic strain with fast geographic spreading. We could show that 027 rates recently decrease in our previously high-prevalent region. We hypothesize that the rates of epidemic strains and presumably also strain specific virulence may change over time e.g. due to host adaptation and possibly also in different regions. Emergence of new epidemic *C. difficile* strains may occur at any time again. Therefore, detection of changing *C. difficile* epidemiology requires regular genotyping of local or more global CDI without restriction to 027 strains. SlpAST is a well standardized method to identify current epidemic *C. difficile* strains but also strains of still unknown epidemic relevance.

Figure 1

Figure 1: Genotypes of *C. difficile* isolates of adult patients with symptoms of infection



GIP08

CagI, an essential component of the *Helicobacter pylori* Cag type IV secretion system, forms a complex with CagL

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Helicobacter pylori, the causative agent of type B gastritis, peptic ulcers, gastric adenocarcinoma and MALT lymphoma, uses the Cag type IV secretion system to induce a strong proinflammatory response in the gastric mucosa and to inject its effector protein CagA into gastric cells. This protein translocation results in altered host cell gene expression profiles and cytoskeletal rearrangements, and it has been linked to cancer development. The Cag system is only distantly related to other type IV secretion systems, and it contains several essential components without functional counterparts in these other systems. Recently, it has been shown that the Cag type IV secretion apparatus binds to integrin molecules as receptors on host cells, and that several Cag components are involved in this interaction. In this study, we have characterized one of the components with integrin binding capacity, CagI.
Previous studies have identified the *cagI* gene as an essential gene for translocation of CagA into host cells, but there have been conflicting results as to its requirement for stimulation of interleukin-8 (IL-8) secretion by epithelial cells. Since the *cagI* gene overlaps and is co-transcribed with the *cagL* gene encoding another integrin-interacting protein essential for Cag functionality, this discrepancy might be due to transcriptional effects of the resistance markers used for mutagenesis. Using a marker-free gene deletion approach and genetic complementation, we show here that CagI is required for both CagA translocation and IL-8 induction. The absence of several components of the secretion apparatus has an impact on CagI and CagL protein levels, indicating that (partial) assembly of the apparatus is required for their stability. Our data provide evidence for a localization of both CagI and CagL at the bacterial outer membrane. Furthermore, we demonstrate by co-immunoprecipitation that CagI and CagL interact with each other. Taken together, our results are consistent with the assumption that CagI and CagL form a complex at the bacterial cell surface.

GIP09

Still increasing incidence of *Clostridium difficile*-infections in Germany-need for action

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Introduction: Since 2003, the United States, Canada and various countries in Europe have reported an increasing incidence of individual cases and outbreaks of *Clostridium difficile* infections (CDI). This was thought to be associated with the emergence of a more virulent strain, *Clostridium difficile* PCR ribotype 027, which was first reported in Germany in 20007. Since then severe cases of CDI (definition below) are notifiable according to the Protection against Infection Act. In order to gain a more comprehensive picture of all CDI over time an analysis of various data sources is presented here.
Methods: We described the incidence of CDI in Germany over time using 4 different data sources:
a) notifications of laboratory-confirmed CDI in the federal state of Saxony, identified through additional mandatory surveillance from 2002-2010, b) national surveillance data of severe CDI from 2008-2010 (defined by at least one of the following criteria: 1. readmission to a hospital due to a relapse, 2.admission to an intensive care unit due to CDI or its complication 3. surgery for toxic megacolon, perforation or refractory colitis, 4. death within 30 days after diagnosis and CDI as primary or contributive cause) c) nosocomial infection surveillance system for CDI (CDAD-KISS) from 2007-2010 and d) hospital discharges with diagnosis code A04.7: "enterocolitis due to *Clostridium difficile*" according to the International Classification of Diseases, 10th revision (ICD-10) from 2000-2009. Time trends were analyzed with negative binomial regression using STATA10.
Results: CDI incidences have reached a peak in 2010 with 113 notified cases/100.000 inhabitants in Saxony, 0.6 severe CDI cases/100.000 inhabitants according to the national surveillance system and an incidence density of 0.72 CDI cases/1000 patient days generated by CDAD-KISS. Hospital discharge diagnosis data for 2010 are not yet available (2009: 120.8 CDI diagnoses/100.000 discharged hospital patients).
The negative binomial regression models revealed a significant yearly increase in CDI incidence rates for hospital discharges (Incidence rate ratios (IRR) 1.43, p= 0.000), for CDI notifications in Saxony (IRR 1.39, p=0.000), and for the national surveillance of severe CDI (IRR 1.16, p=0.003).
Discussion: The continuing increase of CDI cases that peaked in 2010 shows the urgent need for action. The nature of this increase is not yet understood. An improved characterization of cases is therefore needed. This includes the monitoring of disease origin like proportion of healthcare- and community-associated CDI, the distribution and characterization of strains and a continuing evaluation of risk factors. This will help to focus preventive measures.

GIP10

Characterisation of *Clostridium difficile* isolates collected from clinical specimens from a large urban area in Germany from 2007 to 2011

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Introduction: *Clostridium difficile* associated disease has, in the last few years, increased in incidence and in severity world wide. The "epidemic strain" 027/BI/NAP1 causing severe disease in North America and parts of Europe was not observed in large numbers in Germany although the incidence of CDAD has greatly increased. Ribotype 001 has been implicated in severe disease in some studies in Germany.
Methods and Materials: Over the period from 2007 to 2011 a large number of isolates has been collected from the greater Düsseldorf area and subjected to ribotyping, PCR analysis of the presence of the toxin genes; *tcdA*, *tcdB* and *cdtA* and mutation causing truncation of the *tcdC* gene as well as resistance to erythromycin and moxifloxacin.
Results: A total of 254 isolates were analysed for the above characteristics. Most of the isolates (221, [87 %]) possesed both *tcdA* and *tcdB* genes; 20 [7.8 %] possesed *tcdA* but not *tcdB* and none possesed *tcdB* but not *tcdA*. The presence of the binary toxin *cdtA* was found in only 32 [12.6 %] isolates; 20 [60.6 %] of which also possesed *tcdA* only, and 11 [33.3 %] both *tcdA* and *tcdB*. The *cdtA* gene was found in one isolate negative for both *tcdA* and *tcdB* genes. A mutation of the *tcdC* gene leading to the expression of a truncated TcdC protein was found in 237 [93.3 %] of the isolates.

Resistance to erythromycin was observed in 132 [53.7 %] isolates and resistance to moxifloxacin in 194 [78.9 %]. Resistance to both antibiotics together was detected in 122 [49.6 %] isolates and no resistance to either substance in 42 [17 %] isolates.
Ribotyping of the isolates is ongoing. Preliminary analysis of the data showed that the isolates can be grouped into approximately 50 distinct ribotypes. One ribotype contained approximately 80 isolates and the next largest ribotype approximately 20 isolates. Confirmation of the ribotyping is awaited.
Discussion: Analysis of a large number of *c. difficile* from an expansive urban area has not been described in Germany. It is interesting to scrutinise such data as one may be able to determine if the increasing incidence of CDAD is perhaps, unlike the case in North Anmerica, not associated with a specific clonal spread and therby also determine if the change is perhaps due to changes in medical practice rather than a shift in the bacterial virulence. Our results show that the vast majority of the isolates possesed both toxin A and B genes and that only few [12.6 the binary toxin gene, a disproportionate number of these [60.6 %] however were located in isolates positive for tcdA and negative for tcdB genes, which only accounted for 7.8 % of the total number of isolates tested. As previously reported most isolates were resistant to erythromycin and moxifloxacin.

GIP11

Giardia duodenalis Arginine deiminase: Exploring its function for host-parasite interplay

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The protozoa *Giardia duodenalis* (*Gd*) is an intestinal parasite of humans. It causes diarrhoeal and gastrointestinal disease, named giardiasis. Annually, around 300 million people become infected worldwide. *Gd* is ubiquitously distributed and also infects other vertebrates, including companion animals. Thus, giardiasis is a major public and veterinary health concern globally. It remains unclear to what degree it is a zoonosis and what determines its pathogenicity to humans. To assess these issues a *Gd* biobank shall be generated to link epidemiological data with functional characteristics of clinical isolates and as a means to identify virulence associated genes. We hypothesize that potential virulence markers are enzymes released by *Giardia* upon colonization of the small instestine and interaction with the host epithelium. Especially the *Gd* arginine deiminase (ADI) that converts arginine to citrulline and ammonia shall be investigated as possible virulence and pathogenicity factor of *Gd*, because arginine plays a critical role in antimicrobial mechanisms and immune response. The sequence-variation of ADI will be determined by cloning *adi* from *Gd* isolates being collected in the biobank and sequence variants will be functionally characterized by expressing the respective recombinant ADI proteins. Finally, correlation between enzyme function and clinical pathology shall be investigated on basis of *Gd* isolates related epidemiological data. We will present the establishment of this workflow. *Gd* clone WB 6 ADI as well as a mutated ADI (C424A) were cloned, recombinantly expressed and purified by affinity chromatography. An enzyme activity test was adapted for colorimetric measurement of arginine to citrulline conversion to verify active and inactive ADI. Subsequently, *in vitro* generated human dendritic cells (DCs) were treated with recombinant ADI or *mut*ADI (C424A) in absence and presence of Toll-like receptor (TLR) ligands. Investigations of surface molecule expression and cytokine release shall give first evidences for ADI and its role for host-parasite interplay.

GIP12

What makes *Campylobacter jejuni* thrive: the contribution of amino acid metabolism to the efficient proliferation of a wide-spread food-borne pathogen

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Campylobacter jejuni is a major cause of food-borne disease in industrialized countries. We found recently that the highly pathogenic strain *C. jejuni* 81-176 has an extended capacity to metabolize amino acids in comparison to several *C. jejuni* isolates including the reference strain NCTC 11168. The amino acids aspartate, glutamate, proline and serine are growth-promoting substrates for both isolates, but only *C. jejuni* 81-176 possesses the additional capability to utilize asparagine, glutamine, and glutathione. Strikingly, the additional glutamine or glutathione metabolism of *C. jejuni* 81-176 contributes to its enhanced capability to colonize the intestine in a mouse model for colonization, whereas the asparagine utilization results in a more efficient persistence of *C. jejuni* 81-176 in the liver of infected mice (Hofreuter *et al*., 2008).
We have continued our studies and examined the effect of amino acids on the viability of *C. jejuni*. By transposon and targeted mutagenesis we recognized the involvement of previously uncharacterized genes in the amino acid catabolism as well as amino acid biosynthesis of *C. jejuni* 81-176. We tested the impact of amino acid metabolism on the survival of wild-type *C. jejuni* and several mutants defective in the utilization of amino acids under various stress conditions. Further, the contribution of amino acid catabolism by *C. jejuni* 81-176 on its interaction with cultured intestinal epithelial cells was investigated. Using a murine infection model we discovered distinct correlations between the metabolism of the tested amino acids and the persistence of *C. jejuni* in different tissues. The results broaden our knowledge about the amino-acid-driven growth of *C. jejuni* and its role during the colonization of various hosts.

GIV01

Gaussia princeps luciferase (Gluc) can be used to monitor type I protein secretion in *Salmonella enterica* serovar Typhimurium

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Introduction: *Gaussia princeps* luciferase (Gluc) is a highly active reporter exhibiting approximately 1000-fold higher luminescence compared to *Renilla* luciferase. Data about the applicability of Gluc in bacteria is very limited. Given the small size, high activity and co-factor independence of Gluc, it might be especially suited to monitor secretion of bacterial proteins. *Salmonella* Pathogenicity Island 4 (SPI-4) encodes for SiiCDF which forms a type I secretion system (T1SS) and the secreted adhesin SiiE. SiiE mediates intimate contact of *Salmonella* to polarized epithelial cells, thus facilitating the subsequent invasion process.
Methods: A codon-optimized Gluc gene was efficiently expressed in *Salmonella enterica* sv. Typhimurium (*S*. Typhimurium). Fusion proteins of Gluc and different C-terminal portions of the SPI-4-encoded adhesin SiiE were generated. After transfer in *S*. Typhimurium WT and *siiF*, luminescence was detected in bacterial lysates, supernatants and whole cultures.
Results: We detected luciferase activity of Gluc-SiiE fusion proteins in supernatants as well as whole cultures depending on a functional T1SS. We could show that a C-terminal moiety of SiiE including immunoglobulin (Ig) domain 53 is essential and sufficient mediating type I-dependent secretion of Gluc. Secretion efficiency of fusion proteins increased with increasing length of the C-terminal SiiE portion.
Discussion: In conclusion, Gluc can be used to monitor protein secretion via T1SS. This is the first demonstration of enzymatic detection of secreted proteins without stable periplasmic intermediates. Gluc might open a venue for the systematic identification and analysis of type I-secreted proteins and represents a valuable addition to the toolbox of modern molecular biology and microbiology.

GIV02

Helicobacter pylori BCS 100 genome variability and potential virulence determinants during an experimental human infection

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Introduction: *Helicobacter pylori* is one of the bacterial species with highest allelic diversity, due to a high mutation rate and frequent recombination during mixed infections. In a recent whole genome comparison study we demonstrated extensive genomic changes in four sets of sequential isolates obtained from chronically infected Colombians (Kennemann et al., PNAS 2011). Much less is known about genomic adaptations that may occur in the first months of infection of a new human host. Here, we present the complete genome sequence of *H. pylori* strain BCS 100 (H1), a cagPAI deficient isolate that has been used for several human challenge studies in the US and Germany, and draft sequences of reisolates obtained from four volunteers after 3 months of infection.
Results: The genome sequence of *H. pylori* challenge strain H1 was obtained by 454 sequencing and subsequent gap closure by targeted Sanger sequencing. Draft genome sequences were obtained by 454 sequencing of one additional input isolate (H3) and four isolates from four volunteers belonging either to the control group or to the Ty21a(UreA/B) vaccinated group. Additional reisolates from 29 volunteers were analyzed at selected loci by Sanger sequencing.
Conclusions: In contrast to the sequential isolates from Colombia, no evidence of recombination (import) was detected in the reisolates from infected volunteers. 1-2 novel SNPs were identified in each of the reisolated genomes, permitting the calculation of a robust estimate for the *H. pylori in vivo* mutation rate. Unexpectedly, a previously unknown variant of the input strain was detected in several volunteers which differed from the 454

sequenced input clone H1 by 86 SNPs and three clusters of nucleotide polymorphisms (CNPs). This variant was commonly detected among the additional reisolates from the study, suggesting strong *in vivo* selection favoring this subpopulation in some human hosts.

GIV03

Helicobacter pylori Type IV secretion system exploits distinct domains of the human β1-Integrin for CagA translocation

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Translocation of the cytotoxin-associated antigen A (CagA) via the Type IV Secretion System (T4SS) into the host cell is a major risk factor for gastric diseases, like chronic gastritis or gastric cancer. The immunodominant protein CagA is encoded, together with the *cag*-T4SS, on the *cag* - pathogenicity island (*cag*PAI). After translocation into the host cell, CagA subsequently is tyrosine-phosphorylated and modifies different eukaryotic signalling pathways in phosphorylation-dependent and independent ways.
We and others showed that CagA translocation is dependent on interaction of the pilus-like needle structure of the *cag*-T4SS with the β1-Integrin (ITGB1) on the host cell surface. Integrins are a family of αβ heterodimeric cell surface adhesion molecules, which mediate cell-cell, cell-extracellular matrix and cell-pathogen interactions. Three components of the *cag*-T4SS, CagY and CagI, as well as the translocated effector protein CagA, are binding to ITGB1, as originally identified in a Yeast Two Hybrid Assay. We attempt to localize the distinct binding regions of these proteins within the receptor and we want to determine, whether binding of CagA to ITGB1 is an essential step in CagA translocation. The N-terminal integrin binding region of CagA was further narrowed down to a stretch of about 100 amino acids and the corresponding domains on the β1-Integrin for CagA binding are verified.
These data can help to propose a new model of interaction between the pathogenic *H. pylori* and the eukaryotic cell, mediated by specific interactions of the *cag*-T4SS and the ITGB1 of the host cell.

HYP01

Outbreaks by multi drug resistant Gram negative bacteria - A systematic review

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Objectives: Multi drug resistant gram negative bacteria (MRGN) represent an increasing problem in many health care setting. Until now there are only few data on sufficient infection control measures that should be applied in the case of an outbreak.
Methods: We conducted a systematic review of the medical literature based on PubMed, the Outbreak Database (www.outbreak-database.com), and on a hand search of so retrieved articles. Pathogens of interest were *Acinetobacter* spp. (ACI), *Pseudomonas* spp. (PAE) and ESBL-producing *Enterobacteriacea* (ESBL). We extracted data on the setting, type of infection, number of patients, duration of the epidemic, and infection control measures.
Results: A total of 59 ACI outbreaks, 109 PAE outbreaks, and 57 ESBL outbreaks were included. As shown in figure 1a, the median duration of the outbreak was 183 days (ACI), 92 days (PAE), and 210 days (ESBL) respectively. The average number of affected patients in the three groups was 25 (ACI outbreaks; thereof infected: 19 patients; thereof died: 4 patients), 23 (PAE; 16; 3), and 57 (ESBL; 46; 2) (figure 1b - d). ACI outbreak specific findings included an association to university hospitals, to intensive care units, and to surgery departments. PAE outbreaks were associated with significantly less use of isolation precautions, less frequently closure of wards, and less use of protective clothing. ESBL outbreaks took place especially in neonatology departments and caused significantly high numbers of urinary tract infections. Additional information on the source of pathogen, its route of transmission, type of infection, age groups of patients, and implemented infection control measures is provided in the table.
Conclusion: The Outbreak Database served as a valuable tool for the characterization of nosocomial outbreaks as several significant differences between the three groups of pathogens examined were detected. Thus, from the findings of the present study we would recommend adjusting the infection control measures specifically on the type of pathogen. However, from our point of view currently there is no MRGN bacterium that should be totally disregarded with this respect.

References
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Figure 1

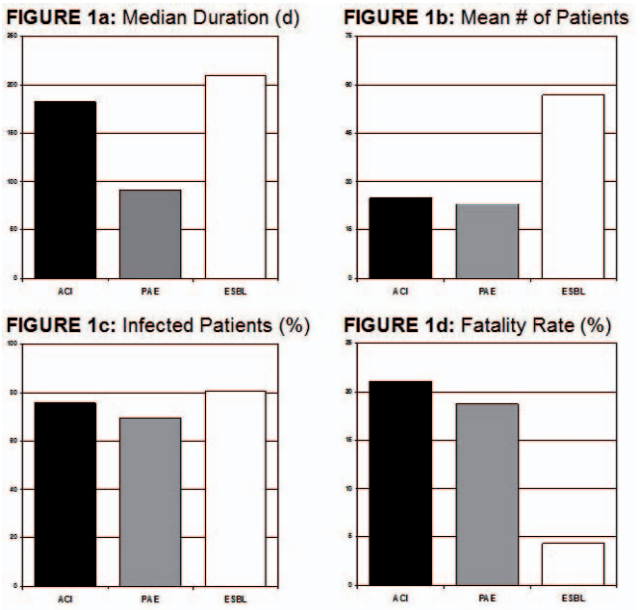

Figure 2

	ACI outbreaks n = 59	PAE outbreaks n = 109	ESBL outbreaks n = 57
type of infection			
blood stream infection	34 (57%)	64 (59%)	42 (74%)
respiratory tract infection	37 (62%)	60 (55%)	32 (56%)
urinary tract infection	17 (28%)	42 (39%)	38 (67%)
wound infection	18 (30%)	44 (40%)	15 (26%)
meningitis	8 (13%)	6 (6%)	11 (19%)
age groups (years)			
<1	10 (17%)	18 (17%)	18 (32%)
1 - 17	10 (17%)	16 (15%)	3 (5%)
18 - 50	20 (34%)	32 (29%)	6 (10%)
>50	14 (23%)	36 (33%)	10 (18%)
not mentioned	29 (49%)	53(49%)	29 (40%)
route of transmission			
contact	16 (27%)	73 (67%)	28 (49%)
unknown	43 (73%)	36 (33%)	29 (51%)
infection control measures			
disinfection / sterilization	29 (49%)	39 (36%)	25 (44%)
environmental screening	34 (58%)	47 (43%)	21 (37%)
hand hygiene	31 (53%)	28 (26%)	24 (42%)
isolation precautions	19 (32%)	15 (14%)	19 (33%)
closure of the ward	9 (15%)	6 (6%)	8 (14%)
screening of patients	19 (32%)	19 (17%)	22 (39%)
screening of personnel	15 (25%)	20 (18%)	8 (14%)
immediate feed back	24 (41%)	39 (36%)	23 (40%)
extensive education of staff	4 (7%)	4 (4%)	7 (12%)
protective clothing	15 (25%)	9 (8%)	17 (30%)
patient-to-patient ratio	1 (2%)	5 (5%)	8 (14%)
medical devices	6 (10%)	25 (23%)	6 (11%)
vaccination	0 (0%)	0 (0%)	0 (0%)
not mentioned	22 (37%)	52 (48%)	27 (47%)
source of pathogen			
index patient	5 (8%)	3 (3%)	11 (19%)
environment	2 (3%)	10 (9%)	1 (2%)
medical devices	7 (12%)	33 (30%)	7 (12%)
multi dose vials	3 (5%)	11 (10%)	1 (2%)
staff	0 (0%)	5 (5%)	2 (3%)
other	2 (3%)	7 (6%)	5 (8%)
remained unknown	40 (68%)	40 (37%)	30 (53%)

HYP02

Surveillance of surgical site infections after open heart surgery

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Introduction: Postoperative wound infections generally cause considerable extra morbidity, mortality and costs. The prevalence of total wound infections after cardiac surgery ranges from 0.47 to 3.5%.
Question: To describe the frequency of surgical site infections (SSIs) following open heart surgery and to identify the most frequent risks factors for these infections.
Methods: A prospective surveillance was performed during the period from January 2011 to April 2011 at the Intensiv CardioSurgery Unit of the Universitätsklinikum Essen, Germany.
Patients were evaluated daily during hospitalization and when re-admitted to Surgical Unit for any SSIs occurring after discharge.
The wound infections were defined according to the Centers for Disease Control and Prevention (CDC) and U.S. National Nosocomial Infections Surveillance (NNIS) system criteria. The descriptive epidemiological method was used.
Results: In total 305 patients were included, mean age was 67.2 years, 25.6 % of patients were female. 82.6% were elective procedures, 61.3% represented by coronary artery bypass grafting (CABG).
Methicillin-resistant Staphylococcus aureus (MRSA) screening was performed by 92.1% of patients, and perioperative antibiotic prophylaxis with a first-generation cephalosporin was administered in 93% of cases.
SSIs were observed in 5 patients (1.6%): 2 (0.6%) were superficial infections and 3 (1%) deep infections.
All infections occurred in CABG procedures and were diagnosed more than 14 days after operation. All patients had BMI >30, ASA score 3. Three patients had blood-glucose levels >180 mg/dl during the postoperative 24hours.
Conclusion: Preventing SSIs in the operating room is a primary goal for the surgical team.
Attention should be paid to antibiotic prophylaxis and Methicillin-resistant Staphylococcus aureus (MRSA) nasal carriage treatment.
In this study BMI >30 and high blood-glucose levels were associated with wound infections.
Overall the rate of SSIs was low, but further surveillance is needed.

HYP03

Changes in hand hygiene compliance: Are professional status and discipline risk factors?

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Background: Hand hygiene is the most effective measure for preventing infections related to healthcare. This study aims to evaluate the hand hygiene compliance in a German university hospital overall and in addiction to the professional status after implementation of the national hand hygiene campaign „Aktion Saubere Hände in 2008.This is the largest nationwide survey on intensive care units (ICU) in a university hospital in Germany.
Methods: During our investigation period (January 2008-December 2010), education and training of hand hygiene were implemented. Consumption rates of hand rub and gloves were collected and evaluated. Changes in healthcare workers behaviour were observed and compared between different professional groups, compliance rates in hand hygiene of Healthcare personnel of twelve ICUs were evaluated before and after implementation of the campaign. Implementation included extensive education and training of all health care workers (at least three teaching units per ward). Five indications of hand hygiene were classified according to WHO definitions, compliance rates were evaluated for these indications. The health care workers were divided in three groups: doctors, nurses and other health care workers.
Results: Consumption rates of hand rub increased from 27 to 32 hand disinfections per patient day. No significant correlation between hand rub consumption and hand hygiene compliance was calculated. No significant change in use of gloves was detected.
Overall 4040 opportunities of hand disinfection were monitored. A significant improvement of hand hygiene compliance was assessed (from 54 to 61%, p>0.05), the risk of no disinfection was reduced significantly (p>0.05, 95%CI 0.74-0.85). The risk of no disinfection decreased significantly in the nurses group, but not in the doctors group (other health care workers were not evaluated due to the small number). There was no significant difference in hand hygiene compliance before the intervention between doctors and nurses, after the intervention, there was a significant difference in hand hygiene compliance (p>0.05, 95%CI 1.42-1.85). Hand hygiene compliance is higher in the nurses group, the risk of no hand disinfection in doctors is decreased, but there is no significance.
Conclusions: A significant change of hand hygiene behaviour was recognized for doctors and nurses. The hand hygiene compliance of nurses increased significantly higher, hence other training strategies for doctors will be essential.

HYP04

Effective and rapid analysis of nosocomial outbreaks using the DiversiLab semi-automated repetitive sequence-based PCR test system

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Objectives: Rapid and sensitive methods are essential for typing and monitoring of hospital associated infections. The new DiversiLab system (BioMerieux) exhibits a semiautomated repetitive- sequence-based polymerase chain reaction (rep-PCR) for typing. Aim of the study was to evaluate the usefulness and accuracy of the DiversiLab system for bacterial strain typing and determination of genetic relatedness of strains associated with nosocomial outbreaks and integration of the system in the workflow of a routine microbiological laboratory.
Methods: For evaluation 20 Methicillin-resistant Staphylococcus aureus (MRSA), 26 multi-drug resistant (MDR)-Acinetobacter baumannii strains and 43 extended-spectrum-ß-lactamase producing Klebsiella spp. strains (30 K. pneumoniae and 13 K. oxytoca strains) from well-defined nosocomial outbreaks were typed using rep-PCR on the DiversiLab system. As reference method for bacterial strains-typing pulsed-field gel electrophoresis (PFGE) was performed.
Results: Concerning the 20 MRSA isolates, there was identical cluster formation in both, the DiversiLab and the PFGE systems. The same result could be observed in the 26 multi-resistant A. baumannii strains, where strains showed identical cluster formation (into 3 separate clusters) and very similar dendrograms. The 30 ESBL- positive K. pneumoniae strains originated from two chronologically separated nosocomial outbreaks: PFGE placed 28 of the ESBL- positive K. pneumoniae strains into the same cluster, whereas PFGE defined all strains as indistinguishable. The DiversiLab-system also formed a cluster of these 28 strains, but the strains in this cluster were not defined as identical, but similar. Both systems identified 2 K. pneumoniae strains as non-outbreak strains. Results from the K. oxytoca outbreak provide similar minor differences between PFGE and results obtained with the DiversiLab-system, whereas rep-PCR on the DiversiLab system exhibits slightly more discriminatory power than PFGE.
Conclusion:
- The DiversiLab system is a rapid, semi-automated repetitive sequence-based PCR test system for typing and analysing bacterial strains, including fungi.
- Compared to PFGE, this study confirms the good discriminatory power of the DiversiLab system.
- Results on the DiversiLab system could be obtained in a short period (8-24h), which is an essential advantage in rapid analysis of hospital associated infections.

HYP05

Use of UV-C light for surface disinfection - Results from a portable sanitizing device

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Background: There are several products that claim to sanitize surfaces quickly and easily by the use of UV radiation. Those products are for use in households or during travel (e.g., on toilet facilities, hotel mattresses, and personal care items). However, often their actual ability of killing germs and their overall practicability in daily life remains unknown.
Method: We tested the disinfection ability of the Verilux® CleanWave® Sanitizing Wand (Verilux Inc., Waitsfield, USA). This is a portable device that emits UV-C radiation from a UV light bulb (3 Watt) when held horizontally. According to the manufacturer's instructions the wand should be used as close as possible and no more than one-half inch above the surface. As stated in the operating manual, a 90% elimination of *S. aureus* would be expected within 12 sec by this procedure.
S. aureus (ATCC 29213), *E. faecium* (ATCC 19434), and *E. coli* (ATCC 25922) were each suspended in sterile 0.9% NaCl solution. A 100 µL sample containing $5*10^6$ CFU were spateled on standard sheep blood agar (surface = 56.7 cm²).

Continuous UV-C radiation was then applied at a distance of 0.7 cm (FIGURE 1A) for various time intervals (10, 20, 30, 45, and 60 sec). Reduction of bacterial growth was determined visually after 18 hours incubation at 37 °C. For this purpose, the number of CFU was counted on a defined area of 4 cm² per plate (FIGURE 1B) and compared to the number of expected CFU in an area of that size. All experiments were performed twice.

Results: The corresponding killing curves are shown in FIGURE 2. A sufficient reduction (≥5 log steps) was achieved for *S. aureus* and *E. coli* after 10 sec already, but 20 sec were needed for the same effect on *E. faecium*.

Discussion: Our results confirm that UV-C light from the device is capable of killing bacteria in principle. However, when using such a device for disinfection purpose in daily life, one has to keep in mind that 20 sec UV-C light exposition time was necessary in order to properly disinfect a rather small surface area from enterococci. Thus, much longer time intervals will be necessary for larger surfaces such as toilet seats. So there might be few opportunities when such a device could be a useful tool, but it seems less helpful on larger exterior due to time necessary for disinfection. Noteworthy, new batteries allowed an overall device working time of less than 15 minutes only.

Figure 1
UV-C exposition of plates (A) and colony forming units (CFU) count (B)

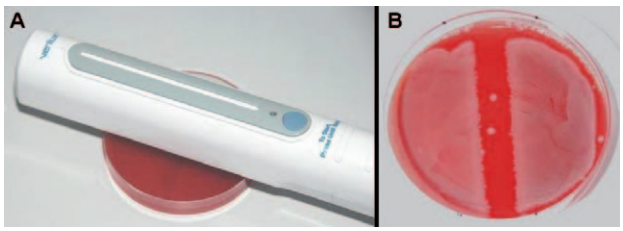
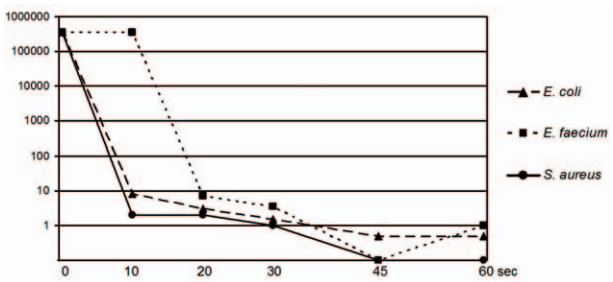


Figure 2
Killing kinetic curves of S. aureus, E. faecium, and E. coli after UV-C exposition over time



HYP06

Influence of water flow rate on the presence of enteric viruses in Ruhr river

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In the last years we have demonstrated the presence of enteric viruses in the Ruhr river, which is a drinking water source for about 5 Mio. people (Hamza et al., 2009, Hamza et al., 2011, Jurzik et al., 2010). Different factors can control the survival of enteric viruses in aquatic environments such as temperature, sunlight, pH, and organic matter. However, little is known about the effect of the flow rate on the concentration of viruses in surface water. Therefore, the present study aims to investigate the impact of flow rate on the presence of enteriv virus in Ruhr river.

Water samples were collected nearly weekly at five different sampling sites from January 2008 to December 2009. Water samples were concentrated using VIRADEL method with a high-salt alkaline phosphate buffer. For the quantification of viruses, qReal-Time PCR was carried out. Somatic coliphages were quantified using the double layer plaque assay. The quantification of *E. coli* and intestinal *Enterococci* were done according to the standard reference method (DIN EN ISO 9308-3 and 7899-1). The flow rate was daily measured at the Ruhr river in Villigst (Schwerte) for the same time period. Results showed that Ruhr river was highly contaminated with human adenovirus ($2.6x10^3$ gen. equ./l, n=175), human polyomavirus ($1.0x10^3$ gen. equ./l, n=123), and human rotavirus ($8.2x10^3$ gen. equ./l, n=93). The median concentrations for bacterial parameters were as follows: *E. coli* ($1.0x10^3$ cfu/l, n=155), total coliforms ($1.4x10^3$ cfu/l, n=177), and intestinal *enterococci* ($6.2x10^1$ cfu/l, n=172). Moreover, no correlation between microbiological parameters and flow rate was found. Further investigations are needed to characterize hydrological factors influencing enteric viruses in river water.

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HYP07

Homogeneous distribution of phenotypic characteristics in clonally unrelated *Pseudomonas aeruginosa* isolates from technical water systems

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P. aeruginosa is an opportunistic pathogen ubiquitously found in water environments where it can persist in biofilms. Cells released from biofilms contaminate the water phase and pose a potential threat to human health, especially when occurring in man-made water systems. The genotypic and phenotypic variability of *P. aeruginosa* in technical water systems is unknown.

During a period of seven years (2003 - 2010), 177 *P. aeruginosa* strains were isolated from technical water systems (drinking water distribution systems, plumbing systems, public swimming pools, and industrial water systems). Using pulsed field gel electrophoresis of SpeI restricted genomic DNA of these isolates and seven *P. aeruginosa* reference strains 42 different clonal variants were identified. For 77 representative strains and all seven reference strains the following characteristics were analyzed in order to reveal possible correlations between water source and genotypic as well as phenotypic characteristics: colony morphology, pigment production, hemolysis, cell surface hydrophobicity, biofilm formation, resistance against antibiotics and occurrence of virulence genes.

64% of these 77 strains and the seven reference strains showed the typical colony morphology for *P. aeruginosa*, 92.8 % produced at least one of the pigments pyocyanin, pyoverdin, pyorubin and pyomelanin, 98.8 % displayed b-hemolysis and 90.5 % had a hydrophilic cell surface. All strains were sensitive to ciprofloxacin, imipenem, meropenem, piperacillin-tazobactam, gentamycin, and except of one strain to ceftazidime. An intermediate sensitivity to aztreonam was determined for all strains and except of one strain all strains were resistant to ampicillin. Compared to reference strain PAO1, 53 strains (63 %) showed a higher biofilm formation on abiotic (polystyrene) surface, with 13 strains (15.5%) forming a cluster of strong biofilm formers. In all 84 strains, the virulence gene *lasB* was detected as well as either the virulence genes *exoS* (63 %) or *exoU* (38 %); only one strain contained both *exoS* and *exoU*.

For the first time an extensive pool of *P. aeruginosa* isolates from different types of technical water systems were characterized phenotypically and genotypically. No correlation between phenotypic and genotypic traits, and between these characteristics and the origin of the strains were detected. The phenotypic characteristics and the occurrence of virulence genes seemed to be distributed in a relatively homogeneous way among the clonally unrelated strains from diverse man-made water systems. There seem to be no selection of a specific genotypic or phenotypic variant of *P. aeruginosa* in technical water systems.

HYV01

Point prevalence study of nosocomial infections in a university hospital

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Background: The incidence of nosocomial infections (NI) increased considerably during the last years. Knowledge about the real frequency of nosocomial infections in Germany is rare.

The purpose of this study was to investigate the current frequency of nosocomial infections in a university hospital by a point-prevalence study.

Methods: In March 2010, a systematic investigation was conducted in all adult in-patients: Patients suffering from nosocomial infections were detected via an intensive chart review and interviews of physicians. A standardized protocol was used for definition and categorization of all nosocomial infections. Nosocomial infections were categorized by using CDC-definitions. Information about using of antimicrobial agents, invasive devices, causative organism, comorbidities and invasive procedures was recorded.

Results: Overall, 1,047 in-patients could be included in the study. 125 NIs were detected in 118 patients for a prevalence of 11.3 per 100 patients. The majority of NIs (37%) were identified in surgery, followed by intensive care medicine (30%) and general medicine (14%). Surgical site infection was the most common NI (36 (29%) of all NI), followed by gastrointestinal tract infection (32 (27%) of all NI), respiratory tract infection (24 (19%) of all NI), urinary tract infection (20 (16%) of all NI), bloodstream infection (5 (3%) of all NI), and other infections (8 (6%) of all NI). *Escherichia coli* was isolated most frequently (16%) followed by coagulase-negative Staphylococci (14%), *Enterococcus faecium* (9%), *Enterococcus faecalis* (8%) and *Pseudomonas aeruginosa* (8%).
Furthermore, the multivariate analysis identified gastrointestinal disease (OR=2.5; CI95% 1.6-3.8), disturbances of the electrolyte and fluid balance (OR=3.3; CI95% 1.1-10.5), history of hospital admission within 12 months (OR=1.6; CI95% 1.0-2.5), history of surgery within 12 months (OR=1.8; CI95% 1.1-3.1), history of antibiotic therapy within 6 months (OR=2.7; CI95% 1.4-5.1), and for each device (OR=1.4; CI95% 1.2-1.7) as independent risk factors for occurrence of nosocomial infections.
Conclusion: This study provides basic information about the current situation of NIs in our hospital. This data is useful to provide an estimate of the health burden of NI and will also be helpful to initiate infection prevention and control measures.

HYV02

Hand Hygiene Compliance: Is there a Difference in Treatment of Special Patient Groups?

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Background: Hand hygiene is the most effective measure for preventing infections related to healthcare. This study aims to evaluate the hand hygiene behaviour of health care workers in a German university hospital in treatment of special patient groups. In addiction compliance rates of different professional groups (doctors, nurses, other health care workers) were investigated.
Transplanted patients were chosen for this investigation. They are high risk patients for developing healthcare associated infections due to the high number of devices, the long hospital stays and immunosuppression.
Methods: During our investigation period (January 2008-December 2010), extensive education and training of all health care workers was implemented (at least three teaching units per ward).Consumption rates of hand rub and gloves were collected and evaluated. Changes in healthcare workers hand hygiene compliance were calculated and compared between transplanted and not transplanted patients. Therefore, the five WHO-indications of hand hygiene were investigated.
Results: Overall, 1607 opportunities of hand disinfection were evaluated.
A significant difference in hand disinfection behaviour was detected between the occupational groups. Nurses hand hygiene compliance was significantly higher. There was no significant difference in hand hygiene compliance between transplanted or not transplanted patients (OR 1.16, 95%CI 0.95-1.42). The same effect was calculated for all healthcare workers and for the professional groups. The disinfection was performed significantly more often after intervention than before (p>0.05, 95% CI 1.24-1.84).
Conclusion: There is no difference in hand hygiene behaviour of health care workers between transplanted and not transplanted patients. Nurses hand hygiene compliance was significantly higher. The risk of no hand disinfection was significantly higher before the intervention, therefore more education and training will be needed to protect this special patient group.

HYV03

Knowing where your infectious patients reside: An early warning infection information system for hospitals.

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Infectious disease outbreaks in hospitals are a severe threat to patients' safety. Once the number of infected persons including hospital staff climbs to a threshold hospital departments may be closed or even the whole hospital. Medical and administrative staff members especially those responsible for hospital hygiene management and infection control have a need to know where infectious patients are situated or should be transferred to. An Infection Information System (IIS) was developed for the hospital operator MTG Malteserträgergesellschaft to support hospital professionals to monitor infected patients. In case of an outbreak admission, discharge and transfer (ADT) operations can be monitored, controlled and analysed retrospectively over time. The core component is a monitoring dashboard which visualizes all beds in hospital on a single PC screen. Infections of patients are presented in one hospital with a colour code, in two others with a text code. The text code allows for displaying multiple infections and is likely to become the preferred standard of the hospital operator. So far Norovirus, ESBL, MRSA, Clostriods and other infections can be differentiated in the dashboard. The data basis of IIS is collected data in its own data warehouse loaded from the central hospital information system (HIS). Every 30 minutes a complete copy of the ADT situation is loaded into the database of the IIS including the notes on infections entered permanently by the hospital staff. The IIS is operational since january 2011 at MTG and first feedback from users was encouraging to continue the development. Especially the linkage between types of infection, localization of the patient and the overview in one screen is seen as a major benefit in daily work.

Figure 1
Monitoring dashboard using colour code to visualize infected patients.

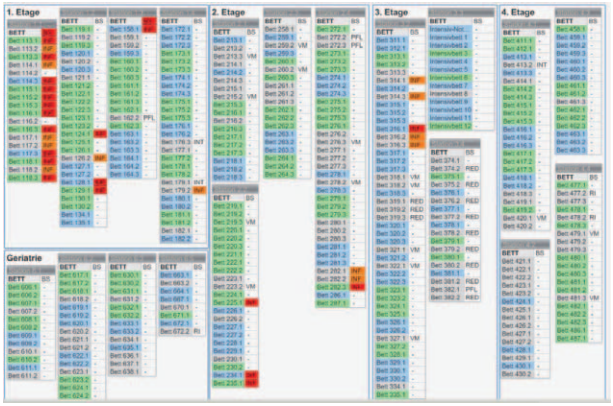
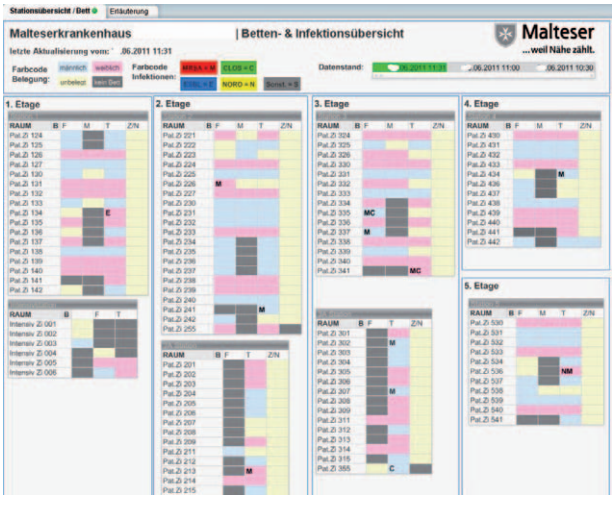


Figure 2
Monitoring dashboard using text code to visualize infected patients.



HYV04

Review of European guidelines for the prevention of *C. difficile* infections results form PROHIBIT WP2

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Objectives: The European Commission (FP-7) funded project "Prevention of Hospital Infections by Intervention and Training" (PROHIBIT; www.prohibit.unige.ch) was established in 2010. PROHIBIT aims to analyse existing guidelines and practices to prevent healthcare associated infections (HAI) in Europe, to identify factors that influence compliance with best practices and to test the effectiveness of interventions of known efficacy. WP 2 provides a review of current national and subnational (regional) European guidelines for the prevention of *C. difficile* infections (CDI) as well as an overview on surveillance systems and public reporting of CDI.
Methods: HAI surveillance National Contact Points (NCP) of the ECDC and other HAI experts in 34 countries (27 EU member states - whereby UK counts as 4 countries, Croatia, Iceland, Norway and Switzerland) were asked to provide their national/subnational guidelines for CDI prevention. A review of the guidelines as well as a comparison with existing ECDC guidance was conducted. In addition a questionnaire was developed to gather information about national surveillance systems and practices of public reporting.
Results: Eighteen of 34 European countries have a national/subnational guideline for CDI prevention. England, Northern Ireland and Wales follow the same guideline. The oldest guideline dates back to 1995, all others were published within the past 4 years. Seven of the 16 guidelines rate the scientific level of supporting evidence and strength of recommendation. All but one guideline explicitly recommend placing symptomatic patients in a single room whenever possible. Cleaning or disinfection of frequently touched surfaces is recommended in all guidelines. However, only 13 include specific recommendation about disinfectants; out of these 11 prefer chlorine based solutions as specified in the ECDC guidance of 2008. There is variation in hand hygiene recommendations. Most guidelines favour hand washing with soap and water. Alcoholic hand rub is mostly mentioned as an additional task to eradicate other pathogens.
Thirteen countries providing a CDI guideline also run a national surveillance system for the disease. In Belgium, England, Northern Ireland, Scotland, Wales and Ireland participation is compulsory for hospitals. In the UK and Ireland public reporting of CDI rates of individual hospitals has been established since 2007 and 2008, respectively.
Conclusions: Although CDI is recognized as a major challenge in infection control only half of the European countries presently provide a guideline for CDI prevention. Furthermore, there is important variation in scope and detailing of the recommendations. The majority of guidelines do not rate the scientific level or strength of recommendation. Countries with mandatory participation in a national surveillance system have introduced public reporting of CDI rates for hospitals.

HYV05

Blended learning Kurs für Hygienebeauftragte Ärzte - innovativ, aktiv, zertifiziert

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Wirksame Prävention nosokomialer Infektionen setzt qualifiziertes Hygienefachpersonal voraus. Neben der Hygienefachkraft und dem Krankenhaushygieniker ist es wichtig, dass auch in den klinischen Abteilungen erfahrene und in Hygiene fortgebildete Ärztinnen und Ärzte Ansprechpartner für Kollegen und Mitarbeiter sind. Aufgrund ihrer besonderen Fachkenntnis in Hygienefragen können sie das Bewußtsein für die Relevanz der Einhaltung der geltenden Hygienestandards immer wieder schärfen und die Umsetzung fördern.
Mit dem von einem interdisziplinären Team vom Hygienikern, Hygienefachkräften, Klinikern, Pädagogen und Kommunikationswissenschaftlern neu entwickelten Blended Learning Kurs erwerben die Teilnehmer die Qualifikation Hygienebeauftragter Arzt. Interaktives E-Learning ist der erste Schritt zum Zertifikat. Die Grundlagen werden im Selbststudium in überschaubaren Modulen erarbeitet. Filme und Links sowie der Online-Wissensverstärker QUADIO vertiefen die Kenntnisse. Lernkontrollfragen schließen jedes Kapitel ab.
In einer Präsenzveranstaltung werden über zwei Tage relevante Hygienethemen von Experten erläutert und zur Diskussion gestellt. Im kollegialen Dialog können dabei die wichtigsten Aspekte der Infektionsprävention vertieft werden.
Der Kurs ist durch die Ärztekammer Hamburg und durch die Staatliche Zentralstelle für Fernunterricht (ZFU) zertifiziert. Im 1. Halbjahr 2011 haben wir den ersten Kurs in Hamburg erfolgreich durchgeführt. Im November 2011 finden die Präsenztage für unseren zweiten Kurs statt.
Neue gesetzliche Anforderungen von Bund und Ländern stellen die medizinischen Einrichtungen vor große Herausforderungen, da derzeit Hygienefachpersonal nicht in dem geforderten Umfang zur Verfügung steht. Mit unseren Blended Learning Kurs leisten wir einen innovativen Beitrag zur Qualifikation von Hygienebeauftragten Ärztinnen und Ärzten.

HYV06

Surface disinfection with Wipe Systems - Efficacy testing and practical indications

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Introduction: There is a world-wide trend using wipes systems for the surface disinfection in health care settings. However, some technical challenges have to be taken into consideration to assure a safe use of these products. Recently, it has been shown, that active ingredients of surface disinfectants e.g. quaternary ammonium compounds can adsorp to the fabrics of wipes and impair the efficacy of the disinfectants significantly (1).
Currently only methods are published testing the efficacy of liquid surface disinfectants (2, 3). Therefore, it was our aim to develop an easy and reproducible test method for this product type.
Material and Methods: Basis of the new test method are the DGHM-standard method 14 (2) and the EN 13697 (3). OR-tiles according to DGHM (2) were used as test surfaces and a standardized technique for direct application of the soaked wipes has been established. In addition, the amount of disinfectant applied to the surface has been investigated
Results: Our results show that the suggested assay is suitable to test the practice like efficacy of disinfectant wipe systems.
In addition, the results show that not only the type of fabric (1) but also the amount of impregnation solution is determining the sufficient efficacy of the products. There is a strong correlation between the amount of impregnation solution and the amount that can be transferred on the surface which is mandatory for the efficacy of the product. We were able to show that the amount of disinfectant that can be transferred from a wipe onto a surface is significant lower than the amounts that are applied on the test carriers in the current published standards (2, 3).
Discussion: Now, a reproducible and easy standard method is available that can be used to show the microbicidal efficacy of wipe systems. The authors strongly recommend testing the microbicidal efficacy of the wipe systems before practical use and before introducing these systems in positive lists such as VAH and SFHH. Only wipes with the correct choice of fabrics (1) that are thoroughly impregnated with disinfectant solution are able to assure a sufficient efficacy and a safe use of the disinfecting wipe systems.

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3 Anonymus: DIN EN 13697: Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements without mechanical action (phase 2/step 2), 2001.

HYV07

Successful management of STEC diagnosis and infection control during a large outbreak caused by ESBL-producing STEC (O104:H4)

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Introduction: Shiga toxin-producing *E. coli* (STEC) are pathogens causing foodborne diarrhea, Hemorrhagic Colitis (HC), and the Hemolytic Uremic Syndrome (HUS). In May 2011, a large outbreak of an O104:H4 STEC strain (HUSEC 41) expressing an ESBL-positive phenotype occurred with a hot spot in Schleswig-Holstein leading to great challenges in the laboratory diagnosis and infection control in the hospital.
Laboratory diagnosis: From May 17th patients with STEC diarrhea and HUS were admitted to the University Hospital Schleswig-Holstein, Campus Lübeck. Having confirmed an ESBL-producing and Sorbitole-fermenting STEC clone as the causing agent within 24 hours, a chromogenic selective media for ESBL screening was immediately introduced in the diagnostic

algorithm. Shiga toxin-expression was analyzed from all *E. coli* isolates grown on media selective for ESBL. For all stool specimens negative for ESBL-producing *E. coli,* Shiga toxin-expression was analyzed from an enrichment broth incubated in parallel. From 80 of 129 (62 %) of patients with probable STEC infection stool specimens were tested in our laboratory. In 58 stool specimen (72.5 %) STEC ESBL was confirmed as the causative agent. None of the ESBL *E. coli* culture negative stool specimens was positive for Shiga toxin from the enrichment broth. For all patients displaying symptoms of HUS, STEC ESBL infection could be confirmed (18/18). Norovirus (3), Rotavirus (1) and Campylobacter jejuni (1) were detected in five patients. No causative pathogen could be identified in 16 specimens.

Infection control measures: A regular ward was immediately defined as quarantine ward for the management of affected patients. Only few severely ill patients were treated on an ICU under barrier precautions. During the peak of infections patients were directly examined at the quarantine ward to unburden the emergency department. For patient management, barrier precautions with single usage coats and gloves for direct medical care procedures were established. Medical face masks were suggested only for procedures with potential aerosol production. No nosocomial transmission of STEC ESBL was observed indicating successful infection control measures.

Conclusions: Introduction of selective chromogenic media for ESBL-producing *Enterobacteriaceae* proved to be a rapid and highly sensitive diagnostic procedure for the detection of the outbreak causing STEC clone. Timely infection control measures circumvented nosocomial transmission of STEC.

HYV08

Rapid screening method for multiple gastro-enteric pathogens also detects novel EHEC O104:H4

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Introduction: Since early May 2011 an increasing number of cases presenting with bloody diarrhoea was reported. The outbreak started in northern Germany and is caused by a novel E. coli variant, namely *EHEC O104:H4*, meanwhile cases are reported all over Germany and Europe.

Patients suspected to be infected with ***EHEC O104:H4*** and suffering from bloody diarrhoea bear the risk of developing the serious and life threatening haemolytic-uremic syndrome (HUS) and thus need a rapid differential diagnosis. To avoid misdiagnosis potentially guiding false initial therapy or infection control measures rapid screening diagnostic methods are required and have been developed by specialized laboratories. However, taking into account the inflation-like increasing number of newly suspected cases, the majority of laboratories has become overloaded, and the risk of delayed diagnosis increases proportionally.

Methods: We thus tested the novel xTAG GPP assay from Luminex (Toronto, Canada). This assay was developed to simultaneously detect the most important and serious viral, bacterial and parasitic pathogens in a single reaction. Pathogens to be detected include Shiga-like Toxin producing *E.coli*, thus we assumed that also the novel variant which is Shigatoxin 1 negative but Shigatoxin 2 positive could be identified.

Results: Until now a total number of 20 patients suffering from bloody diarrhoea or suspected to be infected with the novel EHEC variant were tested. The multiplex testing revealed 4 patients positive for the novel EHC O104:H4 variant of which two were already independently confirmed by an external laboratory. Two further patients suffered from severe Campylobacter infections and were tested negative for EHEC, one patient was tested positive for another EHEC variant that produced Shigatoxins 1 and 2.

Conclusion: We conclude that the assay is useful to pre-screen patients suffering from the novel EHEC variant. Patients can be monitored more closely by the clinicians and test results associated to the clinical course of HUS. Additional beneficiary effects are that only a pre-selected cohort of clinical samples has to be analysed by the specialized laboratories and that those patients that are negative for EHC but positive for pathogens like *Campylobacter spec.* or *Clostridium difficile* can be administered the correct antibiotic therapy. The Luminex technology is widely available and the assays procedure is rather simple with a total hands-on time of less than 5 hours and a sample capacity of up to 96 samples per run, the assays is suitable for high throughput analyses and thus will cover the peaks in the epidemiological outbreak situation.

HYV09

Management and epidemiology of an outbreak caused by exfoliative toxin A positive *Staphylococcus aureus* in a neonatal department

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Introduction: Staphylococcal Scalded Skin Syndrome (SSSS) is a toxin-mediated disease caused by exfoliative toxins A and B (ETA and ETB). In December 2010/January 2011 five neonates developed bullous skin lesions in our neonatal department within a week. Index patients were twins one of whom suffered from generalized exfoliation and thus had to be transferred to our neonatal intensive care unit (NICU). The other one showed a localized desquamation at the rump and the occipital region and remained on the neonatal ward (NW). Two additional cases occurred on the NICU thereafter with localized bullous lesions periorally as well as periorally, in the groin, and hands, respectively. On the NW, one further case was identified presenting with a desquamation around a peripheral venous access. In 4 of the cases microbiological samples showed *Staphylococcus aureus* isolates, which were sensitive to all antimicrobials tested.

Methods: Contact precautions were initiated for the children affected. Swabs of the nose and perineum were taken from all patients on both wards at three different time points within ten days in order to screen for *S. aureus*. Environmental samples were performed on different sites and items on both wards (e. g. skin care products). *S. aureus* isolates were sent to the German Reference Centre for Staphylococci for further characterization and typing. They were subjected to susceptibility testing by microbroth MIC. The presence of the genes for the exfoliative toxins A and B (*eta* and *etb*) was analyzed. Furthermore the spa-type was determined.

Results: All skin lesions resolved without any scarring. No more newborns with desquamations of the skin were observed after precautions had been expanded. No *S. aureus* was detected in environmental samples. 36 children were hospitalized on both wards within the screening period. Among those 12 *S. aureus* carriers were identified, two isolates of whom showed the same phenotype compared to the cases. All four available isolates from the children affected and one isolate detected by screening a child without any symptoms were tested positive for *eta*. All *eta*-positive strains belonged to the spa-type t084.

Conclusions: We observed an outbreak of SSSS caused by an exfoliative toxin A producing *S. aureus* strain within our institution. The phenotype with all antimicrobials tested sensitive may indicate a community rather than a nosocomial primary source. We assume that after introduction of the strain - maybe by the family of the twins first affected - further nosocomial transmissions have occurred. However, the parents were not tested. After initiation of expanded infection control measures and screening and eventually discharge of the last child that carried the strain no more cases have been observed.

HYV10

Contaminated cheesecakes sold in hospital-near cafes as cause of suspected nosocomial salmonella infection

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Background: Salmonella species are commonly isolated as pathogens of gastroenteritis in hospitalised patients. Mostly, salmonella infection is community-acquired.

Objective: To perform a prospective in-hospital surveillance of salmonella infection in order to detect possible nosocomial cases in an acute hospital setting.

Methods: In three tertiary care hospitals all patients with detection of salmonella species were investigated in respect to whether the detection occurred later than 48 h after hospital admission. Patients´charts were investigated for clinical symptoms and patients with nosocomial acquisition were interviewed. Health care authorities (HCA) were informed about relevant interview information.

Results: 2010 in 20, 4 and 31 patients Salmonella species were detected in hospital A, B or C. In March 2010 two nosocomial cases with *S. enteritidis* occurred in hospital A and C. Both hospitals were served by different canteen kitchens. Patients reported having eaten a cheesecake in two different hospital-near cafés. Informed HCA detected the use of raw eggs in the bakery serving both cafés and stopped it. In September 2010 two nosocomial cases occurred in hospital A. Again, patients reported having eaten cheesecake. Involved HCA identified further two cases reporting

consumption of cheesecake during a visit of a relative in hospital A. The same bakery as in March had used raw eggs. The baker and one person in the café were tested positive for *S. enteritidis* in stool samples. For a defined period both persons were forbidden to work in food production. No further cases occurred since then.
Conclusion: A systematic surveillance of all salmonella-cases identified rare nosocomial cases. Food available at food providers near the hospital should be considered if nosocomial salmonella infection occurs. The interaction with HCA is essential for the identification and control of pathogens transmitted to hospitalised patients if acquired from outside the hospital.

HYV11

Cluster of Adenovirus in a pediatric haemato-oncology ward: Is this an outbreak?

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Objective: Human Adenovirus (HAdV) can cause severe infections especially in haemato-oncologic pediatric patients. In our hospital contact- and droplet-precautions are recommended when infections with HAdV are suspected. Despite this recommendation HAdV was identified in stool and nasopharyngeal secretion samples of five children in a pediatric haemato-oncology ward within a two week period in February 2010. Nosocomial transmissions were suspected and precautions expanded. As a high compliance with precautions in ward staff was observed, the theory of an outbreak with patient to patient transmissions was questioned.
Method: A retrospective epidemiological investigation was conducted. All children seen in the children's hospital and tested positive for HAdV in a four week period (one week before and after the cluster on the haemato-oncology ward) were included. HAdV was diagnosed via realtime-PCR. Molecular typing was conducted with type-specific sequencing.
Result: A total of eight children between seven months and 14 years of age were tested positive for HAdV in this four week period. All had symptoms of gastroenteritis or upper respiratory tract infection (URI). Five of eight children were hospitalized on the haemato-oncology ward, all at least two weeks prior to diagnosis. Two children were seen only in the out-patient services; one child was hospitalized with URI on another ward and developed HAdV-diarrhoea three days after admission. The molecular typing of seven isolates of HAdV showed four different genotyps: 2xC1, 1xC6, 2xA31 und 1xF41. Two children on the haemato-oncology ward had the same genotype A31.
Conclusion: Immediate symptom-related implementation of specific precautions in children is successful in the prevention of patient-to-patient transmissions. Possible reasons for the observed cluster may be transmission from parents or siblings or the reactivation of HAdV under immunosuppression. Improved education and information of parents and other family members might be a useful additional effort in pediatric wards.

HYV12

Nosocomial outbreaks caused by medical staff - A systematic review

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Objectives: The role of infected and/or colonized health care workers (HCWs) as a potential source of a nosocomial outbreak is yet unclear. The present study summarizes data from such outbreaks in order to elucidate areas of special interest and to clarify potential reasons for transmission.
Methods: We conducted a systematic review of the medical literature based on PubMed, the Outbreak Database (www.outbreak-database.com), and on a hand search of so retrieved articles. We extracted data on the setting, type of infection, number of patients, duration of the epidemic, and infection control measures.
Results: A total of 152 outbreaks and 1,449 patients got included thereof 51 fatal cases. The main route of transmission was direct or indirect contact (53% of all outbreaks). Duration of the outbreaks ranged from 1 to 287 weeks (mean: 28 weeks; median: 10.5 weeks). The number of affected patients per outbreaks ranged from 1 to 75 (mean: 9.5; median: 7). Surgical (40%) and neonatological departments (24%) were most often involved in outbreaks primarily caused by staff. Transmission took frequently place in surgical theaters (40%) and on peripheral wards (38%). The primary types of infection were surgical site infections (27%) and hepatitis B (22%). The corresponding causative main agents were Staphylococcus aureus (32%), hepatitis B virus (14%), and Streptococcus pyogenes (12%). As shown in the figure, physicians (59 thereof 30 surgeons) and nurses (56) were the predominant staff spreading the pathogen to patients. Nine per cent of them were aware of their carrier status. When checking for the compliance to hand hygiene (HH) of the person causing the outbreak, we found that HH was considered adequate in 14% of the outbreaks but poor in 10.5% (table). The infection control measures most often implemented in order to terminate the outbreak included screening of personnel (90%) and patients (77%), education of staff and disinfection / sterilization procedures (53% each), application of antimicrobial substances (47%), improvement in HH (46%), and use of protective clothing (39%).
Conclusion: Nosocomial outbreaks caused by staff are an important problem in the field of epidemiology and infection control. In the next step we will check for characteristics of HCWs that may predispose for an increased pathogen spread to patients.

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Figure 1

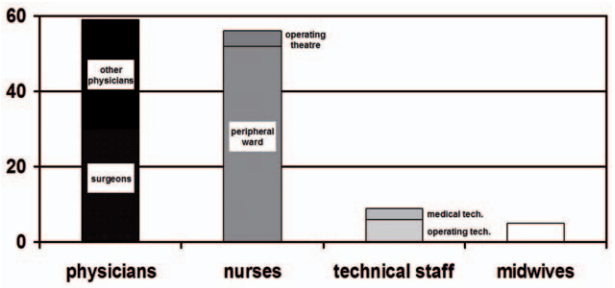

Figure 2

health care worker characteristics		n (%)
hand hygiene	adequate	21 (14)
	poor	16 (11)
	unknown	115 (76)
awareness of carrier status	yes (except viral hepatitis)	4 (3)
	yes (viral hepatitis)	10 (7)
	no	138 (90)
work experience	short	18 (12)
	medium	3 (2)
	long	15 (10)
	unknown	116 (76)

IIP01

The human antibody response to *Staphylococcus aureus* bacteremia

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Staphylococcus (S.) aureus is both a prominent cause of nosocomial infections with significant morbidity and mortality and a commensal with nasal carriage in around 30% of the population. The rapid spread of multi-resistant strains necessitates novel therapeutic strategies; a challenging task, because the species *S. aureus* and the host response against it are highly variable. Although *S. aureus* nasal carriers show an increased risk of endogenous *S. aureus* infection, they have a significantly better outcome. We propose that humoral memory, acquired during colonization, shapes the response to *S. aureus* bacteremia.
In a prospective study among 2023 surgical and non-surgical patients twelve patients developed *S. aureus* bacteremia. The infecting strains were

isolated and patients' sera were obtained at diagnosis and during the course of infection. They were analysed in detail using a personalized approach. For each patient, the extra-cellular proteins of the infecting *S. aureus* strain were identified and the developing antibody response was assessed on 2D immunoblots.
S. aureus carriers showed clear evidence of strain specific pre-immunisation. In all immune-competent bacteremia patients, antibody binding increased strongly, in most cases already at diagnosis. This observation may be exploited for earlier diagnosis. In endogenous infections, the pattern of antibody binding was similar to the pre-infection pattern. In exogenous infections, in contrast, the pre-infection pattern was radically altered with the acquisition of new specificities. These were characteristic for individual patients. Nevertheless, a common signature of ten conserved *S. aureus* proteins, recognized in at least half of the bacteremic patients, was identified. All patients mounted a dynamic antibody response to a subset of these proteins. 2D-analysis of the humoral *S. aureus* immunome proved to be a powerful tool for the coordinated analysis of the variable encounters of *S. aureus* with its human host.

IIP02

Neutrophil cell death induction by *Staphylococcus aureus* from nasal colonization and chronic furunculosis

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Staphylococcus (*S.*) *aureus* is a frequent colonizer and also a common pathogen. Furunculosis, a severe skin infection, is mainly caused by *S. aureus*. Interestingly, the clinical isolates inducing furunculosis frequently harbour the *pvl*-genes, encoding the toxin Panton-Valentine-Leukocidin (PVL). Even at low concentrations PVL is known to induce the lysis of human neutrophil granulocytes.
Neutrophils are essential for the clearance of *S. aureus*. Besides degranulation and phagocytosis, neutrophils are able to kill microbes by releasing their DNA and antimicrobial peptides to form neutrophil extracellular traps (NETs).
To test whether invasive *S. aureus* strains that cause chronic furunculosis differ from colonizing strains we investigated their ability to induce cell death in neutrophils.
Neutrophils were stimulated with living bacteria or bacterial supernatants obtained in different growth media. DNA release indicating cell death was quantified by exclusively staining extracellular DNA. The morphology of cell death was determined by immune fluorescence.
Supernatants from *S. aureus* isolates from furunculosis patients but not from healthy nasal carriers lysed the neutrophils. This was dependent on the presence of the *pvl*-genes and on the bacterial culture medium: Only supernatants obtained in nutrient-rich media contained measurable amounts of PVL and caused necrosis of neutrophils. At sublytic concentrations, PVL did not elicit NETosis (neutrophil cell death during NET formation).
Sublytic concentrations of bacterial supernatants did not induce neutrophil cell death. In contrast, the life span of neutrophils was extended, while their ability of proinflammatory activation was conserved. We can exclude indirect effects by modulating cytokines of contaminating T cells.
When exposed to living *S. aureus* cells, neutrophils died. Depending on the multiplicity of infection we observed rapid DNA release, predominantly necrosis, or delayed cell death, mainly apoptosis or NETosis. However, there were no differences between *S. aureus* cells from colonizing or furunculosis inducing isolates.
Opsonization of the *S. aureus* cells with human serum followed by coculture with neutrophils delayed the DNA release of neutrophils, caused higher IL-8 production and substantially reduced the numbers of vital bacteria.
We conclude that *S. aureus* isolates from nasal carriage and furunculosis infection differed in their ability to cause PVL-mediated neutrophil lysis, but their capacity to induce NET formation or apoptosis in neutrophils was very similar.

IIP03

The *Yersinia* effector protein YopM uses the endocytic pathway to reach its intracellular destination

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Cell-penetrating proteins (CPP) are able to cross cellular membranes, either alone or in association with a bioactive cargo. We identified the Yersinia outer protein M (YopM) as a novel bacterial CPP with anti-inflammatory properties. This implicates a potential use of YopM as an immunmodulatory agent for therapeutic applications. To provide a significant advancement in understanding of YopM´s intracellular and molecular function the routes of internalization were analyzed in this study.
HeLa cells were incubated with YopM and analyzed by cryo-immunogold electron microscopy (EM). For quantification fifty cell profiles were randomly selected and antibody signals were evaluated by number of gold particles linked to certain cellular compartments. Furthermore, colocalization studies with specific marker proteins were performed using confocal immunofluorescence microscopy. Additionally, localization of YopM was confirmed by cell fractionation and Western Blotting.
Cryo-immunogold EM revealed YopM throughout the endosomal compartments, but never in secretory membranes. Free YopM could be detected throughout the cytoplasm and in the nucleus. Additionally, the nuclear localization was confirmed by cell fractionation. To further characterize the nature of the YopM-positive endosomal compartments immunofluorescence studies were performed. We found that YopM colocalizes with specific endosomal markers characteristic for early and late endosomes as well as lysosomes. Additionally, the absence of YopM at secretory membranes was confirmed, excluding the secretory pathway as a YopM target.
Our results show that recombinant YopM enters eukaryotic cells predominantly in an endosome-associated manner. After escaping from the endosomes it localizes to the cytosol and partially in the nucleus, indicating an intracellular endosomal-independent movement of YopM.

IIP04

Long-term response to vaccination against pneumococcal antigens in kidney transplant recipients

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Introduction: Vaccination against Streptococcus pneumoniae is recommended by the German advisory board for vaccination (STIKO) in immunocompromized patients such as kidney transplant recipients. S. pneumoniae mainly infects the upper respiratory tract; it can cause lobar pneumonia or meningitis. Morbidity and mortality are both increased in immunocompromized patients. It was the aim of the present study to define the long-term efficiency of vaccination in clinically stable kidney transplant recipients.
Methods: 49 patients (21 female, 28 male, median age 55, range 29-74 years) were immunized using Pneumovax 23. Antibodies against 14 pneumococcal capsular polysaccharide antigens (serotypes) were determined prior to, one month and 15 months after vaccination by multiplexed bead assay (Luminex).
Results: One month after vaccination, patients displayed a significant increase ($p<0.0001$) in the total antibody concentration against 14 pneumococcal serotypes from a median of 18.2 mg/l (range 2.9-55.5) prior to vaccination to 53.6 mg/l (range 4.5-132.4). Fifteen month after vaccination, the total antibody concentration was still significantly higher ($p<0.0001$) than prior to vaccination (median 41.3, range 4.9-105.0 mg/l). In addition, the kidney transplant recipients showed a significant increase in the number of serotypes recognized from a median of 8 (range 0-13) to 13 (range 0-14, $p<0.0001$) at month 1 and to 11 (range 0-14, $p=0.006$) at month 15. Antibody responses after vaccination were slightly, but significantly ($p<0.0001$, both at month 1 and 15) lower than in a published cohort of vaccinated, healthy controls [14 (3-14), Borgers et al., 2009)].
Conclusion: Our results demonstrate that kidney transplant recipients can produce almost normal concentrations of antibodies against pneumococcal polysaccharides. Fifteen month post vaccination, 77% of the initial antibody response (at month 1) remained detectable.

IIP05

Role of CD95 in *Pseudomonas aeruginosa* infection in Cystic Fibrosis

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Cystic fibrosis is caused by a defective expression of the Cystic Fibrosis Transmembraneconductance Regulator (Cftr) gene. Pulmonary symptoms of the disease are in particularchronic pulmonary inflammation and infections. The pathophysiological mechanisms bywhich these changes are induced in the lungs of patients with cystic fibrosis requiredefinition. This study found that Cftr deficiency in mice results in the upregulation andactivation of CD95. CD95 activation is caused by increased ceramide concentrations in cysticfibrosis lungs, as revealed by genetic modifications that normalize pulmonary ceramideconcentrations. However, the activation of CD95 further increases pulmonary ceramide levelsand results in a vicious cycle of CD95 activation and ceramide accumulation. Genetic studies reveal that CD95 is crucially involved in the induction of aseptic inflammation, an increase inthe bronchial cell death rate, and an increased susceptibility to infection among Cftr-deficientmice. All of these pathologies are at least partially corrected by heterozygosity of CD95 inCftr-deficient mice. These findings identify CD95 as an important regulator of lung functionsin cystic fibrosis and suggest that CD95 may be a novel target for treating cystic fibrosis.

IIP06

Distinct profiles of the human immune response to *Staphylococcus aureus* revealed by immune proteomics of Ig subclasses

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Objectives: *Staphylococcus aureus* plays a role as commensal and as pathogen. About 20 % of the human population is persistently colonized with *S. aureus* in the anterior nares. We have shown that carriage tunes the adaptive immune system and results in a strain-specific humoral response.
The adaptive immune system can mobilize different effector mechanisms to a given antigen. E.g., Ig class switch to IgG1 is driven by pro-inflammatory Th1 cells, whereas IgG4 production requires help from Th2 or Tregs that play a role in allergy and immune tolerance, respectively. We have determined the Ig subclass composition of human anti-*S. aureus* antibodies, focussing on the IgG1/IgG4 ratio.
Methods: Colonizing and invasive *S. aureus* strains and sera of carriers and *S. aureus* bacteremia patients were investigated using a personalized approach. The extra-cellular proteins of each strain were separated by 2D SDS-Page, blotted, decorated with serum and binding of *S. aureus*-specific IgG1 and IgG4 was quantified.
Results: In carriers, the IgG1 and IgG4 binding patterns to the colonizing *S. aureus* strain were highly individual. This applied to the number of protein spots recognized by IgG1 and IgG4 and to the relative intensity of IgG1 versus IgG4 binding. Some *S. aureus* antigens were bound by both IgG-subclasses, whereas others were selectively bound by IgG1 or IgG4. The investigation of the *S. aureus* bacteremia patients showed similar results. Overall, antibody binding increased during infection in immune competent patients, and new anti-*S. aureus* antibody specificities emerged. The relative increase of binding differed between the IgG subclasses and protein spots.
Conclusions: In summary, humans differ strongly in their antibody subclass response to *S. aureus.* On the other hand, individual *S. aureus* antigens elicit heterogenous subclass profiles in the same individual. We are currently determining the antigen signatures of IgG1- versus IgG4-dominance in the antibody response to *S. aureus*.

IIP07

Yersinia-induced apoptosis involves cleavage of MyD88 and concomitant deregulation of TLR signalling

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A wide variety of pathogenic microorganisms has evolved tactics to modulate host cell death or survival pathways for colonizing the host organism and establishing infection. *Yersinia enterocolitica* triggers apoptosis in macrophages by injecting the YopP effector protein through type III secretion into host cells. YopP deactivates several TLR-induced signaling pathways which impedes the development of a coordinated immune response and finally affects cell survival, leading to macrophage apoptosis.
Here, we show that *Yersinia*-induced apoptosis of human macrophages involves caspase-dependent cleavage of the TLR adapter protein MyD88. In the same manner, overexpressed MyD88 is cleaved in HEK293 cells following apoptosis induction. The processing of MyD88 occurs in the advanced stages of apoptosis when caspase-3 is activated. Accordingly, caspase-3 cleaves MyD88 in *in-vitro* protease assays. The processing of MyD88 is not restricted to *Yersinia* infection, but is also observed during staurosporine-induced apoptosis. It appears that the cleavage of MyD88 uncouples the activation of TLR4 from NF-κB signaling. Thus, the disruption of MyD88 signaling in the execution of apoptosis may render the dying cells less sensitive to TLR stimulation. This suggests that the cleavage of MyD88 is implicated in apoptotic death signaling by conferring immunogenic tolerance to the dying cell in response to microbial stimulation.

IIP08

Investigation of the mucosal immune response against *Mycoplasma pneumoniae* infections after immunization with a new vaccine candidate in combination with chitosan

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The cell wall-less bacterium *Mycoplasma pneumoniae* is a leading cause of both upper and lower respiratory infections in humans. A crucial step in host colonization is the adhesin-mediated binding of this pathogen to the cells of the respiratory epithelium. For cytadherence *M. pneumoniae* exhibits a complex membrane extension called attachment organelle which includes amongst other proteins the major adhesin P1 and the adherence-associated protein P30.
In the present study we investigated the immune response of guinea pigs against *M. pneumoniae* over a time period of 21 days after a single intranasal infection. ELISA, Western blot and FACS analysis have shown an early and strong serum immune response against the major adhesin P1 and a time-dependent reduction of adhesion of mycoplasmas to HeLa cells after pretreatment of the bacteria with the different antisera. In a previously published work we created a chimeric protein (HP14/30) derived from functional adhesion regions of proteins P1 and P30. A polyclonal antiserum against HP14/30 was able to decrease *M. pneumoniae* adherence to three different human cell lines to around 5%. We used this chimeric protein as antigen for an immunization strategy to elicit an antibody response in the respiratory tract of guinea pigs which can reduce the adhesion of *M. pneumoniae*. For this purpose, we combined the adherence inhibitory properties of HP14/30 and the mucoadhesive characteristics of the polysaccharide chitosan as adjuvant. Guinea pigs were immunized intranasally (i.n.) with an optimized mixture of HP14/30 and chitosan. Laboratory experiments confirmed the stability of the protein-chitosan mixture and the time-dependent release of the antigen. The immune responses in the i.n. immunized animal group were compared with those obtained by subcutaneous (s.c.) immunization. Preliminary tests have shown that i.n. immunization with chitosan and HP14/30 was able to induce higher mucosal IgA titre than s.c. immunization alone. The results are promising to achieve increased levels of *M. pneumoniae*-specific secretory IgA in comparison to the conventional systemic immunization by using the advantages of chitosan for the nasal vaccination of host organisms with the characterized antigen.

IIP09

The fibrinogen binding protein Fbl of *Staphylococcus lugdunensis* is not involved in the invasion into epithelial and endothelial cells.

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Bacterial invasion of non-phagocytic host cells is an important pathogenicity factor for escaping the host defense system. Several Staphylococci, such as *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis*, are internalized in eukaryotic cells and this mechanism is discussed as an important part of the infection process. Fibronectin binding proteins have been discussed as an important prerequisite for internalization described for *S. aureus*. For *S. epidermidis* a fibronectin independent mechanism via HSP70 has been also recently described. Two fibrinogen binding adhesins have been previously described for *S. lugdunensis*. Fibrinogen binding proteins support the adhesion of bacteria to eukaryotic cells, but were not considered as prerequisite for the internalization process for *S. aureus.* To date a fibronectin binding protein for *S. lugdunensis* has not been described. We sought to investigate whether *S. lugdunensis* is internalized into the epithelial and endothelial cell line cells (5637 and EA.hy 926) using a previously described FACS-based invasion assay.
We could show that clinical isolates of *S. lugdunensis* were internalized into human urinary bladder carcinoma cell line 5637 and the endothelial cell line EA.hy 926. Interestingly, all strains that invade into human urinary bladder carcinoma cell line 5637 bound to fibronectin. In contrast to this, strains not binding to solid-phase fibronectin were not internalized. The fibrinogen binding protein Fbl was not involved in this internalization process, since an isogenic fbl knockout mutant also invade into eukaryotic cells and unaltered bound to fibronectin.
The discovery of the internalization attribute of *S. lugdunensis* and its linkage to fibronectin binding suggests that the internalization of this important pathogen also occurs via a fibronectin mediated surface contact.

IIP10

Temporal decline of serum-bactericidal activity after immunization with Meningococcal ACWY Polysaccharide vaccine in adults

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Laboratory staff working with *Neisseria meningitidis* should be offered immunization with available vaccines against serogroups A, C, W-135, and Y. As part of our biological safety management we have performed a cross-sectional study with 20 individuals between 18 and 50 years, who were vaccinated once with quadrivalent polysaccharide vaccine; the aims of the study were: estimation of the duration of protection by polysaccharide vaccine; and assessment whether revaccination with newly available quadrivalent conjugate vaccine was necessary. Timepoints of immunization were retrospectively obtained from immunization cards of participants. Serum-bactericidal activity testing is the most widely accepted surrogate parameter for assessment of vaccine effectiveness against meningococci; we tested sera using the tilt method (Maslanka et al., 1997) with reference strains M99 243594 (“CDC strain F8238”), C11, M01 240070, and M00 242975 for serogroups A, C, W135, and Y, respectively. The minimal duration of protection was estimated by the intersection of the lower 95% prediction band of a linear model describing the temporal decline of the titre’s logarithm (to the base 2) with a horizontal line representing the protective titre of 1:8. Minimal duration of protection was 33.7, 25.2, 0.0, and 55.2 months for serogroups A, C, W135, and Y, respectively. Mean protective duration, however, exceeded 100 months for all serogroups. Individuals with titres below 1:8 were vaccinated with a quadrivalent conjugate vaccine and protective titres were restored after 4 weeks (data not shown). In conclusion, our analyses might point to less effective immunization against serogroup W135 by a polysaccharide-only vaccine. To corroborate this finding, additional measurements of the concentration of anticapsular IgG present in the sera of vaccinees are underway.

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IIP11

Haemophilus influenzae uses the PE surface protein to acquire human plasminogen and to evade innate immunity

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Haemophilus influenzae is an important respiratory pathogen and is responsible for a variety of infections in humans. The nontypeable *H. influenzae* (NTHi) strain 3655 binds human plasminogen and we identify the adhesion protein Protein E (PE) of NTHi 3655 as an new and major plasminogen binding protein. Plasminogen is a 92 kDa human plasma protein that is expressed by liver cells and is the key enzyme in fibrinolysis. Plasminogen is composed of five homologous triple loop “kringle” domains and a C-terminal serine protease domain. Plasminogen bound dose dependently to intact bacteria and also to recombinant PE of NTHi 3655. Plasminogen also bound to ten different tested clinical *H. influenzae* isolates. The plasminogen-PE interaction is mediated by lysine residues and is affected by ionic strength. Plasminogen binding to the PE knock out strain (NTHi 3655Δ*pe*) is reduced by 65 %, as compared with the wild-type NTHi 3655. In addition, plasminogen bound to PE which was ectopically expressed on the surface of *E. coli*.
The zymogen plasminogen can be converted by specific activators to the serine protease plasmin. Activated plasmin controls complement attack by degrading C3b and also degrades extracellular matrix components, such as fibrinogen. Plasminogen, when bound to intact NTHi 3655 or to PE is converted by human uPa to active plasmin which cleaved the synthetic substrate S-2251 and also the natural substrates C3b and fibrinogen. The plasminogen-binding region were localized within the PE protein by using synthetic linear peptides and the major plasminogen-binding region was localized to a linear peptide that represents the 28 aa of the N-terminus, i.e. aa 41-68. PE binds plasminogen as well as vitronectin and the two human plasma proteins compete for PE binding. Thus, PE of *H. influenzae* is a major plasminogen-binding protein and PE bound human plasminogen and when converted to plasmin aids in immune evasion and contributes to bacterial virulence.

IIP12

Hepatocyte-specific knockout of A20 augments defense against malaria liver stage infections

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Introduction: During a malaria infection, *Plasmodium* sporozoites migrate through several host cells before finally infecting hepatocytes, where they develop into erythrocyte-infecting merozoites. Cytosolic factors released from the traversed hepatocytes activate the NF-κB pathway in adjacent hepatocytes, which, in turn, leads to the reduction in parasite load by inducing the expression of TNF and iNOS. A20, an ubiquitin-editing enzyme, can block the proximal NF-κB signalling pathway under various stimulations and A20 knockout mice succumb prematurely from uncontrolled hyperinflammation. In order to explore the function of A20 in *Plasmodium* liver stage development, hepatocyte-specific A20-null mice were generated and investigated for malaria infection.
Material and methods: Hepatocyte-specific A20 knockout mice were generated by utilizing the Cre/loxP system under control of the albumin promoter. A20 was efficiently and specifically deleted in hepatocytes of the Alb-creA20fl/fl (KO) mice, while its expression was not affected in A20fl/fl (control) mice. We applied blood stage parasitemia and quantitative RT-PCR of liver parasite loads as two complementary primary endpoints of a malaria sporozoite challenge infection. Infiltrating leukocytes were isolated from livers and subpopulations were investigated by FACS. Expression of cytokines and chemokines in infected livers was also assessed by qRTPCR.
Results: Forty-two hours post infection, KO mice had lower liver parasite load as compared to A20fl/fl mice. In the first few days after infection, parasitemia of KO mice was also significantly lower as compared to control mice, but this difference gradually disappeared as the disease progressed. FACS analysis showed more infiltrating CD8 T cells, B cells, macrophages and inflammatory monocytes in KO mice. Besides, higher levels of TNF, IL6, IP10, MCP1 and MCP5 were also detected in the liver of KO mice indicating a stronger inflammatory response. Interestingly, mRNA levels of iNOS, which is responsible for producing anti-malarial NO, were also significantly higher in infected KO mice.

Conclusion: Our study demonstrates that A20 deletion in hepatocytes is beneficial for parasite clearance during malaria liver stage infection, presumably by inducing a stronger local inflammatory response. Augmenting anti-liver stage defense mechanisms may further improve experimental malaria vaccine strategies.

IIP13

A phenolic glycolipid from virulent mycobacteria causes suppression of pro-inflammatory cytokines through inhibition of innate immune system pathways

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Introduction: Tuberculosis (TB) still is a major threat to global health. According to the WHO nearly 1.7m people died in 2009 whereas nearly 10m people are infected with TB worldwide. Since drug-resistant strains of *Mycobacterium tuberculosis* (Mtb) continue to rise, new ways of treating this disease have to be established. Therefore a basic understanding of the infection mechanisms of TB is needed.
It has been shown that Mycobacteria infect their host by various mechanisms, eg. phagosome maturation inhibition (Via et al. 1997) and inhibition of the inflammation after infection (Guenin-Mace, 2009).
Our group has found a lipid (Phenolic glycolipid, PGL) in the outer cell wall of *Mycobacterium marinum* (Mmar, a model organism for the research of Mtb) which might interfere with the innate immune system (Robinson et al., 2008).
We are currently focused on establishing the link between PGL and the pathways of the innate immune system.
Material/Method: PGL was isolated from 7H9 cultures of Mmar and subsequently purified. Human monocyte derived macrophages (HMDM) were isolated from Buffy Coats and cultured over 6 days in RPMI, 10% FCS.
PGL was coated onto RPC18 paramagnetic beads or heat-killed Bacillus Calmette-Guerin (HK-BCG) and chased into HMDM for 5 hours. In case of the magnetic beads HMDM were co-stimulated with LPS. After that the RNA was extracted. The cytokines IL-6, IL-12p40 and TNF-alpha were analysed by qRT-PCR.
HMDM were transfected with a luciferase based reporter system for promotors of different signaling pathways of the innate immune system. After infection with PGL-coated HK-BCG the luciferase activity was analysed.
Results: Upon infection of HMDM with PGL-coated HK-BCG the amount of IL-12p40 RNA is greatly reduced after prolonged incubation. Other cytokines like IL-6 or TNF-a show the same effect. Even after the stimulation of HMDM with LPS and PGL at the same time cytokine RNA levels are significantly reduced.
The promotor activity for NF-kB and AP-1 after stimulation of HMDM with PGL-coated HK-BCG is greatly reduced in comparison to the activity after stimulation with uncoated HK-BCG.
Discussion: Our findings suggest that PGL is indeed able to interfere with the signaling pathways of the innate immune system.
We could show that PGL supresses the production of IL-6, IL-12p40 and TNF-a RNA in HMDM after stimulation with HK-BCG or LPS.
The reduced promotor activity of NF-kB and AP-1 indicates that PGL interferes with pathways of the innate immune system. It still remains elusive where exactly PGL interferes with these pathways.
These results show the importance of PGL for Mycobateria to be able to undermine the response of the immune system. Our work could therefore help to complete the picture of the infection mechanisms of Mycobacteria.

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IIP14

Adhesion and degranulation-promoting adapter protein is needed for the protective immune response against *Toxoplasma gondii*

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The intracellular protozoan parasite *Toxoplasma gondii* can invade the brain and cause a persistent infection, toxoplasmic encephalitis (TE). IFN-γ producing $CD8^+$ T cells, activated microglia, macrophages, and astrocytes appear to be the major effector mechanisms in host immunity. Infiltration of the brain depends on the expression of different adhesion molecules and on the integrity of the blood brain barrier. The adhesion and degranulation-promoting adapter protein (ADAP) is expressed in T cells, myeloid cells, and platelets. This adapter protein is involved in T cell receptor (TCR)-dependent inside-out signaling and thereby regulates the activation and adhesion of T cells. Little is known about the role of ADAP in brain cells. Since we could demonstrate that ADAP-deficient ($ADAP^{-/-}$) mice showed ameliorated disease progression in the experimental autoimmune encephalomyelitis (EAE) model, a function of ADAP in brain cells was hypothesized.
In this study, we performed low dose infection of $ADAP^{-/-}$ mice and wild-type mice with *T. gondii* and monitored survival. Furthermore, we examined the cyst load and inflammatory infiltrates using immunohistochemistry as well as the distribution of microglia cells, macrophages, and lymphocytes in the brain by FACS analysis.
Strikingly, $ADAP^{-/-}$ mice showed significantly reduced survival in line with increased cyst load at day 44 after infection compared to wild-type mice. Although the number of IFN-γ producing lymphocytes was increased in the brains of $ADAP^{-/-}$ mice, all $ADAP^{-/-}$ mice died until 54 days post infection, while all wild-type mice survived. In contrast, the number of activated microglia cells and astrocytes was reduced in the brain of $ADAP^{-/-}$ mice.
These data indicate an important role of ADAP for the activation of microglia cells during toxoplasmosis, whereas the integrin-mediated migration of T cells through the blood-brain-barrier seems to be independent of ADAP. ADAP contributes to survival from TE and therefore plays a major role in maintaining immune homeostasis in the brain.

IIP15

The staphylococcal protein Efb binds plasminogen and enhances plasmin-mediated C3 degradation

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Staphylococcus aureus is a human pathogen that lives as a human commensal but can also cause superficial and life-threatening infections. Upon infection, *S. aureus* induces host innate immune reactions, such as the activation of the complement system, recruiting of phagocytic cells and the secretion of antimicrobial peptides. However *S. aureus*, as master of immune evasion, interferes particularly with the actions of the complement system. Complement activation normally leads to opsonization and lysis of pathogenic microorganisms as well as inflammatory reactions. *S. aureus* has developed multiple strategies to target and inhibit C3, the central molecule of the complement system. *S. aureus* secretes Efb (extracellular fibrinogen binding protein), which directly binds the precursor C3 and the opsonin C3b to block the C3 convertase activity. Upon binding to C3, Efb induces a conformational change in C3 leading to an enhanced susceptibility to degrading proteases such as trypsin. Since *S. aureus* also recruits the human serum protein plasminogen, and subsequently activates plasminogen by Staphylokinase (SAK) to the C3/C3b-degrading plasmin, we asked whether Efb modulates the plasmin-mediated C3 cleavage. Here we show that Efb recruits plasminogen. Efb bound plasminogen is accessible for the human plasminogen activator uPa as well as for the bacterial SAK, both generating proteolytic active plasmin. The interaction of Efb with C3 and plasmin leads to an enhances C3 degradation by plasmin. Thus Efb targets C3 in two ways, directly by binding and inducing a conformational change in C3, and indirectly by facilitating the proteolytic activity of plasmin.

IIP16

Critical role of type III interferons during rotavirus infection

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Type I and type III interferons (IFN) bind to different cell surface receptors but induce identical signal transduction pathways leading to the expression of antiviral host effector molecules. Although IFN-l has been shown to predominantly act on mucosal organs such as the lung and gastrointestinal tract, studies using IFN-l receptor-deficient mice have failed to define a non-redundant function. Here we show a protective role of IFN-λ signalling during rotavirus infection using suckling wild-type, type I IFN receptor-deficient, IFN-l receptor-deficient and double-knockout mice. Enhanced virus replication and tissue damage was found in the absence of IFN-l signalling, whereas no significant contribution of IFN-a/b signalling to the antiviral host defence was detected. Quantitative RT-PCR and simultaneous immunostaining of virus-infected cells and the antiviral Mx1 protein highlighted the distinct responsiveness of intestinal epithelial cells to IFN-l and its critical role to restrict epithelial viral replication *in vivo*. Thus, the antiviral host protection of the intestinal epithelium in response to rotavirus infection relies on intact IFN-l signalling.

IIP17

Potentiation of epithelial innate host response by intercellular communication

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Recognition of pathogenic microbes by intestinal epithelial cells results in transcriptional activation and secretion of soluble mediators that attract professional immune cells to the site of infection. This defence mechanism works very efficiently despite the often low number of pathogens and the limited amount of mediators secreted per epithelial cell. We have recently identified horizontal intercellular communication as critical component of an efficient epithelial innate host defence. Chemokine production after L. monocytogenes infection was primarily observed in neighboring, non-infected cells despite the invasion-dependent nature of Listeria-induced epithelial activation. Cell-to-cell communication was independent of gap junction formation, cytokine secretion, ion fluxes or nitric oxide synthesis, but required NADPH oxidase (Nox) 4-dependent oxygen radical formation. In conclusion, our results provide a novel concept of a coordinated epithelial host response upon microbial challenge to maintain mucosal homeostasis and provide an efficient protection from infection with enteropathogenic microorganisms.

IIP18

Influence of toxic kynurenines on the viability of microorganisms and tumor cells

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In human cells stimulation with IFN-γ results in a strong activation of the tryptophan degrading enzyme Indoleamine 2,3-dioxygenase (IDO) which is an important antimicrobial- and immunoregulatory effector mechanism. IDO converts the essential amino acid tryptophan to formyl-kynurenine, which is channelled into kynurenine pathway. IDO mediates its antimicrobial effects mainly by a starvation of tryptophan. However, tryptophan metabolites produced in the kynurenine pathway are able to inhibit the growth of immune cells. In this study we analysed the effect of kynurenine (Kyn), 3-hydroxy-kynurenine (3-HK), 3-hydroxy-anthranilic acid (3-HAA) and of quinolinic acid (QA) on the proliferation of T-cells, *Staphylococcus aureus*, *Toxoplasma gondii* and of tumor cells. We found that Kyn, 3-HK and 3-HAA do not influence the growth of *S. aureus*, while these metabolites are able to inhibit the proliferative response of T-cells. Additional experiments showed an influence of these metabolites on the growth of *T. gondii* in glioblastoma cells. Detailed analyses indicate that this antiparasitic effect is due to a toxic effect of the tryptophan metabolites on the host cells. This astonishing result was furthermore investigated, using different types of tumor cells. We found that the strongest toxic effect was mediated by 3-HAA followed by 3-HK and Kyn, while QA did not influence the growth of all tumor cells tested. In addition we detected a synergistic effect of Kyn and 3-HAA on tumor cell viability. Quantitative analysis of the toxicity indicates that some tumor cells e.g. A549 cells (lung cancer cells) are more resistant against 3-HK and 3-HAA than T-cells. In vivo many human tumor cells express IDO activity and are therefore able to produce kynurenines. These metabolites might inhibit the activity of tumor specific T-cells, while the enhanced resistance of some tumor cells against the toxic effect of tryptophan metabolites might represent an immune escape mechanism.

IIP19

Involvement of the hyaluronan metabolism in microbial – fibroblast interaction

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Background: Fibroblasts regulate epithelial cell differentiation, are responsible for wound healing, control inflammation process and seem to play an important role in cancerogenesis. Bacteria and their products play as well are involved in prevention/promotion of chronic inflammatory diseases, in wound healing and in cancer. The linkage might be the hyaluronan (HA) metabolism. Bacteria produce hyaluronidases and bacterial products interact with Toll-like-receptors (TLRs) which also seem to be linked to HA metabolism. We investigated how far microorganisms/microbial products influence the metabolism of HA in human dermal fibroblasts through TLR activation.
Methods: We stimulated human dermal fibroblasts with the staphylococcal toxins TSST-1 and enterotoxin B (SEB), with lipopolysaccharide (LPS), peptidoglycan (PGN) as well as with soluble products from clinical Staphylococcus aureus, S. epidermidis and Lactobacillus crispatus isolates. The release of the chemokine IL-8 was analyzed by ELISA as well as by quantitative real-time PCR (RT-PCR). Activation of TLRs (TLR 2-5, 8-9), hyaluronan acid synthases (HAS 1-3) and hyaluronidases (HYAL 1-3) was analyzed by RT-PCR.
Results: Fibroblasts responded to LPS, TSST-1, to soluble products of S. epidermidis and of L. crispatus with the release of high amounts of IL-8 and an up to 200-fold increase in mRNA levels. The most pronounced changes in TLR expression were seen for TLR 4, TLR 5 and TLR 9 by S. epidermidis, for TLR4 by the staphylococcal superantigens TSST-1, SEB and by PGN, for TLR-2 and TLR-4 by LPS. PGN activated IL-8 release predominantly through NOD1 activation, while the additional stimuli used MYD88 signalling as well. In unstimulated fibroblasts the highest mRNA expression was seen for HAS 2 and HYAL 2, moderate expression was seen for HAS 3 and HYAL 3, no signal was obtained for HAS 1 and HYAL 1. However, all microorganisms and the microbial products under study did not modulate HA metabolism. Breakdown of hyaluronan by staphylococcal hyaluronidases did not change mRNA expression of HAS 1-3 or HYAL 1-3 as well.
Conclusion: Our data clearly show that fibroblasts interact with microorganisms/microbial products to a different degree, e.g. through TLRs. However, a possible role of HA metabolism in host-microorganism interacting needs further studies.

IIP20

Quantification of regulatory T cells in septic patients, a comparison of a methylation-sensitive qRT-PCR and flow cytometry ($CD4^+CD25^{++}CD127^-$)

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Regulatory T cells play a pivotal role in establishing a peripheral immunotolerance. An imbalance between regulatory T cells (T_{reg} cells) and effector T cells is accused to influence the origin and course of several chronic infections, autoimmune diseases and certain tumours. There is also evidence, that in septic patients, the percentage of T_{reg} cells referred to all T cells increases during sepsis and that these patients show a sepsis-induced immunoparalysis and have a poor outcome. Besides, an exact quantification of T_{reg} cells is of great interest for elucidating the role of these cells for development and progress of many diseases. There are even concepts of T cell therapy for appointed diseases to re-establish a balance between T_{reg} cells and effector T cells. Up to now, most studies quantified T_{reg} cells by the analysis of surface markers and of the transcription factor Foxp3 using flow cytometry. As to date, a constitutive expressed specific unique marker for human T_{reg} cells is not available, an exact quantification on protein or RNA level is imprecise. Recently it has been found that epigenetic differences in DNA methylation between T_{reg} cells and effector T cells exist and that these differences can be used for quantification of T_{reg} cells. T_{reg} specific demethylated region (*TSDR*) of *FOXP3* has been demonstrated to be an applicable genomic region for T_{reg} quantification. Here, we present data of the quantification of T_{reg} cells by a newly developed methylation sensitive real-time PCR assay using *FOXP3-TSDR* and compare these data with the analysis of T_{reg} cells by flow cytometry ($CD4^+CD25^{++}CD127^-$). Data of the quantification of T_{reg} cells from blood of septic patients are compared with quantified T_{reg} cells from blood of healthy people.

IIP21

Salmonella enterica as a carrier for vaccination

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Salmonella enterica serovar Typhimurium is a facultative intracellular pathogen which can enter host cells and replicates within a specialized membrane compartment. Salmonella is also an interesting vehicle for the display of recombinant antigens to professional antigen presenting cells (APC). Heterologous antigens can be expressed in Salmonella as fusions with recombinant or native proteins. This approach has been used mainly to direct the expression of the desired antigen to a particular location of the bacterial cell and to increase the immunogenicity of foreign antigens by fusing them to proteins that could exert a carrier effect. The type III secretion system (T3SS)-mediated translocation by Salmonella can be used for efficient delivery of heterologous antigens into the cytosol of APC, leading to stimulation of both CD4 and CD8 T-cells. In this work, we investigated the use of a subset of effector proteins of the SPI2-encoded

T3SS as SseJ, SifA, SseL and SteC. These effectors proteins are associated with endosomal membranes after translocation. Our In-vitro and in-vivo experiments for vaccination show that effector SseJ is the most suitable fusion partner. In previous work it was shown that sifB promoter was the most efficient in-vivo inducible promoter. Here we show that SseJ antigen fusion protein under control of the sifB promoter is most efficient in comparison with effector fusions under control of other in-vivo inducible promoters. By comparison of various attenuated carrier strains, we observed that the htrA/purD double mutant strain can be used efficiently as attenuated carrier for vaccination and observed the efficient stimulation in-vitro T-cell proliferation by the delta sifA mutant strain as carrier.

IIP22

Role of the MAPK phosphatase Dusp16/MKP-7 in innate immunity

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The production of cytokines in response to microbial danger signals is regulated at the level of MAPK activation. Dual specificity phosphatases (Dusp) control MAPK activity by dephosphorylating threonine and tyrosine residues. Differential expression and inducibility of Dusp gene expression suggest that these phosphatases specify the outcome of MAPK activation in terms of cytokine production. Here, we have investigated the role of Dusp16 (also known as MKP-7) in the immune system. Dusp16 expression in macrophages and dendritic cells was inducible by TLR stimuli in vitro. In vivo, Dusp16 expression was constitutive in some organs and in B lymphocytes; in contrast, LPS challenge up-regulated Dusp16 mRNA in the spleen. A gene trap ES cell clone, bearing an insertion in the Dusp16 locus that abrogates the expression of full length Dusp16 mRNA, was used to generate mice lacking Dusp16. Matings of heterozygous Dusp16trap/+ mice failed to yield homozygous Dusp16trap/trap mice at weaning. However, embryonic development of homozygous mice was grossly normal until E18.5, indicating that Dusp16trap/trap mice die perinatally. Fetal liver cells were used to generate macrophages in vitro and to reconstitute lethally irradiated mice for in vivo analysis. Dusp16trap/trap macrophages responded normally to TLR stimulation for most cytokines analysed, but showed a significantly higher production of IL-12p40. In vivo, Dusp16trap/trap fetal liver cells reconstituted T and B cell compartments similar to WT. Following injection of LPS, Dusp16trap/trap-reconstituted mice significantly over-produced IL-12p40, consistent with the phenotype of Dusp16-deficient macrophages in vitro. Taken together, the production of the cytokine IL-12p40 in response to TLR stimulation in vitro and in vivo is selectively controlled by Dusp16. Ongoing work will address the functional consequences of Dusp16-deficiency in models of inflammation and infection.

IIP23

The cellular response of human monocyte derived macrophages to infection with genetically distinct *M. tuberculosis* complex strains

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Aim of the study: The *Mycobacterium tuberculosis* complex (*MTBC*) was initially regarded as a highly homogeneous population; however, recent data suggest the causative agents of tuberculosis are genetically and functionally more diverse than appreciated previously. The impact of this natural variation on the virulence of the pathogen and clinical manifestations remains largely unknown.
Methods: In this study we analyzed the host response of human primary monocyte derived macrophages upon infection with genetically distinct *MTBC* strains. The study included strains that have caused larger outbreaks in Hamburg, Germany as well as attenuated strains, all of which belonging to the Haarlem genotype. Supernatants were collected after 16h and monitored for the release of pro- and anti-inflammatory mediators.
Results and Conclusions: All strains induced a comparable release of Tumor necrosis factor (TNF)-alpha, Interleukin (IL)-12, IL-6, IL-10 and RANTES. A significant difference between clustered isolates and attenuated strains was not observed. These data demonstrate that differences in strains success monitored in a longitudinal epidemiological study are not reflected at the level of mediator release after infection of human monocyte-derived macrophages. However, additional data also demonstrate that a particular *MTBC* genotype translates into a distinct biological response profile when getting into contact with human immune cells.
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IIV01

Suppressor of cytokine signaling 1 (SOCS1) limits proinflammatory NFκB p65 signaling within the cell nucleus

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Activation of innate immune cells during infections has to be tightly controlled to avoid overshooting reactivity. Suppressor of cytokine signaling (SOCS) proteins are well known feedback inhibitors of janus kinases (Jak) and signal transducer and activator of transcription (STAT) signaling pathways. Unexpectedly, we observed that SOCS1, but no other members of the SOCS family, localized predominantly in the nucleus. Nuclear localization was due to a so far unknown bipartite nuclear localization signal (NLS). Using photoactivable SOCS1 constructs we confirmed rapid translocation from the cytoplasm to the nucleus. Within the nucleus SOCS1 was highly mobile as demonstrated by FRAP experiments. To further characterize the novel function of SOCS1 within the cell nucleus we generated NLS-mutants whose expression was restricted to the cytoplasm. Thereby we identified genes that were differently regulated depending on the nuclear availability of SOCS1. We were able to identify the NFkB component p65 as novel interaction partner for SOCS1. SOCS1 bound to p65 within the nuclear compartment and increased p65 ubiquitination and degradation. The SH2 domain of SOCS1 contributed to p65 binding whereas ubiquitination was mediated through the SOCS box. Limitation of p65 availability resulted in reduced expression of a subset of NFkB dependent genes. Mass spectrometry identified further interaction partners. The results show that SOCS1 regulates the duration of NFkB signaling by means of its ubiquitin ligase activity thereby exerting a so far unrecognized function within the cell nucleus. The nuclear function of SOCS1 is important to limit proinflammatory stimulation of innate immune cells by microbial products.

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IIV02

Differential transcription factor expression and activity by CLR and TLR ligands in macrophages: dissecting gene expression induced by the mycobacterial cord factor

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The mycobacterial cord factor trehalose-6,6-mycolate (TDM) is a major virulence factor of *M. tuberculosis* that is recognized by innate immune cells in a Syk-Card9 dependent manner. Recently, we identified the C-type lectin receptor (CLR) Mincle as the receptor for the TDM and its synthetic analogue trehalose-6,6-dibehenate (TDB). These two glycolipids are potent adjuvants that elicit mixed Th1/Th17 responses, whereas Toll-like receptor ligands such as CpG DNA induce exclusively Th1 responses**.** CLR and TLR ligands trigger distinct profiles of cytokines and effectors in antigen presenting cells. How these unique transcriptional responses are generated, and which role they play for Th1 and Th17 induction is incompletely understood. TLR and CLR signaling activates the MAP-Kinase pathway and the transcription factor (TF) NF-kappaB. In contrast, the latent TF NF-AT is activated upon CLR but not upon TLR stimulation. However, little is known about the TF profile induced downstream of these constitutively expressed TFs. Here we assessed the expression kinetics of inducible TFs in macrophages after TDB and CpG stimulation to understand similarities and differences between expression profiles elicited by the two types of PRRs. TDB rapidly and directly induces early growth response genes Egr1, Egr2 and Egr3 expression dependent on Mincle. Cebpbeta and Hif1alpha are upregulated at protein level after stimulation with TDB or CpG. In ChIP experiments we observed Hif1alpha recruitment to the iNOS promoter. Furthermore Hif1alpha deficiency in macrophages results in a strong reduction of NO release after TDB but not CpG stimulation. Taken together, our data shed new light on the regulatory network controlling transcriptional activation induced by the CLR Mincle in response to microbial Mincle ligands.

IIV03

The type I interferon induced protein IFIT-2 contributes to type I interferon mediated proinflammatory immune response

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IFIT-2, also called ISG54, is a type I interferon induced protein and belongs to the family of tetratricopeptide repeat proteins. IFIT2 is upregulated in various virus infections, as well as it is known that upregulation of IFIT2 leads to better survival of tumor patients after resection of tumors [1]. It was reported that IFIT2 binds to the translation initiation factor eIF3c and therefore inhibits translation of proteins [2]. Specifically IFIT2 may recognize and may prevent translation of capped viral mRNA lacking 2'-O methylation [3].
Stimulation of interferon α/β Receptor $(IFNAR)^{-/-}$ bone marrow derived macrophages (BMDM) demonstrates that TLR3 and TLR4 ligands as well IFN-γ induced IFIT-2 protein expression is type I interferon receptor dependent, indicating that IFIT-2 can be directly induced by type I interferons only. In this study we want to elucidate the role of IFIT-2 as an effector of type I interferon induced biological processes. For this purpose IFIT-2 knockout mice have been generated.
In order to investigate a potential role of IFIT-2 in inflammatory responses, $IFIT\text{-}2^{-/-}$ and $IFIT\text{-}2^{+/+}$ BMDM were stimulated with LPS. $IFIT\text{-}2^{-/-}$ BMDM show significantly reduced TNF-α, IL-6, MCP-1 and MIP-2 secretion, indicating that IFIT-2 contributes to type I interferon mediated amplification of the LPS induced proinflammatory response. In line with these findings, it could be demonstrated that $IFIT\text{-}2^{-/-}$ mice are less susceptible to LPS mediated endotoxin shock compared to littermate wildtype controls. Taken together, this data demonstrate, that IFIT-2 contributes as an effector to type interferon I mediated amplification of inflammatory responses.

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IIV04

A novel role for coagulation factor XIII in the innate immune response against *Streptococcus pyogenes*

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The vertebrate coagulation system plays a major role in hemostasis and wound healing. Coagulation factor XIII (fXIII), a plasma transglutaminase, finally crosslinks a formed fibrin network to generate and stabilize a plasma clot and seal the injured site. Evidence is accumulating which implies that coagulation can also contribute to the early innate immune response against pathogens. Activation of the intrinsic pathway of coagulation, also known as the contact system, seems to play an important role in these processes. Previous work has shown that it can be activated on the surface of many pathogenic bacterial species, which in turn leads to the generation of antimicrobial peptides and bacterial killing.
Here, we present data which demonstrate that contact system activation and especially human fXIII have beneficial effects during infections with *Streptococcus pyogenes*. We find that the system is activated on the surface of these bacteria after exposure to human plasma, which is followed by an activation of thrombin and fXIII. As a consequence of this interaction bacteria are immobilized and killed within the clot. In vivo experiments using a streptococcal skin infection model revealed that $fXIII^{-/-}$ mice develop more severe signs of pathologic inflammation at the focus of infection when compared with wildtype animals. When analyzing skin biopsies from infected wildtype mice, bacteria were found clustered within the fibrin network at the local site of infection. However, in infected $fXIII^{-/-}$ animals, bacteria were widespread and scattered over a large affected area. Treatment of wildtype mice with a human fXIII-concentrate three hours after bacterial inoculation dampened the systemic dissemination of *S. pyogenes* during the early phase of infection. To proof that our in vitro and in vivo results also apply to the clinical situation we analyzed tissue biopsies of patients suffering from streptococcal necrotizing fasciitis. As seen in the murine biopsies, we detected fXIII-mediated bacterial crosslinking to the fibrin network in these biopsies supporting the concept that coagulation and fXIII contribute to the early innate immune response against this pathogen.

IIV05

A role for the lysosomal phospholipase A2 in immune responses to intracellular bacteria

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Phospholipases form a ubiquitous class of enzymes that catalyse the cleavage of membrane phospholipids into smaller bioactive molecules. Recently, a lysosomal phospholipase A2 (LPLA2) has been identified and shown to be highly enriched in alveolar macrophages, where it plays an important role in surfactant degradation. LPLA2 knockout (KO) mice develop a phospholipidosis phenotype as demonstrated by a marked accumulation of phospholipids in alveolar and peritoneal macrophages, and the spleen. Given the high expression of LPLA2 in macrophages and its importance in membrane homeostasis and phospholipid turnover, we hypothesized that LPLA2 contributes to host responses against intracellular bacteria.
In order to study the role of macrophage LPLA2 in response to intracellular bacteria, we performed functional *in vitro* analyses with peritoneal macrophages from wild type (WT) and LPLA2 KO mice. In addition, we study the role of LPLA2 in antibacterial host responses against lung tuberculosis *in vivo*.
While LPLA2 deficient macrophages do not show defects in the killing of intracellular bacteria such as non-pathogenic *E. coli* or pathogenic *Mycobacterium tuberculosis*, we found that they are impaired in digesting intracellular bacteria *in vitro*. Even non-pathogenic *E. coli* are not readily cleared and persist long after those in WT macrophages have been degraded. Furthermore, absence of functional LPLA2 strongly affects the ability of macrophages to stimulate T cells *in vitro.* In line with this, adaptive immunity is impaired in LPLA2 deficient mice upon infection with the lung pathogen *M. tuberculosis in vivo*. In the absence of LPLA2, both the number and activation of *M. tuberculosis* specific CD4 T cells as well as Th1 cytokine levels are significantly reduced upon *M. tuberculosis* infection. LPLA2 deficient mice present with a mild pathology in both lungs and spleens due to reduced cellular infiltration in both organs while bacterial loads are significantly increased.
Taken together, our data indicate that LPLA2 is involved in the clearance of bacteria by macrophages and the processing of bacterial antigens for subsequent generation of T cell responses.

IIV06

The importance of IL-17A and IL-17F during *Mycobacterium tuberculosis* infection

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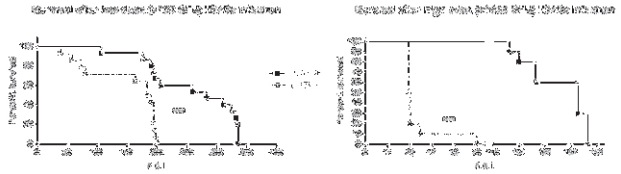
Though (IL)-17A/F-producing T helper (TH)17 cells are potent inducers of tissue inflammation and have been associated with several chronic and autoimmune inflammatory diseases, the main function of TH17 cells appears to be the clearance of pathogens, which are not appropriate affected by TH1 or TH2 cells. To evaluate the role of IL-17A or IL-17F and its protective role during *Mycobacterium tuberculosis* (*Mtb*) infection, we analyzed the outcome of experimental tuberculosis (TB) in IL-17A-deficient ($^{-/-}$) and $IL\text{-}17RA^{-/-}$ mice, which is the receptor for IL-17A and IL-17F. Interestingly, *Mtb*-infected $IL\text{-}17A^{-/-}$ mice efficiently generated interferon-gamma (IFNgamma)-producing TH1 cells and IFNgamma-dependent effector responses after low dose (100 CFU) infection. However, $IL\text{-}17A^{-/-}$ mice were not able to control mycobacterial replication during the chronic phase of experimental TB after low dose infection and died earlier (200 days post infection (dpi)) than corresponding wildtype mice. After high dose infection (1000 CFU), $IL\text{-}17A^{-/-}$ mice rapidly died already during the early stage of *Mtb*-infection at 30 dpi. The breakdown of controlling mycobacterial replication after low and high dose infection was associated with a modulation of gene expression of pro-inflammatory cytokines, chemokines and their receptors. Especially the highly increased gene expression of IL-17F in $IL\text{-}17A^{-/-}$ mice lead to the hypothesis that hypercompensation of IL-17A deficiency by IL-17F induce neutrophilia and susceptibility to *Mtb* infection. In fact and in contrast to $IL\text{-}17A^{-/-}$ mice, mice deficient for IL-17RA (the receptor for IL-17A and F) were resistant

to high dose infection with *Mtb*. So far, our findings lead to the conclusion that IL-17F accounts for susceptibility of *Mtb*-infected IL-17A$^{-/-}$ mice. Hence, IL-17A appears to play no direct role for protection against *Mtb* infection. (Supported by the Inflammation Research Excellence Cluster)

Figure 1



IIV07

Intravital two-photon microscopy of *Staphylococcus aureus* skin infections

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Staphylococcus (S.) aureus is a frequent cause of severe skin and soft tissue infections in humans, the treatment of which is complicated by the emergence of multi-resistant and hyper-virulent strains. The ability to control the infection is largely dependent on the rapid recruitment of polymorphonuclear neutrophilic granulocytes (PMN). To gain more insight into PMN migration and host-pathogen interactions *in vivo*, we investigated a mouse model of *S. aureus* flank skin infection using intravital two-photon microscopy. For this purpose we generated *S. aureus* reporter strains, which expressed codon-adapted fluorescent proteins. PMN migration in response to infection *in vivo* was then visualized using LysM-EGFP transgenic mice.
After injection of the bacteria, we observed the rapid appearance of PMN in the extravascular space of the dermis and their directed movement towards the focus of infection. This resulted in the accumulation of large numbers of cells, which led to the delineation of an abscess within one day. Surprisingly, the direct encounter of bacterial cells by PMN did not always lead to an uptake of the pathogen. Depletion of PMN or blocking G-protein coupled receptors (GPCR) lead to an uncontrolled proliferation of the bacteria. Moreover, tracking of transferred labeled bone-marrow derived neutrophils showed that PMN recruitment to the site of infection is dependent on GPCR on the cells themselves, whereas IL-1-receptor was required on host cells other than PMN. Finally, by using a reporter of the staphylococcal Agr quorum-sensing system, we were able to follow bacterial gene regulation in vivo on a single cell level. Here, we observed that Agr activity could be localized to small areas at the margin of the infection focus.
Our results establish that two-photon microscopy is a powerful tool to investigate the dynamics of the immune response, bacterial cell location, and gene expression in vivo on a single cell level during *S. aureus* infections.

IIV08

Absence of CYLD is essential for survival during murine listeriosis

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Introduction: In infectious diseases, activating as well as inhibitory transcription factors are required to enable pathogen control and to prevent over-shooting pathological immune reactions. CYLD is a deubiquitylating enzyme which plays a pivotal inhibitory role in immune responses. CYLD downregulates NF-kB activity by the proteolysis of K63-linked ubiquitin from signal transducing molecules like TNFR, TLR2, TLR4 and CD40. In addition to NF-κB, CYLD is also known to inhibit MAPK and the tissue protective factor plasminogen activator inhibitor (PAI-1).
Materials and Methods: To gain insight into the function of CYLD in listeriosis, we infected C57BL/6 CYLD$^{-/-}$ and wildtype (WT) mice with a lethal dose of *Listeria monocytogenes*. The bacterial burden in both the mice groups were determined on days 1, 3 and 5 p.i., On day 5 p.i., tissues from liver and spleen were obtained for RT-PCR and WB analysis. To further characterize the role of CYLD, functional assays like inhibition of fibrin production by Warfarin and in vivo IL-6 neutalisation were performed. Finally CYLD was knocked down in WT mice by siRNA to show its importance in listeriosis.
Results and Discussion: Whereas all WT mice succumbed to the infection up to day 7 p.i., CYLD$^{-/-}$ mice survived the infection indicating that CYLD inhibits protective host responses. A macroscopic examination revealed that WT mice suffered from multi-organ bleeding and succumbed to liver failure as shown by increased AST and ALT levels compared to the CYLD$^{-/-}$ mice. In contrast, the bacterial burden was only partially increased WT mice. Additional WB analysis showed an increased activation of NF-κB and MAPK as well as an increased production of protective IL-6 and IFNγ in CYLD$^{-/-}$ mice. Importantly, CYLD$^{-/-}$ mice had increased fibrin deposition in the liver and upon inhibition of fibrin production by warfarin CYLD$^{-/-}$ mice succumbed to listeriosis indicating that (i) CLYD inhibits fibrin production via inhibition of PAI-1 and (i) illustrating the importance of fibrin in limiting the spread of listeriosis. In good agreement, IL-6 stimulation of CYLD$^{-/-}$ hepatocytes resulted in a stronger activation of the transcription factors STAT1 and STAT3 and fibrinogen production as compared to WT hepatocytes in CLYD$^{-/-}$ hepatocytes upon IL-6 stimulation. Neutralization of IL-6 abolished the protective effect of CYLD-deficiency and resulted in multi-organ bleeding in CYLD$^{-/-}$ mice.
Conclusion: Absence of CYLD is essential for survival during murine listeriosis. Our observation that neutralisation of CYLD in WT mice by siRNA partially protected WT mice from lethal listerioses identifies CYLD as a potential therapeutic target in listerioisis.

IIV09

A new role of the complement system in the interaction with intracellular bacteria: Complement drastically increases survival in *Chlamydia Psittaci* lung infection

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The complement system directs immune effector functions and modulates the intensity of innate and specific immune responses. It is activated by extracellular pathogens and damaged cells. Almost nothing is known about the role of the complement system in infections with intracellular bacteria.This project demonstrates its important role in *C. psittaci* lung infection using several complement factor and receptor knock-out (or deficient) mice.
Mice were intranasally infected with the non-avian *C. psittaci* strain DC 15 and sacrificed on days 4, 9, and 21 for detailed analysis.
Weight and clinical score were determined daily. Complement activation occurred before symptoms of pneumonia became apparent. Surprisingly, following a phase of partial protection, all complement factor C3$^{-/-}$ mice died until day 17 post infection. Compared to wildtype controls, the mice lacking most complement effector functions were 100 times more susceptible to the intracellular bacteria. During the phase of partial protection of the C3$^{-/-}$ mice until day 9, more granulocytes migrated into their inflamed lungs, proinflammatory cytokines were more elevated, Th1-polarization seemed to be even stronger. The bacterial load in the lung of the knock-out mice was significantly decreased, they lost less weight and their clinical score was better. Experiments on additional knock-mice are performed to identify the responsible complement effector function down-stream of C3.
In summary: Our data indicate that C3-effector functions are harmful early in *C. psittaci* infection; increased Chlamydia uptake mediated by C3b might be the reason for that. However, later in infection, when specific immunity becomes essential for defence, complement activation seems to be necessary for the fine-tuning of an otherwise deleterious immune response. Our data show for the first time the strong influence of complement activation on the outcome of an infection with intracellular bacteria.

IIV10

Lipoamide dehydrogenase (Lpd) of *Pseudomonas aeruginosa* binds vitronectin, the human terminal complement pathway regulator for complement evasion

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The Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic human pathogen and a major cause of hospital-acquired infections, particularly in immuno-compromised individuals. In order to cross the first line of host innate immune defence, *P. aeruginosa* has developed multiple strategies to escape host complement attack. Complement resistance is crucial for bacterial virulence and several pathogens acquire host complement inhibitors such as vitronectin and Factor H to modulate this attack. In the present study we show that *P. aeruginosa* binds human vitronectin, a plasma protein that regulates the terminal complement pathway and when bound to the surface of *P. aeruginosa*, vitronectin blocks the insertion and assembly of the terminal complement complex and thereby increases bacterial survival in human serum. Upon incubation in vitronectin-depleted, complement active human serum, survival of *P. aeruginosa* was decreased as compared to bacterial survival in normal human serum. Thus, confirming the protective role of vitronectin at the bacterial surface. We identified Lipoamide dehydrogenase (Lpd) as the first vitronectin binding protein of *P. aeruginosa*. Although Lpd was initially identified as a cytoplasmic protein, flow cytometry and electron microscopy experiments revealed that Lpd is localized on the bacterial surface. In addition, Lpd was found in the outer membrane fraction of twenty different *P. aeruginosa* strains when analyzed by Western blotting using a specific Lpd antiserum. Lpd bound vitronectin in a dose-dependent manner and heparin inhibited the Lpd/vitronectin binding, suggesting that Lpd binds one of the heparin binding domains of vitronectin. By using recombinant fragments, the major Lpd interaction site within human vitronectin was located to amino acid residues 353-363, which are positioned within the C-terminal heparin binding domain of vitronectin. Taken together, the surface exposed Lpd of *P. aeruginosa* binds human vitronectin, the terminal complement pathway regulator and thereby protect the bacteria from complement-mediated attack.

IIV11

Innate immunity controls Yersinia enterocolitica infection in the mouse model

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Yersinia enterocolitica causes an enteric food-borne disease. In the mouse model of infection, Yersiniae cause systemic disease with replication in the small intestine, invading Payer's Patches (PPs) of the distal ileum, and disseminating to liver and spleen. Y. enterocolitica carries a 70kb-virulence plasmid encoding for Type III secretion system (T3SS) and Yersinia outer Proteins (Yops), which paralyse phagocytes of the innate immunity enabling Yersiniae to survive and replicate extracellularly. Further Yersinia is expected to release typical pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), peptidoglycan fragments (PG), lipoprotein (LP) and the Yersinia V antigen (LcrV), which are recognized by the respective host receptors (PRRs) such as diverse TLRs or NOD1/2 receptors. PRR signaling leads to release of pro-inflammatory cytokines (TNF, IL-6) as the first step in host defense activation followed by an anti-inflammatory response such as secretion of IL-10 and IL-1Ra. From this, we suggest that the infection process can be separated in two different modes of pathogen - host cell communication: short-distance effect = silencing of contacted cells and long-distance effect = release of PAMPs with recruitment and activation of phagocytes. With the aim to unravel which PAMP-receptor is involved in control of Yersinia-infection, we used different ko-mice with defined defects in innate immunity (TLRs- und NODs - signaling pathway and defects in IL-10 and IL1-Ra production) for susceptibility testing towards Y. enterocolitica. The results enable us to define 3 different groups of susceptibility: high-susceptible (Ye-HS: MyD88-/- and TLR2-/-/4-/-), susceptible (Ye-S: C57BL/6, TLR2-/-, TLR4-/-, CD14-/-, TLR2-/-/CD14-/-, NOD1-/-, NOD2-/-, NOD1-/-/NOD2-/-, RIP2-/-) and high-resistant mice strains (Ye-HR: IL10-/- and IL1Ra-/-). Histological analysis of spleen cryosections of C57BL/6 and IL10-/- mice revealed that Yersinia microcolonies grow within marginal zone of the white pulp in the T-cell areas and are tightly surrounded by infiltrated neutrophils, what leads to abscess formation. Histological analysis of spleen cryosections of MyD88-/- mice revealed a homogenous bacterial spread through the whole red pulp and damage of the splenic architecture. Further more, we obtained evidence that Yersinia susceptibility of mice is closely associated with dynamics of neutrophil recruitment to the site of infection due to host cell chemokine production. IL10-/- mice recruit high amount of neutrophils into the peritoneal space in the first 4h of infection due to the high chemokine production (G-CSF, KC, MIP-2) what leads to initial inhibition of Yersinia replication. TLR2-/-/4-/- and MyD88-/- mice are characterized by delayed neutrophil infiltration what leads to uncontrolled Yersinia replication. These findings elicit the decisive role of PRR-signalling in Yersinia-infection model.

IIV12

Peptides derived from virulence factor of E.coli inhibit TLR signaling and are new promising candidates for treatment of autoimmune diseases and sepsis

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Toll like receptor signaling is known to be part of the first line defense of the innate immune system. Over the last decade, the importance of TLR7 and 9 in autoimmune diseases, such as systemic lupus erythematosus (SLE), psoriasis and rheumathoid arthritis, has been revealed. In SLE, inappropriate or excessive activation of TLR7 and 9 by self RNA or DNA sequences is involved in the observed activation of the pDC IFN-alpha pathway and autoreactive B-cell mediated autoantibody production. Furthermore, the stimulation of TLR4 plays a key role in hyper inflammatory situations such as systemic inflammation and sepsis. Up to date, there are rather unspecific immune modulators available for treatment, such as corticosteroids and cytostatic reagents (e.g. cyclophosphamide), which are limited in their efficiency and cause immense side effects. Therefore, there is a need for new therapeutic strategies with a more specific inhibition of certain TLRs.
With TcpC (TIR domain containing protein C), we identified a virulence factor of uropathogenic E.coli (CFT 073) inhibiting MyD88-dependent TLR responses.[1] To make it suitable for therapeutic approaches, we developed peptides derived from exposed loop regions of the TIR-domain of TcpC, mainly the BB-loop and the DD-loop peptide. The BB-loop of other Tcps is known to inhibit TLR-signaling and the DD-loop is a highly conserved domain among bacterial pathogens harboring Tcps. Upon stimulation of TLR4, TLR7 and TLR9, both peptides inhibit cytokine production, including TNF-alpha, KC, and IL-12, in bone marrow derived-macrophages (BMDM) and -dendritic cells (BMDC) in vitro without showing any toxicity. Moreover, peptides show promising effects in a murine LPS shock-model leading to significantly reduced TNF-alpha serum levels.
In conclusion, the TcpC-derived peptides are able to inhibit TLR signaling without showing any side effects and are promising candidates for new therapeutics in autoimmune diseases and sepsis.

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IIV13

In vitro effects of *Aspergillus fumigatus* on human invariant natural killer T Cells

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Objectives: Invariant human Natural Killer T Cells (iNKT-Cells) are innate immune effector cells. They are characterized by their expression of both, T- and NK cell markers and therefore they are a connecting link between innate and adaptive immunity. These cells are further defined by their T-Cell receptor, consisting of a specific, invariant α-chain and a β-chain derived from a limited repertoire. They strongly react upon stimulation with lipid antigens presented in the context of the MHC-like molecule CD1d on dendritic cells (DCs) and thus they can be expanded *in vitro* by stimulation with their prototypic agonist α-galactosylceramide (α-GalCer). A protective role of iNKT cells in autoimmune diseases and cancer immunity, as well as their action against different pathogens is well documented. However, little is known about their direct interaction with fungi, especially with *Aspergillus fumigatus*. *A. fumigatus,* an omnipresent mold, is the most prevalent cause of a highly devastating opportunistic infection, affecting mainly immunocompromised patients.
Methods: In order to expand human iNKT cells ($iNKT^+CD3^+$), PBMCs were treated with α-GalCer and rhIL-2 for 15-20 days. After expansion, purity of the iNKT+CD3+ fraction was >90%. To further purify this fraction, a positive selection with CD3 microbeads was performed. After that the purity increased above 95%. iNKT cells were cocultured with different morphologies *of A. fumigatus,* resting conidia and germlings. Gene induction was evaluated by microarray analysis (Affymetrix U219) and the protein release by using multiplex ELISA assays (BioRad Bio-Plex). Additionally, the induction of IFN-γ, a major Th1 cytokine, was analyzed

by flow cytometry. Finally, XTT assays were used to examine the toxic effects of iNKT cells on *A. fumigatus*.
Results: Stimulating iNKT cells with different *A. fumigatus* morphologies, we observed a time- and morphotype-dependent induction of INF-γ, with germlings to be shown more immunogenic than conidia. Furthermore, challenging iNKT cells with *A. fumigatus* germlings at an MOI=1 for 6h, we found that iNKT cells caused a significant fungal damage. We could also observe defined patterns of gene induction, especially regarding cytokine and chemokine gene expression profiles. By multiplex ELISA assays gene expression patterns could be confirmed.
Conclusion: We found that there is a specific interaction of iNKT cells with *A. fumigatus* revealed, leading to defined cytokine induction and a fungicidal effect. INF-γ, a cytokine, which has a known protective role against IA, is produced by iNKT cells when confronted with *A. fumigatus.*

IIV14

The course of *Plasmodium yoelii*-infection is affected by $CD4^+Foxp3^+$ regulatory T-cells

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Regulatory T-cells (Tregs) play a critical role in the control of a variety of different immune responses. However, studies on the impact of Tregs during the course of *Plasmodium*-infection have shown conflicting results. Here we demonstrate that *Plasmodium yoelii* (*P. yoelii)*-infection of BALB/c mice leads to an expansion of highly suppressive $CD4^+Foxp3^+$ Tregs, while its diphtheria toxin-mediated depletion in *P. yoelii*-infected DEREG mice resulted in a significant increased activation of $CD4^+$ and $CD8^+$ T-cells, accompanied by a significant decrease in parasitaemia. Gene expression analysis of $CD4^+$ T-cells from *P. yoelii*-infected mice revealed that molecules described in the context of Tregs are not only up-regulated in $CD4^+CD25^+Foxp3^+$ Tregs but also in $CD4^+CD25^+Foxp3^-$ T-cells, e.g. CTLA-4, PD-1, Lag-3 or IL-10. Due to the fact, that *P. yoelii*-infection induce a strong up-regulation of anti-inflammatory IL-10 in both $CD4^+CD25^+Foxp3^+$ Tregs and $CD4^+CD25^-Foxp3^-$ T cells, we analyzed the impact of $CD4^+$ T-cell derived IL-10 in more detail by using the $IL\text{-}10^{fl/fl}$ x CD4cre (CD4IL10-KO) mice. Interestingly, *P. yoelii*-infection of CD4IL10-KO mice leads to a reduced suppressive capability of $CD4^+CD25^+$ Tregs and a significant enhanced inflammatory $CD8^+$ T-cell response compared to WT mice, whereas the parasitic burden is only marginallyl affected.

IIV15

IL-23-dependent IL-17A production is required for protective immune responses against *Trypanosoma cruzi* infection

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The protozoan parasite *Trypanosoma cruzi*, the causative agent of human Chagas' disease, is an obligatory intracellular parasite that replicates in the cytoplasm of the parasitized cell. Although *T. cruzi* is able to infect almost any nucleated cell in the body, macrophages represent an important site for *T. cruzi* replication in the acute phase of infection. However, when a T helper (Th)1-based immune response develops, interferon (IFN)-gamma-activated macrophages are the primary immune cells that control *T. cruzi* replication by the production of reactive nitric intermediates (RNI). In this study, we analyzed the role of interleukin (IL)-23 in protection against *T. cruzi* infection in a mouse model of Chagas' disease. Compared to wild-type mice, $IL\text{-}23p19^{-/-}$ mice developed a higher parasitemia and an increased mortality. However, this susceptibility was not due to an impaired Th1 immune response. Because IL-17A production was reduced in $IL\text{-}23p19^{-/-}$ mice, we infected $IL\text{-}17A^{-/-}$ mice with *T. cruzi* to study the relevance of this cytokine for protective immune responses. Like $IL\text{-}23p19^{-/-}$ mice, $IL\text{-}17A^{-/-}$ mice exhibited a higher parasitemia, an elevated mortality and an altered liver pathology. Moreover, Th1 immune responses were not affected by the absence of endogenous IL-17A. *In vitro* studies revealed that IL-17A directly stimulated trypanocidal activities in macrophages leading to the eradication of internalized parasites. In contrast to unstimulated macrophages, in which trypanosomes quickly escaped from the parasitophorous vacuole into the cytoplasm, IL-17A-treatment of macrophages led to a prolonged residence of parasites in endosomal/lysosomal compartments, where they were vulnerable to the lytic activity of the macrophage. Taken together, our results suggest that the IL-23-IL-17A-axis is necessary for host protection against *T. cruzi* infection by stimulating antimicrobial mechanisms in macrophages.

IIV16

Magic compounds: Ingredients of red wine, curry wurst and cholesterol-lowering drugs ameliorate acute small intestinal inflammation in mice

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Background: The health beneficial effects of Resveratrol and Curcumin, compounds found in the red wine grape and curry spices, respectively, as well as of the cholesterol-lowering drug Simvastatin have been demonstrated in various experimental models of inflammation. We investigated the potential anti-inflammatory and immunomodulatory mechanisms of the above mentioned compounds in a murine model of hyper-acute Th1-type ileitis following peroral infection with *Toxoplasma gondii*.
Methodology/Principal Findings: Here we show that after peroral administration of Resveratrol, Curcumin or Simvastatin, mice were protected from ileitis development and survived the acute phase of inflammation whereas all Placebo treated controls died. In particular, Resveratrol treatment resulted in longer-term survival. Resveratrol, Curcumin or Simvastatin treated animals displayed significantly increased numbers of regulatory T cells and augmented intestinal epithelial cell proliferation/ regeneration in the ileum mucosa compared to placebo control animals. In contrast, mucosal T lymphocyte and neutrophilic granulocyte numbers in treated mice were reduced. In addition, levels of the anti-inflammatory cytokine IL-10 in ileum, mesenteric lymph nodes and spleen were increased whereas pro-inflammatory cytokine expression (IL-23p19, IFN-g, TNF-a, IL-6, MCP-1) was found to be significantly lower in the ileum of treated animals as compared to Placebo controls. Furthermore, treated animals displayed not only fewer pro-inflammatory enterobacteria and enterococci but also higher anti-inflammatory lactobacilli and bifidobacteria loads. Most importantly, treatment with all three compounds preserved intestinal barrier functions as indicated by reduced bacterial translocation rates into spleen, liver, kidney and blood.
Conclusion/Significance: Oral treatment with Resveratrol, Curcumin or Simvastatin ameliorates acute small intestinal inflammation by down-regulating Th1-type immune responses and prevents bacterial translocation by maintaining gut barrier function. These findings provide novel and potential prophylaxis and treatment options of patients with inflammatory bowel diseases.

IIV17

Infection with the filarial nematode *L. sigmodontis* improves the immune response during sepsis

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Helminths modulate the immune system of their hosts and induce a regulatory, anti-inflammatory milieu that enables not only parasite survival within the host, but also benefits the host. Thus, helminth infections prevent or ameliorate autoimmune diseases like Type I diabetes, rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease (IBD) (1, 2). With regard to IBD, this concept led to the FDA approval to treat patients with eggs of the porcine whipworm *Trichuris suis*.
As sepsis is one of the major causes of mortality that is partly due to an exaggerated pro-inflammatory immune response (3), we tested whether natural infection with the rodent filarial nematode *Litomosoides sigmodontis* ameliorates a systemic inflammatory immune response that is induced via i.p. injection of a sublethal dose of *E. coli* LPS in C57BL/6 mice 35 dpi or vital *E. coli* bacteria 90 dpi in BALB/c mice.
L. sigmodontis infected C57BL/6 mice had a reduced hypothermia 6 hours post LPS injection compared to uninfected mice. This correlated with significantly increased amounts of peritoneal bioactive TGFβ and IL-10 in infected mice compared to uninfected controls, whereas levels of the pro-inflammatory cytokines IL-6, IL-1β, IFNγ, and MIP2 were not decreased, rather trended to be increased in *L. sigmodontis* infected mice. Similarly, increased expression of CD80 and CD86 on peritoneal macrophages and neutrophils suggest a stronger activation of these cell types in *L. sigmodontis* infected mice 6 hours after LPS challenge. Using a lethal dose of LPS, survival rate in *L. sigmodontis* infected mice was improved. As *L. sigmodontis* infected animals had a stronger initial pro-inflammatory immune response, we evaluated whether challenge with vital *E. coli* may be more efficiently controlled in infected mice. Indeed, *L. sigmodontis* infected BALB/c mice regained their initial body temperature 3h post *E. coli*

challenge, whereas uninfected controls had a progressive drop of their body temperature and a significantly increased bacteremia 6h post *E. coli* challenge.
Our results suggest that *L. sigmodontis* induced modulation of the immune response may increase the survival rate after bacterial challenge by inducing a strong pro-inflammatory immune response that controls bacteremia, followed by a potent anti-inflammatory mechanism that reduces pathology due to exaggerated inflammation.

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KMP01
Serologic evaluation of Hepatitis B and D in patients with Cirrhosis

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Background: Fifteen million people in the world have chronic hepatitis D. This research is implemented on a number of cirrhotic patients with the aim to assess the prevalence of hepatitis D and B.
Materials and Methods: This study included 60 cirrhotic patients. The level of Anti HDV, AntiHBc (IgM) and HBsAg were measured by method of ELISA.
Results: In this study 60 cirrhotic patients were evaluated. Sixteen patients were HBsAg positive. In total of 16 HBs Ag positive patients, 13 patients were Anti HBc positive (IgM) and in total 13 HBc Ab positive patients, 8 patients were HDV Ab positive.
Conclusion: In present study 50% of cirrhotic patients with HBsAg positive, were Anti HDV Ab positive indicating concurrent infection with HBV and HDV in these cirrhotic patients. By timely screening of cirrhotic patients for HDV, patients could undergo additional management of treatments.

KMP02
(1→3)-β-D-Glucan kinetics for the assessment of treatment response in patients with *Pneumocystis jirovecii* pneumonia

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Introduction: Effective therapy is vital in reducing mortality of *Pneumocystis jirovecii* pneumonia (PCP). Because of the inability to grow the fungus *in vitro* there are currently no reliable methods of assessing *P. jirovecii* antimicrobial sensitivity. To identify treatment failure, physicians have to rely solely on the clinical presentation, radiology results and unspecific parameters such as LDH levels.
(1→3)-β-D-Glucan (BG) is a cell wall component of *P. jirovecii* and of various other fungi. In a previous study we have shown that BG is an excellent biomarker for PCP with a sensitivity of 98% and a specificity of 94%. However, at the moment nothing is known about its usefulness for monitoring the patient's clinical course or for identifying treatment failure.
Methods: We conducted a retrospective case control study to investigate whether consecutive serum BG-measurements can be used to assess treatment response in PCP. All patients presenting at the University Medical Centre of Freiburg between January 2003 and July 2010 with a confirmed PCP and ≥ 5 sera during follow-up were enrolled. Serum BG levels were measured by use of the Fungitell assay. The study patients were grouped according to the pattern of their BG kinetic and independently by their clinical course. BG kinetic and clinical course were then compared for each patient.
Results: 18 patients (7 HIV-patients, 11 non-HIV patients) fulfilled the inclusion criteria and were enrolled in the study. A median of seven follow-up sera (IQR 6-9) per patient were tested. The median serum BG level was 2353 pg/ml (IQR 790-3710) and therefore was highly elevated (cut-off 85 pg/ml). During follow-up 8/18 patients had constantly decreasing BG levels, 6/18 patients had increasing BG levels and 4/18 patients had complex BG kinetics with one or more episodes of decreasing and increasing BG concentrations.
Comparison of the BG kinetic with the clinical course gave an overall correlation in 61% of patients. Separate analysis of the different subgroups showed that decreasing BG-levels are associated with a favourable outcome in 88% of cases (Fig. 1). In contrast, increasing BG-levels reflected death or treatment failure in only 44%, while the remaining 56% responded well to PCP-therapy. One patient with a complex BG-kinetic had a clinically, radiologically and microbiologically proven treatment failure and his BG level increased 7 fold at the same time (Fig. 2).
Conclusion: Decreasing BG-levels show a good correlation (88%) with a favourable treatment response. This may assist in deciding whether to continue first-line therapy in cases of missing clinical improvement. Under special circumstances, consecutive BG-measurements might be suitable to identify treatment failure. However, the overall correlation of increasing BG-levels with an adverse outcome is poor (44%) and its usefulness for follow-up in severe PCP limited.

Figure 1

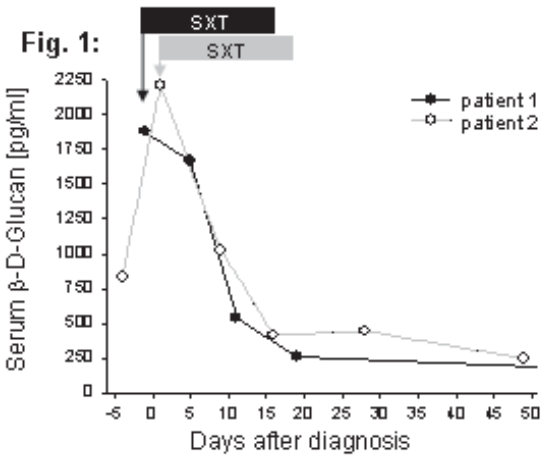

Fig. 1: BG kinetics of 2 patients with good treatment response show constantly decreasing BG levels after start of SXT-therapy.

Figure 2

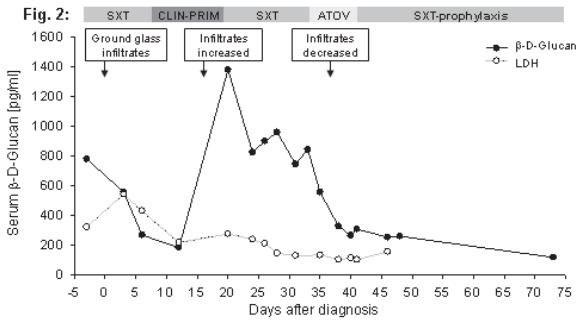

Fig. 2: Patient with a clinically, radiologically and microbiologically proven Clindamycin-Primaquine treatment failure. BG levels increased 7 fold at the same time and started to decrease again after the therapy was changed to SXT.

KMP03
Characterization of MRSA and MSSA strains of a federal state multicentre screening by spa typing, Maldi-TOF mass spectrometry, and microarray-based genotyping

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Introduction: Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), ranging from skin and soft tissue infections to sepsis and toxic shock, have become a worldwide challenge in hospitals. 2010, a prospective multicentre prevalence screening of patients admitted to 24 hospitals was performed in the region of Saarland, Germany (MRSAarNet).
Methods: In the present evaluation 47 MRSA-isolates collected in the study period at the University Hospital of Saarland were selected for a more detailed phenotypic and genotypic characterization. As a control group, 47 matched methicillin-sensitive isolates (MSSA) of colonized patients of the same risk-factors treated at the University Hospital of Saarland were selected (according to patient characteristics such as time period since hospitalization, gender, and age). Isolates were collected by nasopharyngeal swabs and of infection sites. Spa types were assigned using the Ridom StaphType spa server. Matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI-TOF MS) based spectra were used to detect discriminative protein profiles of different MRSA and MSSA strains. Antimicrobial resistance genes, pathogenicity associated genes and virulence factors were examined using a microarray based system which covers 333 *S. aureus* gene sequences and alleles.

Results: The spa typing of the MRSA collection analyzed here identified spa type t003 as predominant spa type which was found in 28 isolates (60%), followed by t504 which was found in 11% (5 isolates). 38 isolates (81%) were positive for the ß-lactamase structural gene (*blaZ*) and its transcriptional regulators *blaI* and *blaR*. The resistance gene *ermA*, conferring resistance to macrolide-, lincosamide-, and streptogramine antibiotics, was found in 42 isolates (89%). Nearly all (96%) of the strains possessed a capsule type 5 genotype, and only one strain exhibited a capsule of type 8. The predominant accessory gene regulator (*agr*) type identified in this collection was type 2, which was found in 42 MRSA isolates (89%). 5 MRSA isolates were positive for *agr* type 1 (11%). All isolates were negative for Panton-Valentine leukocidin (PVL) ant *tst* (encoding the toxic shock syndrome toxin), but harbored the genes for the staphylococcal superantigen-like proteins C, 6/SSL1 and 7/SSL7. The enterotoxin gene cluster *egc* (comprising the genes *seg*, *sei*, *sem*, *sen*, *seo*, *seu)* as well as genes coding for enterotoxins M, N and U were found in 44 isolates (94%).
The results of the genotypic and phenotypic characterization of the MRSA collection analyzed here will be compared with those of matched controls of patients of the same risk-factors colonized with methicillin-susceptible *S. aureus* strains.

KMP04

Diagnostic performance of multiplex PCR for diagnosis of bloodstream infections in liver transplant recipients with suspected sepsis

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Backround: Rapid detection of causative microorganisms in patients with bloodstream infections is crucial for the early initiation or adaption of adequate antimicrobial therapy.
Objective: The goal of this prospective study was to evaluate a commercial PCR-based kit (SeptiFast, SF) for the detection of 25 clinically important pathogens in septic patients after liver transplantation (LTX) and major abdominal surgery (non-LTX).
Methods: Overall 225 blood samples from 170 patients (46.5% after LTX and 53.5% non-LTX) with suspected sepsis were obtained in parallel for blood culture and SF over a period of three years at a tertiary care centre.
Results: In total, SF and BC yielded concurrent results, negative in 110 (48.8%) samples and positive in 51 (25.3%). 35 (15.5%) blood samples were SF positive/BC negative. 23 (10.2%) samples were BC positive/SF negative of which in twelve cases the detected pathogens were considered to be contaminants. Using BC as gold standard the sensitivity, specificity, positive and negative predictive value of SF was for LTX 81%, 70%, 68%, 82% and for non-LTX 58%, 80%, 53%, 83%, respectively.
Conclusion: In LTX and non-LTX patients SF yielded a higher positive rate than BC. The SF performance had a higher sensitivity and positive predictive value for the LTX patients, but lower specificity and negative predictive value compared to non-LTX patients.

KMP05

Bacterial diversity of dental intra-implant contamination

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Objective: There is growing evidence that microgaps between dental implants and their abutments lead to bacterial contamination of the interior of the implants that might provide a reservoir for pathogens causing periimplantitis. The aim of this study was to investigate the intraimplant bacterial spectrum of two stage implants, and to compare the results of two different sample collection methods.
Methods: Ten healthy patients without clinical symptoms of periimplantitis and with two-stage implants on each side wearing ball-attached removable prosthodontics took part in this investigation. Abutments were removed 56-105 months (median 63.1 months) after connection, and samples were obtained by i). collecting the intra-implant fluid (planctonic bacteria) and ii). by brushing the cavity (biofilm growth). Bacterial colonization was evaluated by aerobic and anaerobic incubation on agar and broth media. Isolated bacterial colonies were identified by means of MALDI-TOF identification, classical biochemical differentiation tests, and 16S rDNA sequencing.
Results: Specimen from a total of 20 implants were investigated, resulting in 3 - 25 (median: 16) different bacterial species per implant, representing a total of 105 different taxa. *Streptococcus anginosus*, *Actinomyces naeslundii*, and *Veillonella parvula* were found most frequently while periimplantitis marker bacteria such as *Prevotella intermedia* or Fusobacteria were only sporadically detected or not at all (*Aggregatibacter actinomycetemcomitans*). Comparison of specimen collection by rinsing and by brushing showed a low consistency of results (median: 21 %). Most taxa per implant found were detected by brush collection (median: 62 % of species), however, only combination of both methods allowed complete analysis of the intra-implant microbiom.
Conclusions: Our results suggest ubiquitous heavy contamination of internal spaces of dental implants, revealing a broad spectrum of bacterial species. For analysis of the intra-implant microflora the combined examination of both intra-implant fluid and cavity surface samples is recommended in order to cover biofilm-grown as well as planktonic microorganisms.

KMP06

Genomic *Staphylococcus aureus* adaptation to the cystic fibrosis lung

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The airways of cystic fibrosis (CF) patients are frequently colonized and infected by *Staphylococcus aureus*, often accompanying severe course of disease. Many patients are persistently colonized with one predominant *S. aureus* clone. Although we do not have detailed knowledge about genomic alterations, *S. aureus* long-time persistence in the CF lung assumedly selects for phenotypes that are tightly adapted to this specific environmental niche.
A unique CF-derived *S. aureus* collection allows us to track genomic adaptation due to persistence in the CF lung. Clonal early and late isolates from three CF patients, isolated 12-14 years apart, were sequenced on a genome analyzer (Illumina, Solexa) and screened for SNPs, insertions, and deletions. We identified several mutations in the late *S. aureus* isolates compared to the respective early isolates. By Sanger sequencing we could confirm mutations in a number of genes that are associated with virulence or antibiotic resistance, such as protein A (*spa*), the autoinducer sensor kinase (*agrC*), the allelic variants of bone sialoprotein-binding protein (*bbp/sdrE*), and DNA topoisomerases type II and IV (*gyrA* and *parC/grlA*). All of these mutations are associated with amino acid changes and we currently analyze the potential outcome. The altered *gyrA* corresponds to an acquired resistance towards fluoroquinolones, whereas altered *agrC* is assumedly associated with different characteristics affecting *S. aureus* virulence. We furthermore include the sequencing of intermediate *S. aureus* isolates to determine the time when the mutation occurred.
Our preliminary data suggest that adaptation of persistent CF isolates include changes on DNA level and the regulation of protein expression. However, detected mutations did not occur in all patients indicating that the host environment promotes individual adaptational processes. Resulting altered virulence may favor precise camouflage in response to the hostile environment under a specific clinical situation. Our results may help to understand the complex pathogenesis of chronic infection against the background of host-pathogen interactions.

KMP07

Staphylococcus pettenkoferi: pathogen or colonizer

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Staphylococcus pettenkoferi is a coagulase-negative staphylococcus (CNS) that has been recently described as a rarely isolated new species. Whether this new species is a harmful pathogen or an harmless bystander could not be answered yet. Nevertheless, a few case reports suggested an invasive role of *S. pettenkoferi. Staphylococcus lugdunensis* and *S. pettenkoferi* have been described as potentially related species as shown by biochemical methods. To determine whether this new species could be a putative pathogen similar to *S. lugdunensis*, the strains were characterized for the presence of fibrinogen and fibronectin binding adhesins. In addition, the internalization of the bacteria into eukaryotic cells were invesitgated using a previously published FACS-based internalizatio assay. The occurrence of putative *S. lugdunensis* adhesin and hemolysin genes were evalutated using primer pairs designed for *S. lugdunensis*.
An estimated 30 % of the *S. lugdunensis* strains bound to fibrinogen and none of the *S. pettenkoferi* isolates (n=23) bound to fibrinogen or fibronectin. In addition, an estimated 30-40 % of *S. lugdunensis* isolates were internalized into bladder carcinoma 5637 cell line cells. In contrast to this, only one out of 23 isolates of *S. pettenkoferi* were internalized.
The lack of fibrinogen and fibronectin binding adhesins, and the lack of an internalization ability indicate that *S. pettenkoferi* is more likely a harmless colonizer of the human skin flora more than a harmful infective pathogen.

KMP08

Phenotypic characteristics of *Chlamydia pneumoniae* isolated from different origins

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Introduction: *Chlamydia pneumoniae* (*Cpn*) is an obligate intracellular bacterium and causes mild-to-moderate pharyngitis and bronchitis. In addition, *Cpn* infection are supposed to play a role in chronic diseases like asthma, COPD and atherosclerosis. Systemic dissemination of *Cpn* from the lung to peripheral organs and arteric vessels has been shown to be mediated by blood mononuclear cells (PBMC). Using whole genome sequencing we recently found genetic differences between isolates that were cultivated from different tissues. It has been shown previously that intracellular morphology and growth characteristics of chlamydiae signficantly differ between human monocytes and epithelial cells. However, phenotypic differences between isolates from different anatomical origins have not been investigated so far.
We therefore compared growth characteristics and progeny of *Cpn* isolates from three different infection sites.
Material and methods: *Cpn* strains, CW029, CV6 and PB2 which were isolated from the respiratory tract, coronary arteries and PBMC, respectively were used in this study. Infection doses for HEp-2 cells of the different isolates were adjusted by determination of the amount of genome encoded 16S ribosomal RNA. Rate of infection, number of intracellular inclusion, production of infectious progeny, and developmental pace of each isolate were investigated by fluorescence microscopy, transmission electron microscopy and recovery assays.
Results: The coronary artery strain CV6 showed the highest infection and multiple inclusion rates among the three different isolates.The isolate PB2 that was derived from PBMC showed dramatically less production of infectious progeny and slower developmental speed compared to the respiratory strain CWL029.
Conclusion/Discussion: Clinical isolates of *Cpn* from the bood circulation (PBMC, coronary arteries) can be differentiated from lung isolates by non-synonymous SNPs in 9 ORFs related with chlamydial metabolisms, bacterial transition, inclusion membrane formation, LPS synthesis and regulation of sigma factors. Further studies will have to show whether these genetic differences are responsible for the observed phenotypic differences between the isolates with regard to intracellular growth and progeny.

KMP09

Gram-negative bacteria with carbapenemases in Hamburg, Germany

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Detection of carbapenemases has only very rarely been reported from Germany. In order to assess the presence of carbapenemases in Hamburg a number of carbapenem resistant gram-negative bacterial isolates collected during a period of eight month were analysed. VIM-1, VIM-2 and KPC-2 were detected in diverse enterobacterial species and *Pseudomonas aeruginosa* isolates suggesting a more widespread prevalence of carbapenemases in Germany than currently believed.

KMP10

Proteomic analysis in early and late *Staphylococcus aureus* isolates from cystic fibrosis patients

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Due to mutations in the gene encoding the *cystic fibrosis transmembrane conductance regulator*, lungs of cystic fibrosis (CF) patients exhibit dehydration of the airway mucus leading to decreased mucociliary clearance and chronic infections. One of the earliest pathogens which can be isolated from lungs of CF patients is *Staphylococcus aureus*. Often the lungs of CF patients are infected by a predominant *S. aureus* clone for many years. During long-term colonization the pathogen needs to adapt to the environment of the lungs. This project is aiming to further reveal the mechanisms underlying the adaptation of *S. aureus* during persistent colonization and infection to the hostile niche of CF patients using proteomic analysis. In addition the virulence potential was assessed in *in vitro*, as well as *in vivo* assays.
From a unique longitudinal collection of *S. aureus* strains isolated from the airways of CF patients early and late *S. aureus* isolates of three patients were chosen. The comparison of the proteome of early and late isolates was performed using 2D-gelelectrophoresis followed by MALDI-TOF mass spectrometry. *S. aureus* strains were cultured in artificial sputum medium in order to mimic the *in vivo* situation. Virulence potential of the strains was investigated *in vitro* by means of invasion assays using the epithelial cell line A549. Furthermore, the virulence potential was studied *in vivo* in a *Caenorhabditis elegans* killing assay.
Preliminary results of invasion assays revealed different alterations in invasive properties comparing early and late isolates. For late isolates of two patients an increase of invasive properties compared to the early isolates could be observed whereas the properties of the late isolate of one patient were decreased. First results using isolates of one patient in the *C. elegans* killing assay revealed no significant difference in virulence between early and late isolate. The investigation of the proteomic changes during long-term colonization is still in progress.
In this project we studied the adaptive mechanisms of *S. aureus* during persistent colonization within the airways CF patients on a functional level to gain insights into the interaction between the pathogen and the host.
Since adaptation of bacteria can occur on genomic as well as on regulatory levels, it is of importance to investigate the alterations in late isolates compared to early isolates in a proteomic approach which allows the analysis of differences in protein expression as well as of post-translational modifications.

KMP11

Orthopedic device-related infections with Enterococcus faecalis

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Introduction: Orthopedic device-related infections (ODRI) are increasing as the number of patients to retrieve an orthopedic implant rises. The main etiologic agents in orthopedic implant infections are staphyloccoci.
Here we describe the therapy and outcome of ODRI with a difficult-to-treat organism: Enterococcus faecalis.
Patient and methods: Five patients with a chronic hip implant infection caused by E. faecalis were treated by a two-stage replacement and with use of antimicrobial cement and long term treatment with antimicrobial agents. All five isolates were sensitive to ampicillin and four out of five isolates were high-level resistant to gentamicin.
All patients recieved a systemic therapy with ampicillin or amoxycillin for six weeks plus a vancomycin containing cement. The second device implantation took place after a mean time of 3,5 months.
Outcome: After 24 months there were no further signs of infection in all five patients. Two patients needed revision surgery because of surgical site infections prior to second device implantation.
Conclusion: Chronic infections with E. faecalis are difficult to treat. As most patients recieve gentamicin containing cement for their first implantion many E. faecalis isolates show a high-level gentamicin resistance. The combination of vancomycin containig cement with systemic ampicillin therapy might be a useful option for these patients.

KMP12

Attacking the oxidative defense system of *Mycobacterium tuberculosis* by antimicrobial compounds

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Introduction: In the infected host, *Mycobacterium tuberculosis* (*Mtb*) faces oxidative and nitrosative stress. Thus, the pathogen needs an effective antioxidative system to maintain the infection. Due to the lack of the glutathione system in *Mtb* the thioredoxin reductase (TrxR) system is an essential mechanism to reduce reactive oxidative and nitrosativ species and assure the bacterial survival. Similarity between the mycobacterial and the eukaryotic TrxR system is low, therefore it may be used as a novel drug target to fight tuberculosis.
Methods: To identify new substances which inhibit the interaction between thioredoxin (Trx) and TrxR an *in silico* high throughput screening was conducted. Four altered low mass scaffolds were chosen to be tested *in vitro* to examine their inhibitory effect on *Mtb* TrxR. After optimization of these compounds, whole cell testing was performed in *Mtb* liquid cultures using the MGIT 960 system (Becton Dickinson).
Results: The interference of 170 low mass compounds with the mycobacterial TrxR was tested *in vitro*. 10 percent of the chosen low mass compounds showed activity in low µM concentrations. After optimization of these compounds some of them showed bacteriostatic effects on *Mtb* cultures even within a low µM range.
Conclusion: Effecting the interaction between Trx and TrxR with small molecule inhibitors at micromolar levels leads to bacteriostatic effects in *Mtb* cultures.

KMP13

Characterization of atypical uropathogenic *Escherichia coli* isolated from hospital patients

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Urinary tract infection represents the most common type of bacterial infection in industrialized countries. Almost 90 % of UTI are caused by uropathogenic *Escherichia coli* (UPEC), a subtype of extraintestinal pathogenic *E. coli* (ExPEC). Although several important UPEC virulence factors and their role during pathogenesis have been described, many UPEC isolates cannot be unambiguously distinguished from commensal *E. coli* based on the presence of UPEC virulence-associated genes. In contrast, intestinal pathogenic *E. coli* (IPEC) which cause diarrhoea are characterized by typical sets of virulence factors which are usually absent from UPEC. The majority of virulence-associated genes of ExPEC and IPEC are located on mobile and accessory genetic elements, e.g. plasmids, bacteriophages or pathogenicity islands and can thus be horizontally transferred.
We analyzed 283 *E. coli* isolates from hospital patients with urinary tract infection. These isolates were screened by multiplex PCR for important virulence determinants of UPEC and IPEC. Interestingly, about 10 % of these isolates carried one or more known IPEC virulence genes, sometimes in combination with classical UPEC virulence genes. In addition, molecular epidemiological and phenotypic analyses were performed to assess the relatedness and similarity of these isolates. Notably, among the atypical urine *E. coli* isolates were strains carrying marker genes of enteroaggregative *E. coli* (EAEC). In addition, isolates were typed as atypical enteropathogenic *E. coli* (aEPEC), and Shiga toxin-producing *E. coli* (STEC).
Our results indicate that nosocomial UTI due to *E. coli* may be caused by a diverse group of *E. coli* variants, some of which represent IPEC or carry virulence properties of diarrheagenic *E. coli*. The combination of typical ExPEC and IPEC virulence determinants also further supports the finding that *E. coli* exhibits a marked genome plasticity and that gene transfer may promote an evolutionary transition from IPEC to ExPEC - or vice versa. The relatively high prevalence of EAEC marker genes among urine isolates also demonstrates the heterogeneity of this pathotype and raises the possibility that some EAEC strains may have the potential to be uropathogenic.

KMP14

Predominant genotypes among CTX-M-15- producing *Escherichia coli* isolates in Nigeria

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Background: We investigated the association between the prevalent extended-spectrum beta-lactamase and the genetic lineage of *Escherichia coli* from two tertiary hospitals in Lagos, Nigeria within a period of 8 months in 2009.
Methods: Identification and susceptibility testing of *E.coli* isolates (*n*=109) were by Vitek 2 and E-test. Extended-spectrum beta-lactamase and quinolone resistance genes (the *aac-(6')-lb-cr* and plasmid-mediated quinolone resistance [PMQR] genes) were characterized using PCR and sequencing. Clonality was determined by ERIC PCR and multilocus sequence typing (MLST).
Results: Of 109 isolates, 14 (12.8%) were found to be ESBL-producers. All the ESBL isolates were resistant to ceftazidime and cefotaxime with MIC of >16µg/ml. Resistance to ciprofloxacin (MIC≥32µg/ml) and an aminoglycoside co-existed also in these ESBL isolates. They were however, all sensitive to the carbapenems. The only ESBL gene detected in these isolates was CTX-M-15. All the CTX-M-15 producers carried the insertion sequence IS*Ecp*I which has recently been demonstrated to mobilize 3'-adjacent genes to transfer between DNA replicons and is involved in spread of such ESBLs.
ERIC-PCR identified 11 types among these ESBL-producers. A diversity of MLST types was also observed. Clonal group ST131 (35.7%) was the predominant ST, closely followed by the clonal complex ST10 (28.6%). The other sequence types established in these strains included ST23 complex (*n*=1), ST 448 complex (*n*=1), ST295 (*n*=1) and ST 501 (*n*=1). Plasmid-mediated quinolone resistance *qnrA1* gene (*n*=3) and *qnrB1* (*n*=1) were detected among the ESBL isolates of ST131. All the ESBL isolates with ST131 harboured the *aac-(6')-lb-cr* gene.
Conclusion: This is the first report of *E. coli* ST131 and clonal complex 10 in West-Africa (Nigeria) found predominant among hospital isolates of CTX-M-15-producing *E. coli*. The high diversity of STs is remarkable among the CTX-M-15 positive isolates. This study highlights the need to adopt appropriate control measures to overcome the ESBL dissemination in Nigeria.

KMP15

Bacteriocin production by staphylococcal nasal isolates

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Staphylococcus aureus is a major pathogen in hospital- and community-acquired infections. Colonisation of the anterior nares in about 30% of the population is a major risk factor for *S. aureus* infections. Recently the composition of the nasal microbiota has been inverstigated. Interestingly, the bacterial diversity in the human nose reaches from aerobic to strictly anaerobic bacteria. The most frequently occurring species are *Corynebacterium accolens, C. macginleyi, Staphylococcus. epidermidis* and *Propionibacterium acnes.* In order to investigate if bacteriocin production might play a role during nasal colonisation we analysed the bacteriocin production of nasal *Staphylococcus* strains.
The test strains (*Micrococcus luteus* or *S. aureus*) were casted in an agar plate and the nasal isolates were stamped on the plate. Various isolates showed growth inhibition zones of the test strains indicating baceriocin production. Transposon plasmids could be transformed into various strains and mutagenesis was performed to identify corresponding bacteriocin-biosynthetic genes.
Analysis of 93 stapylococcal nasal isolates revealed that various strains produce bacteriocins against *M. luteus or S. aureus*. The bacteriocin production of some nasal isolates turned out to be inducible by certain stress conditions.
One of these bacteriocins, produced by an *S. epidermidis* strain, could be characterized as a nukacin-like lantibiotic with activity against *Micrococcos luteus*.
Extending our knowledge about the various interactions between staphylococcal and other nasal species could be important for finding new effective anti-*S. aureus* strategies.

KMP16

Humanisation of the murine anti-IsaA antibody - a potential target for an antibody-based therapy against multi-resistant *Staphylococcus aureus* strains

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Background: The Gram-positive bacterium *Staphylococcus aureus* is the major cause of nosocomial infections. In particular, diseases caused by methicillin-resistant *S. aureus* (MRSA) are associated with higher morbidity, mortality and medical costs due to showing resistance to several classes of established antibiotics and their ability to develop resistance mechanisms against new antibiotics rapidly. Therefore, there is an urgent need for novel treatment options. Immunotherapy has the potential to close the gap in the treatment of infections caused by multi-resistant *S. aureus*. The immunodominant staphylococcal antigen A (IsaA) has been identified as a putative target for immunotherapy due to its expression by all clinical strains in *vivo*, and the surface exposure. Preclinical experiments revealed protective properties of a monoclonal mouse anti-IsaA antibody (UK-66) in different experimental models of *S. aureus* infections. In addition, *in vitro* phagocytosis assays showed enhancement of opsonophagocytic killing of *S. aureus* in the presence of UK66. Therefore, the mouse monoclonal antibody was selected for humanization as a prerequisite of clinical studies in humans.
Results: The hybridoma clone UK-66 was the basis for the identification of the antibody binding domain against IsaA. The coding sequence was used to construct recombinant scFv and scFvFc fragments towards IsaA (Fig. 1) and to humanised the murine antigen binding domain. The murine and humansed scFv fragment was expressed in *E.coli* and the murine and humanised scFvFc fragment was expressed in eucaryotic HEK293 cells. The scFv- and scFvFc fragments were characterized in their function and specificity by Western Blot analysis, ELISA-studies, immuno-fluorescence analysis and FACS experiments. The results revealed that all constructed fragments showed a high specificity towards IsaA and the property of the antigen binding fragments to detect IsaA on the cell surface of different *S. aureus* strains.
Conclusion: IsaA is an antigenic structure expressed in *vivo* in different *S. aureus* strains. The murine anti-IsaA antibody is protective in animal models. The murine and humanised scFv- and scFvFc fragments showed a high specificity towards IsaA.

KMP17

Rapid dose-response investigation of binary compound mixtures

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For an improved treatment of infectious diseases the identification of optimized combinations of antibiotics is a promising approach. A major requirement for such investigations is the rapid testing of a moderate number of different compound combinations with a limited number of infectious agents.
We developed a device for the combinatorial generation of compound mixtures by diffusion with concentrations over several orders of magnitude. Diffusion takes place in cell compatible polymer matrices such as agarose, gelatine or AB3 agar. Mixtures of test compounds are generated by a superposition of concentration gradients (2D-gradient). Only two pipetting steps are required to generate a series of assays that only depends on the spatial resolution by which the diffusion gradient is converted into a biological assay. The diffusion device is compatible with 12-well microtiter plates enabling the efficient testing of combinations of different compounds. The device is applicable for all diffusible compounds such as small molecules, peptides, lipopeptides and antibodies.
Here we present the investigation of antibiotic combinations against different staphylococcal strains to define antibiotic combinations with additive and synergistic effects. The miniaturization, parallelization and especially the reduction of the number of steps required for the preparation of samples enabled the rapid and efficient screening of drug combinations on different microbial strains.

Figure 1

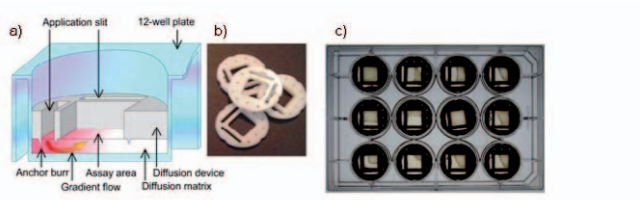

Figure 1: a,b) Scheme and photograph of the diffusion device placed on an AB3 agar (1.5 %) in a well of a 12-well plate. Staphylococcus suspension in an AB3 agar (0.75 %) is poured into the central opening. c) Biological activity by combination of two different antibiotics.

KMP18

A new class of antimycotic (S)-2-aminoalkyl benzimidazoles exhibits potent antifungal activity against clinically relevant and against fluconazole resistant strains of *Candida spp*

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Invasive fungal infections are a main cause for morbidity and mortality in transplant recipients and immunocompromised patients in the clinic and thus a growing health problem. Regarding the constantly growing number of resistant strains the discovery of new classes of antimycotic drugs is highly necessary.
In a high throughput activity-selectivity screening a series of (S)-2-aminoalkyl benzimidazole derivatives with strong antimycotic activity against *Candida albicans* was identified. A collection of analogues for a SAR-study was prepared in order to evaluate and improve the antimicrobial properties.
We examined the activity of five of the (S)-2-aminoalkyl benzimidazoles against a set of twenty-one fungal strains including different type strains and significant clinical iso-lates of *Candida spp*. and confirmed the antimycotic activity. The most active com-pound 120B12 in this study showed potencies equal to that of fluconazole. Consequently, the novel non-toxic (S)-2-aminoalkyl benzimidazoles represent promising candidates for future developments in antimycotic drug discovery. Currently, studies with *Caenorhabditis elegans* are carried out.

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[2] Bauer, J., Kinast, S., Burger-Kentischer, A., Finkelmeier, D., Kleymann, G., Abu Rayyan, W., Schröppel, K., Singh, A, Jung, G., Wiesmüller, K.-H., Rupp, S. Eickhoff, H., *J. Med. Chem.*, submitted

Figure 1

Test strains	MIC [µg/ml] of test compounds	
	Fluconazole	120B12
ATCC control strains		
C. albicans DSMZ 11949	2	2
C. glabrata ATCC 90030	0.125	0.125
C. parapsilosis 22019	1	2
C. parapsilosis 90018	0.25	0.5
Clinical isolates		
C. albicans CA20	0.125	0.125
C. albicans CA21	0.125	0.125
C. albicans MY 2902/2008	0.5	16
C. albicans SCS 71865L	0.125	64
C. albicans Jg 32570	128	16
C. albicans AM2001/0007	0.5	0.25
C. albicans AN 1699	8	32
C. albicans AN 1994	128	16
C. tropicalis AN 1946	128	4

KMP19

Laboratory performance and exprerience with the QuantiFERON®-TB Gold in-Tube test during contact investigations in Bavaria

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The Bavarian Health and Food Safety authority (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, LGL) provides several microbiological services for local health authorities in Bavaria. According to the German Protection Act (Infektionsschutzgesetz, IFSG) §§ 29-31 the public health system is responsible to ensure an adequate care of TB cases and in the following the performance of contact investigations to actively identify infected persons. In this context, the LGL maintains a TB-laboratory facility for the diagnosis of *M. tuberculosis* infections. As the interferon-gamma-release-assays (IGRA) have been shown to improve the diagnosis (better specificity and sensibility than TST) especially of latent tuberculosis infections (LTBI) this test has been increasingly requested with more than 8500 samples in 2010 by health care workers and clinicians. IGRAs (e.g. QuantiFeron®-TB Gold In-Tube test and T-SPOT®-TB) measure a person's immune reactivity to specific mycobacterial antigens. Specimens are mixed either with peptides that simulate antigens of *M. tuberculosis*. These *M. tuberculosis* specific antigens (ESAT-6, CFP-10 und TB7.7) are lacking in all BCG strains and almost all non-tuberculous mycobacteria (NTM) except *M. kansasii, M. szulgai* and *M. marinum.* In a person infected with *M. tuberculosis*, the white blood cells recognize the peptides and release interferon-gamma. Test result is based on the amount of interferon-gamma releas. At present the LGL supply the QuantiFeron®-TB Gold In-Tube test (QFT) mainly due to operational advantages regarding the high-throughput of blood samples. The poster will give an overview about laboratory performance of QFT at the LGL.

KMP20

Phenotypic and genotypic characterization of *Salmonella enterica* Serovar Livingstone strains isolated from Tunisia and Belgium

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Salmonella enterica is one of the most common causes of human gastroenteritis worldwide. It is also responsible for nosocomial outbreaks particularly in developing countries. In Tunisia, many nosocomial outbreaks, occurred in pediatric services, have been involved various different serotypes, especially serotype Livingstone. In Belgium, the reported sporadic human cases of S. enterica serotype Livingstone infection is low.

In this study, 43 *S. enterica* serotype Livingstone were analyzed. Most of them were isolated from human (31 strains from Tunisia and 10 from Belgium) and tow strains came from food products (pork and beef meats). Tunisian strains were isolated in the same hospital but during different periods (2002, n=1; 2004, n=1; 2005, n=27 and 2009, n=2).

All strains were characterized by antibiogramm, Multilocus sequence Typing (MLST) and virulotyping. This last technique was carried out by simple PCR of chromosomic genes (*agfA*, *hin/H2*, *iroB*, *phoP/Q* and *sly A*) and plasmids genes (*spvA, spvB* and *spvC*).

Tunisian strains were resistant to amoxicillin, amoxicillin-clavulanic acid, ticarcillin, gentamicin, kanamycin and showed a particular β-lactam resistance phenotype. Belgium strains were susceptible for the majority of the antibiotics.

Further to MLST analyses, tunisian strains belonged to the same sequence type, ST543. For belgium isolates, 3 strains had a ST638 profile, 8 strains had ST543 profile and one strain had an unknown sequence type.

Analyses of the virulence genes content showed that strains isolated in different years, in different origins (human and food products) and in different countries had the same virulence profile. They carried the same chromosomic virulence genes and the plasmidic virulence genes were lacked.

Combination of different typing methods showed that the majority of belgium strains (8/12) and all tunisian strains isolated in different time periods are closely related; they belonged to the same sequence type (ST543) and had the same virulence profile.

KMP21

Does the change in the EUCAST ESBL expert rules help the patient?

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Objectives: ESBL producing E.coli strains have become a serious problem, as these strains often leave few therapeutic options, especially in intensive care units. The therapeutic options seem limited. Furthermore the former EUCAST expert rules were changed so that the definitions of sensible to beta lactams were changed to intermediate and intermediate to resistant whenever an ESBL was recorded for the respective strain. The change for 2010 reads: Cephalosporin breakpoints for Enterobacteriaceae will detect clinically important resistance mechanisms (including ESBL). Some strains that produce beta-lactamases are susceptible or intermediate to 3rd or 4th generation cephalosporins with these breakpoints and should be reported as found, i.e. the presence or absence of an ESBL does not in itself influence the categorization of susceptibility. We analysed isolates in order to see if the change in the EUCAST expert rule opens new therapeutic options.

Methods: The laboratory participating in the network use the automated BD PHOENIX-systems measuring MICs. The BD EPICENTER Data-Management-System is used for evaluation of lab data and for the transfer of the data for joint analysis. For this study we analysed the MICs of ESBL producing E. coli isolates during 2006 to 2010. ESBL production was checked with the associated BD-expert system, as the Phoenix shows excellent performance in ESBL detection (Maurine A. Leverstein-van Hall, et al: J Clin Microbiol. 2002 October; 40(10): 3703)

Copy strains were excluded. Quality control assays were routinely performed.

Results: Using the MICs of strains producing ESBL producing strains to ceftazidime (CAZ), cefotaxime (CTX), cefepime (FMC), and aztreonam (ATM) we calculated the sensitivity using the new EUCAST breakpoints.

Sensitivity of ESBL producing *K. pneumoniae*

	%S	%I	n
ATM		16,1	684
CTX	11	3,4	684
CAZ	2,3	16,8	683
FEP	0,2	23,3	476

Sensitivity of ESBL producing *E. coli*

	%S	%I	n
ATM	0,2	28	1.846
CTX	21,4	4	1.828
CAZ	6	47	1.845
FEP	0,4	28	1.537

Conclusion: There is no doubt that understanding the underlying mechanism is of great value for epidemiological and infection control purposes. However, in the day-to-day routine laboratory, and in the interest of the patient, the new breakpoints together with a reliable system for the detection of ESBLs are of benefit to the patient, as they open treatment alternatives and at the same time give warning signals for the control of infection.

KMP22

Temocillin susceptibility testing in ESBL-producing *Enterobacteriaceae*

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Introduction: The prevalence of infections due to ESBL-producing *Enterobacteriaceae* is increasing. Antibiotics with gramnegative coverage will not be introduced in the near future. Temocillin, a substance with a remarkable β-lactamase stability and resilience to all classical extended-spectrum TEM, SHV and CTX-M enzymes, becomes an attractive and carbapenem-sparing treatment option. Temocillin susceptibility against clinical isolates producing ESBL was investigated in the present study.

Methods: *Enterobacteriaceae* isolates from a University microbiology laboratory were screened for ESBL-production by Vitek 2 system. Positive results were confirmed by phenotypic tests using cefotaxime and ceftazidime susceptibility disks, alone and in combination with clavulanic acid according to CLSI guidelines. Further molecular detection of ESBL enzymes was done by PCR. Isolates were screened for CTX-M ESBL by PCR and thereafter classified as CTX-M and non-CTX-M isolates. Temocillin susceptiblity was determined by Etest (bioMérieux, France) as described by the manufacturer.

Results: *K. pneumoniae* and *E.coli* isolates were chosen for this study on the basis of the prevalence as the most frequent ESBL-producing bacteria. Most ESBL enzymes were CTX-M. More than 60% of the tested ESBL-producing *Enterobacteriaceae* were susceptible to temocillin. Evaluation of further isolates is still ongoing.

Discussion: The results of our study for in vitro susceptibility testing of temocillin against ESBL-producing *Enterobacteriaceae* deliver promising results as a treatment option. The role of temocillin for the treatment of septicemia, hospital-acquired pneumonia and urinary tract infection due to ESBL-producing bacteria has to be evaluated in clinical studies. Temocillin might become an interesting antimicrobial alternative in ESBL infection.

KMP23

VRE outbreak analysis: MALDI-TOF MS as a novel epidemiological tool

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Introduction: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) provides fast and accurate identification of microorganisms. Several works have speculated about its possible use in taxonomy and epidemiology [1]. However, no systematic comparison of MALDI-TOF MS with the gold standard, the pulsed-field gel electrophoresis (PFGE) to type strains has been reported yet. For the first time, this study evaluates the potential use of MALDI-TOF MS for the epidemiological analysis of outbreaks and compares it to PFGE.
Method: During a recent outbreak of vancomycin-resistant enterocci (VRE) on a paediatric intensive care unit at the Heidelberg University Hospital, 5 patients were found positive for VRE. All isolated VRE were Van A positive *E. faecium*. The isolates underwent PFGE as well as MALDI-TOF MS analysis. PFGE results were interpreted following the Tenover criteria [2]. For the dendrogram creation with a Microflex mass spectrometer (Bruker Daltonik, Bremen, Germany) and the MALDI Biotyper 2.0 software, VRE isolates underwent an extraction procedure, as described elsewhere [3]. Spectra were recorded in the positive linear mode (laser frequency 20 Hz; ion source 1 voltage at 20 kV ion source 2 voltage at 18.5 kV; lens voltage 8.5 kV; mass range 2000 - 20137 Da). For each spectrum 6x480 shots from different positions of 6 target spots were collected per isolate. Spectra were cleaned and calibrated with the peak of the 50S ribosomal protein L36 of *E. faecium* at 4427 kDa. Subsequently minimum spanning trees (MSP) were created. The creation of the dendrogram was based on cross-wise MSP matching.
Result: The topology of the dendrogram (Figure 1) generated from the spectra of the VRE isolates was in agreement with that inferred from the PFGE analysis (Figure 2). Three different strains could be clearly identified. C1 was the index case and was held responsible for the transmission to all others beforehand. Epidemiological analysis revealed actually two transmissions; the first from patient C1 to C2 and the second from patient A1 to A2. Patient B was probably an already prevalent case. In the dendrogram, strain B is separated from the others but closer related to strain C than A.
Discussio: MALDI-TOF MS provided results concordant to the PFGE gold standard and that in a fraction of time and costs. These results show great promise for MALDI-TOF mass spectrometry as an epidemiological tool for the real-time management of VRE outbreaks.

References
1. Murray PR. Clin Microbiol Infect **2010**; 16:1626-30.
2. Tenover FC et al. J Clin Microbiol **1995**; 33:2233-39.
3. van Veen SQ et al. J Clin Microbiol **2010**; 48:900-7.

Figure 1
MSP dendrogram of the isolated VRE strains

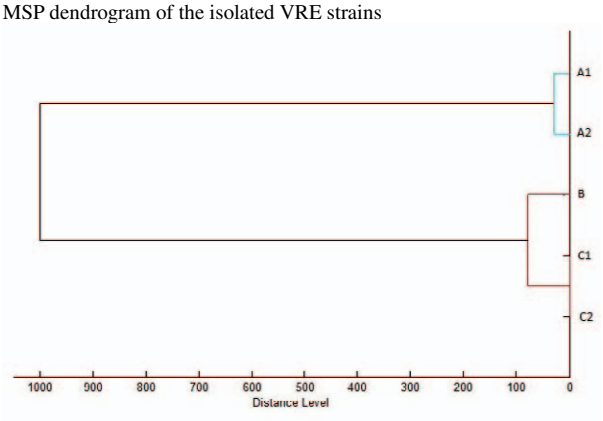
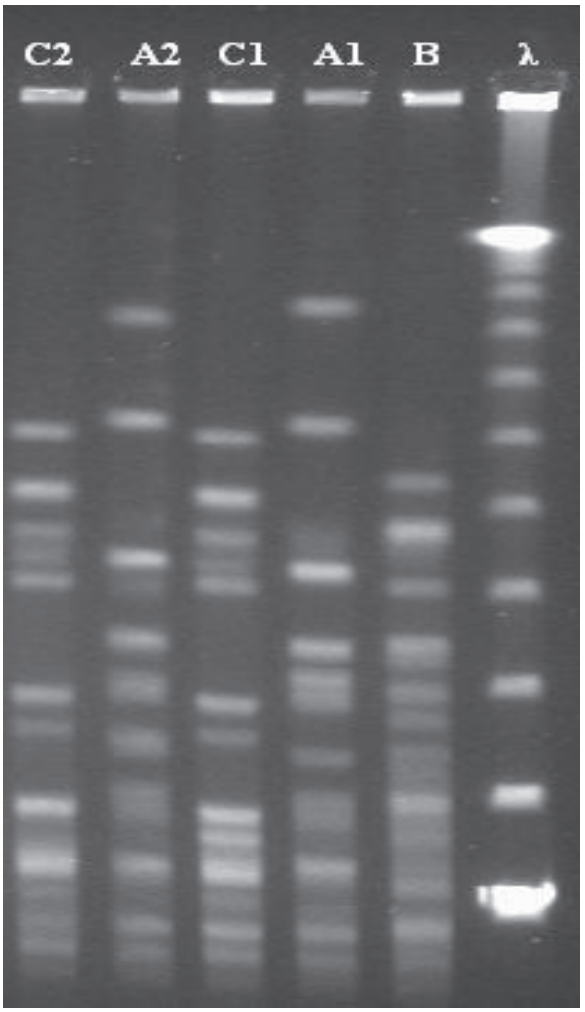


Figure 2
PFGE of the isolated VRE strains



KMP24

Methicillin Resistance and *spa* types in the Invasive *Staphylococcus aureus* Infection Cohort (INSTINCT)

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Background: *Staphylococcus aureus* is a leading cause of community-acquired, healthcare-associated and nosocomial bloodstream infections. The clinical course of *S. aureus* bacteremia (SAB) is very variable. In a number of patients, serious complications (i.e. endocarditis, septic arthritis, vertebral osteomyelitis) and death may result from dissemination and metastatic infection of distant organs. Methicillin-resistant strains (MRSA) complicate treatment and patient care.
Methods: A cohort of 666 consecutive patients with SAB was monitored prospectively at two university hospitals (Freiburg and Cologne) from January 2006 to December 2009. Data was collected based on patient charts, microbiological reports and interviews. Patients were analyzed for clinical features, diagnostic measures, and outcome. MRSA isolates from the University of Cologne were typed by sequencing the spa (*S. aureus* protein A) locus.
Results: The rate of MRSA remained stable from 2006 to 2009 at about 16% (Cologne) and 11% (Freiburg). Of the 666 patients with SAB, 375 (54%) were hospital-acquired infections, 218 (33%) were community-acquired healthcare-associated infections and the remaining 91 (14%) were community-acquired infections. The most common sources of infection were: intravascular catheter (220, 33%), skin and soft-tissue infections (38, 6%), cardiovascular system infection and endocarditis (53, 8%), vertebral and non-vertebral osteomyelitis (56, 9%). The 30-day mortality was 19%.

In Cologne, the most prevalent *spa* type was t003 (32/38); t001, t018, t030, t032, t038, and t068 each in a single patient.
Conclusion: SAB is a common infection in German hospitals associated with a high mortality. In Cologne, the most prevalent *spa* type is t003 which currently is also one of the most prevalent spa-types in Germany.

KMV01

Why Eculizumab as treatment for EHEC-induced haemolytic uraemic syndrome?

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The control of the infectious agent is usually not the determining factor for a favourable outcome of an infectious disease in its final stadium, but rather the control of the over-reactive immune system. Complement is an important part of the humoral innate immune system. Inherited mutations in this system, and particularly in its regulatory proteins, lead to such an over-reactive state and eventually to a haemolytic uraemic syndrome (HUS). This type of HUS is termed „atypical", as it is unrelated to an infection with enterohaemorrhagic E. coli (EHEC). Thus, both HUS types, the typical, as well as the atypical, show similar clinical courses, but differ in their causal origin - an inherited mutation (or an acquired autoantibody) on the one hand, and an EHEC infection on the other.
About 20 years ago we characterised a murine, monoclonal antibody directed against C5, N19-8, which was able to almost completely block the terminal complement cascade, by preventing both C5a and terminal complement complex (TCC) formation (Würzner et al.; Complement Inflamm 1991;8:328-40). These properties are retained in the humanised recombinant single-chain Fv fragment (Evans et al., Mol Immunol 1995;32:1183-95); N 19-8 became the prototype of the licenced medical drug Eculizumab (Soliris).
Eculizumab was recently very successfully used to block complement in the treatment of paroxysmal nocturnal haemoglobinuria (PNH), in which red blood cells are lysed by complement due to an inherited lack of inhibitors on their cell surface. As Eculizumab works for PNH, and atypical HUS also shows an uncontrolled complement activation, this humanised antibody was also successfully used by us in a boy who suffered from an inherited mutation leading to this clinical condition (Zimmerhackl et al., N Engl J Med. 2010;362:1746-8).
Last, but not least we provided the rationale for a treatment of the typical HUS with a complement inhibitory antibody: although it is well established that the released EHEC shiga toxins have a direct deleterious effect on kidney cells, they also activate complement and bind factor H, its main regulator, leading to both a stronger activation and a weaker control of complement (Orth et al., J Immunol 2009;182:6394-400). Several other labs have now corroborated the role of complement in typical HUS (Thurman et al., Clin J Am Soc Nephrol 2009;4:1920-4; Stahl et al., Blood 2011;117:5503-13).
This opened the field for using Eculizumab in typical HUS, as recently and successfully performed by Lapeyraque and coworkers in three patients (Lapeyraque et al., N Engl J Med, 2011, in press). The evaluation of the outcome of the many dozens patients treated during the O104 outbreak will eventually demonstrate the clinical impact of complement in typical HUS.

KMV02

PCR–based rapid sepsis diagnosis effectively guides clinical treatment in patients with new onset of SIRS

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Background: Early detection of the causing microorganism and timely therapeutic intervention is crucial for improved outcome of patients with sepsis. Quite recently we evaluated the technical and diagnostic feasibility of a commercial multiplex real time polymerase chain reaction (PCR)(LightCycler SeptiFast assay) for detection of blood stream infections in a cohort of intensive care unit (ICU) patients with the risk of abdominal sepsis. The PCR positivity rate showed a high coincidence with SIRS (75.8%). In this study we focussed on patients from the same surgery ICU with upcoming SIRS and addressed the utility of the LightCycler SeptiFast assay on therapeutic decision-making.
Methods: In addition to conventional microbiological and laboratory tests we analyzed blood samples by the multiplex-PCR-assy.102 patients on the ICU fulfilling the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) SIRS criteria were included. Blood samples were taken within 24h of upcoming SIRS.
Results: 39.9% (n=59) of the blood samples (n=148) were positive using multiplex-PCR and 20.3% (n=30) using conventional culture. In 11.4% of all samples multiplex-PCR detected more than one microorganism. Among the 77 microorganisms identified by multiplex-PCR, only 25 (32.5%) could be confirmed by blood culture, additional 17 could be confirmed by microbiological test results from other significant patient specimen. Positive blood samples independent of the detection method were characterized by significant elevated levels of procalcitonin (p
Conclusion: Our study demonstrates improved detection of specific pathogens with a high intrinsic resistance and a positive impact on therapeutic decisionmaking by additional multiplex-PCR based analysis of blood samples for infectious agents in patients with new onset of SIRS. Thus, we showed for the first time that PCR test results guide clinical treatment successfully.

KMV03

Acid Sphingomyelinase and Ceramide are Key Regulators of *Staphylococcus aureus* Induced Septic Shock

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Infection with *S. aureus* can result in a clinical condition ranging from Systemic Inflammatory Response Syndrome to Septic Shock with multiple organ dysfunction. One commonality of this spectrum is an evolving and eventually overwhelming host immune response that is often manifested clinically in pulmonary edema. Ceramide is a biologically active sphingolipid created through hydrolysis of sphingomyelin by the inducible enzyme acid sphingomyelinase. As a membrane constituent and signaling molecule, ceramide functions as an integral component in multiple signaling pathways. In the present study we sought to demonstrate that inducible ceramide is an important mediator of the overwhelming immune response leading to septic shock from *S. aureus* bacteremia. C57Bl6 (wt) mice and acid sphingomyelinase knockout (Asm(-/-)) littermates were inoculated intravenously with a clinical isolate of *S. aureus*. Dosing of the organism was such as to induce a state of septic shock and death within 24-48 hours for all mice. Using immunohistochemical techniques, quantification of GR1 positive staining cells in the lung revealed >50% decrease in neutrophil trafficking at 24 hours in Asm(-/-) mice compared to wt controls. Measurement of Evan's Blue dye extravasation in the lung displayed a two-fold reduction of pulmonary edema in Asm(-/-) animals. While sequestering of neutrophils in the lung and pulmonary edema was diminished in Asm(-/-) mice, these animals failed to clear the infection, as indicated by a progressive increase in *S. aureus* CFUs isolated from saponin treated splenic homogenates. Wild type mice, in comparison, began to clear the infection at 6 hours post-inoculation. These data indicate that membrane ceramide is an important mediator of immune system activation and pulmonary dysfunction in *S. aureus* induced septic shock. While the pulmonary manifestations of septic shock are reduced in Asm(-/-) mice, they demonstrate impaired bacterial clearance and ultimately succumb to infection. This outcome implicates acid sphingomyelinase dependent ceramide production as an important component of the innate immune response.

KMV04

Wolbachia endbacteria of filarial nematodes are sensitive to Acyldepsipeptides

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Introduction: Filarial nematodes infect more than 150 million people worldwide. Persons infected with *Wuchereria bancrofti*, *Brugia malayi* or *Onchocerca volvulus* can develop hydrocele, lymphoedema, dermatitis or blindness. *Wolbachia* are obligate endosymbiotic bacteria of filarial nematodes that are essential for embryogenesis, larval development and adult survival. Targeting these endobacteria with doxycycline has proven to be an effective treatment that can replace conventional antifilarial therapy. Since doxycycline is contraindicated for a large portion of the population, new antibiotics would be advantageous. Acyldepsipeptides (ADEPs) interfere with the proteolytic protease ClpP. When ADEPs bind to ClpP, the protease is dysregulated leading to inhibition of cell division and cell death.
Methods: To characterize the activity of ADEPs against *Wolbachia*, we treated the *Wolbachia* infected insect cell line *Aedes albopictus* C6/36 with different ADEP derivatives. Depletion of *Wolbachia* was monitored by quantitative real-time PCR by analysis of gDNA for the *16S-rRNA* gene of *Wolbachia* normalized to the *actin* gene of the C6/36 cells. The *in vitro* activity of ADEPs to the wolbachial ClpP was monitored using beta-casein

and purified recombinant ClpP from the *Wolbachia* of *Brugia malayi* and visualized by SDS-PAGE and silver stain.
Results: With the exception of one derivative, all congeners significantly depleted *Wolbachia* from the C6/36 cells. The most active derivative ADEP 1 depleted 97% of *Wolbachia*, a reduction comparable to the gold standard doxycycline. ADEPs are able to activate wolbachial ClpP which leads to a degradation of casein. The activation is depending on the ADEP derivative used. Thus, ADEPs have anti-wolbachial activity and induce dysregulation of the protease. Additionally, the proper regulation of ClpP is necessary for *Wolbachia* survival and the results have identified ClpP as a promising target for further antibiotic discovery.

KMV05

First detection of IMP-13 metallo-β-lactamase in clinical isolates in Germany

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Introduction: Resistance to carbapenems in Gram-negative Bacteria is increasing worldwide. In *Pseudomonas aeruginosa* resistance can be mediated by production of metallo-β-lactamases (MBL). MBLs are able to hydrolyse penicillins, cephalosporines, and even carbapenems, but not aztreonam. In Germany members of the Verona integron-encoded (VIM) family of MBLs have been reported most frequently. Here we report the detection of an IMP-13-type MBL (IMP for "active on imipenem") in two clinical isolates of *P. aeruginosa* at a university hospital in northern Germany. To the best of our knowledge, this is the first report of IMP-13-producing organisms from clinical specimens in Germany.
Case report: Two *P. aeruginosa* strains were isolated from clinical samples in February and March 2010, respectively. One Isolate was detected in the urine from a 70-year old male patient with a neurogenic disturbance of bladder and rectum. The patient was admitted to our hospital for surgical treatment of a sacral decubital ulcer.
A second *P. aeruginosa* isolate was found in an inguinal wound swab from a 61-year old male patient who was admitted to hospital because of a type B dissection of the aorta. Both patients received multiple antibiotics before detection of the IMP-producing *P. aeruginosa*. More over, both patients developed *Clostridium difficile*-associated diarrhea during their stay at hospital.
Bacterial isolates were cultivated and identified by standard microbiological procedures. Susceptibility testing was done by the Vitek2 automated system. Production of MBL was confirmed by Etest and PCR from whole cell lysates. Amplified PCR-products were sequenced and analyzed using the BLAST algorithm.
The MICs for both *P. aeruginosa* isolates were (isolate 1/isolate2, [µg/ml]): piperacillin/tazobactam ≥ 128/≥128, ceftazidime ≥64/≥64, imipenem 8/≥16, meropenem 4/≥16. The production of an MBL was confirmed by Etest phenotypically. Sequencing of a 661 bp fragment (isolate 1) and a 622 bp fragment (isolate 2), respectively, revealed an IMP-13-type MBL in both isolates.
Discussion: Although IMP-13 has been detected in isolates from other countries like Italy, Argentina or Austria, to the best of our knowledge, this is the first report of detection of IMP-13 in clinical isolates from Germany so far. This report underscores the sustaining spread of multidrug-resistant bacteria producing broad-spectrum beta-lactamases. It stresses the need for more effective infection control measures and development of new antibiotic drugs.

KMV06

The urinary antibiotic nitroxoline blocks septic stone formation by multiple mechanisms: a better and non-toxic alternative to acetohydroxamic acid treatment?

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Urinary tract infections with *P. mirabilis*, *P. vulgaris* or *P. rettgeri* are often complicated and refactory to therapy, as the urease activity of these organisms is unique in mediating urine pH rises to 8 and higher, thereby leading to the formation of precipitates containing viable bacteria. Biofilm formation on catheter or epithelial surfaces will further enhance the retention of these septic crystals. For the treatment of such infections the nickel chelator and urease inhibitor acetohydroxamic acid (AHA) is in clinical use in the U.S.A. However, due to severe side effects AHA has not been approved in Europe. The purpose of this study was to test the urinary antiseptic and cation chelator nitroxoline (NIT) as a possible non-toxic alternative.
UTI isolates of *P. mirabilis, P. vulgaris, P. rettgeri* were collected during routine diagnostic procedures and MIC determined according to CLSI recommendations or in artificial urine with 2% TSB. MIC values for NIT were found to lie between 7.8 and 31.3 µM being slightly lower in urine. In contrast, no inhibitory effect was noted for AHA at concentrations of up to 1 mM. Effects on enzymatic urease activity were examined by suspending bacteria in artificial urine with or without NIT or AHA and taking samples at regular intervals during a one hour incubation period at 37 °C. At 1 mM, the urine concentration found during oral treatment, NIT nearly completely blocked the pH rise caused by ammonia formation (measured by glas electrode and the indophenol method, respectively). The effect was concentration dependent and partially resolved by addition of 100 µM Ni^{2+}, but not by Fe^{2+}. Similarly, precipitate formation was completely blocked by 1 mM NIT (determined by ARS staining and subsequent spectrophotometric quantification). In comparison, AHA was slightly weaker in all assays. When precipitate formation in preformed *P. mirabilis* biofilms was examined, complete blockage was obtained already with 1 µM NIT, the lowest concentration studied. In contrast, results observed with AHA correlated well with the results obtained during urease activity assays. However, when biofilm was preformed in the presence of urea, the subsequent NIT effect on precipitate formation was lower, thus indicating an additional effect of NIT on urease induction. Finally, we observed a reduction of biofilm in the presence of NIT (determined by crystal violet staining and subsequent spectrophotometric quantification).
Taken together, NIT was thus found to be as good or better than AHA in the inhibition of urease activity. Moreover, it was found to exhibit potent antimicrobial effects against all clinical isolates examined, and reduced precipitate formation in *P. mirabilis* biofilms, presumably by a combination of biofilm degradation, inhibition of urease induction and blockage of urease activity.

LMP01

Risk-Assessment of *Cronobacter sakazakii* contamination of spray-drying processes within infant milk formula production: D-value examination, biofilm formation; decontamination models

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Background: This study aimed at assessing the risk of *Cronobacter sakazakii* contamination of spray drying processes within infant milk formula production. Three main aspects were examined: The bacteria specific D-value for thermic sterilisation properties, biofilm formation on certain filter materials, and efficiency of cleaning agents for the cleaning in place system.
Material and Methods: The D-value was analyzed in CASO bouillon and reconstituted infant formula, both were inoculated with *C. sakazakii* (DSM 4485) in a concentration about 10^7 cfu/ml and inoculated at 58°C. Samples were taken every two minutes. The bacteria count was determined by the cast plate method on CASO agar.For optical detection of biofilm formation, cellulose and polypropylene filter were inoculated with *C. sakazakii* and exposed to dry conditions. The filter material was fixed in 4% Glutaraldehyde and then dried in ethanol and in a critical point dryer. The pictures were made by a scanning electron micrograph LEO Stereoscan 420. An agar diffusion test and a qualitative suspension test were made to test the effect of acids and bases to *C. sakazakii*. Sodium hydroxide and nitric acid were used in different concentrations (2 %, 2.5 %, 3 %, 4 %, 5 %, 10 %). The bacteria count was determined by cast plate method on CASO agar with an inactivator.
Results: The $D_{58°C}$ of *C. sakazakii* was 2.2 to 6.6 in CASO bouillon and 1.8 to 4.3 in the infant formula. Influencing factors were heat conductivity of media, culture conditions and state of cell growth. *C. sakazakii* is able to form biofilm on cellulose and polypropylene fibres under dry conditions. On cellulose fibres the bacteria were rod-shaped while on polypropylene coccoid forms occurred. *C. sakazakii* is approvingly sensitive to nitric acid. Sodium hydroxide (2.5 - 3%) treatment leads to aggregate formations of the bacteria and to a total depletion after 60s incubation time.
Conclusion: As *C. sakazakii* adheres to organic and synthetic filter materials, spray drying processes of milk based products are at risk for contamination. Therefore, pasteurisation process validation for all kind of heat exchanger and cleaning regimes with nitric acid and sodium hydroxide reduces this specific contamination risk.

LMP02

Species-specific identification of *Arcobacter* spp. by MALDI TOF MS

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Background: *Arcobacter* spp. are natural inhabitants of intestinal tracts of poultry and warm blooded domestic animals. Identification of species

belonging to the genus *Arcobacter* has become increasingly important since some of them are now proved to be pathogenic for humans and/ or animals. Methods based on phenotypic identification are time-consuming and, due to the occurrence of biochemical atypical strains, inaccurate. Genotypic identification methods are more accurate. Matrix-associated laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) has been shown to be a rapid and sensitive method for identification of bacteria as well.

Methods: In this study a reference database of *Arcobacter* strains for MALDI-TOF MS identification was build up. Subsequently, 203 isolates of *A. butzleri, A. cryaerophilus, A. skirrowii* and *A. cibarius* were identified by real-time PCR and conventional PCR methods and analyzed by MALDI-TOF MS in duplicate.

Results: 193 of the 203 (95%) *Arcobacter* isolates were identified accurately at species level. Ten isolates (5%) had a log(score) value below 2.0. Therefore reliable identification was only possible at genus level. MALDI-TOF MS showed no misidentifications, neither at genus nor at species level.

Conclusions: The discrimination by MALDI-TOF proved to be a useful and reliable tool for species-specific identification of *Arcobacter* species.

LMP03

Food safety: Heat inactivation of *Cronobacter* in powdered infant formula

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Cronobacter bacteria, formerly classified as *Enterobacter sakazakii*, have been implicated in several incidents as the cause of meningitis and enterocolitis with high mortality rates in premature infants resulting from feeding with contaminated powdered infant formula (PIF) [1]. PIF therefore is strictly recommended to be "sakazakii-free" which is defined as the absence of any colony forming unit in 30 samples of 10g of PIF [2].

In cooperation with the Food Process Engineering section of our institute and the Milchwerke "Mittelelbe" GmbH (an industrial producer of PIF), we are developing a modified production process assuring the inactivation of *Cronobacter* by pasteurization of all raw materials. Detection of viable cells is accomplished by quantitative RealTime PCR as well as by selective growth on chromogenic media following enrichment culture.

We are going to present our first results of heat inactivation kinetics of *Cronobacter* species in raw materials of PIF.

Based upon these data, a modified pasteurization process is to be developed.

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LMP04

Identification and disinfectants resistance ability of bacteria isolated from clean rooms of pharmaceutical factories in Iran

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Introduction and Objectives: Clean rooms are essential in aseptic pharmaceutical or food production. Monitoring microbial distribution and identifying the predominant isolates is part of Good Manufacturing Practices. Aims of this research were to screen for the predominant bacteria strains distributed in clean rooms and to analyze their microbiological and molecular characterizations.

Material and Methods: Bacteria strains were isolated in the routine aseptic monitoring courses from the clean rooms of a pharmaceutical factory, using air sampler and with air settle plate method. Representative bacteria strains were selected based on colony morphology. On divergent colonies, Gram staining, as well as oxidize and catalase tests was performed. The method of these tests followed John *et al*. 1994. With this process, and based on the distribution frequencies of these isolates, 3 predominant strains named S1~S3 were selected. Their morphology and physiological/ biochemical characteristics were tested according to John *et al*. (1994). The PCR amplification did for final verification of S2 isolate.

Results: Results showed that all the three isolates belong to Gram positive bacteria, *Staphylococcus*, *Bacillus* and *Microbacterium*, respectively. Sensitivity tests for these bacteria isolates to UV irradiation and phenol treatment showed that isolate S2 the most resistant isolate against commonly disinfectants usable in pharmaceutical units.

Conclusions: Bacteria widely distributed in clean rooms are mainly a group of Gram positive strains, showing high resistance to selected disinfectants. Screening bacteria isolates and identifying them will aid in finding a more effective disinfection method.

Keywords
Clean room, Gram positive bacteria, Disinfectant, Resistant abilities

LMV01

Tenacity of *Listeria monocytogenes* strains in food-grade H1-lubricants

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Strains of the Gram-positive human pathogenic species *Listeria monocytogenes* have caused several food-borne outbreaks of disease including fatalities. The species is characterized by its ubiquitous occurrence as well as a wide range in growth temperature and pH-value. Risk foods are especially raw milk and raw milk products, meat and fish. Especially the ready to eat-foods smoked and graved salmon have been identified as risk products. *Listeria* may be spread through the fish-processing equipment and machinery and may persist there. Today, insufficient data exist whether the H1-lubricants applied in these machines are putative reservoirs and sources of *L. monocytogenes*.

In this project, we investigated the survival of three *L. monocytogenes* strains in H1-lubricants with different chemical compositions applied in the fish industry. In a first step, the presence of *L. monocytogenes* in the native H1-lubricants was assessed. Upon a negative result, in the second step of the studies the lubricants were contaminated with the three *L. monocytogenes* strains and their survival in the lubricants was monitored over a period of 14 days. Furthermore, the influence of the water content of the lubricants (0, 1 and 5%) on the survival of *Listeria* was evaluated.

None of the native lubricants contained *Listeria*. Upon contamination of the lubricants with the three *L. monocytogenes* strains their viable counts decreased significantly by at least 99.995% within seven days. The reduction rates were found to depend on the chemical composition of the lubricant as well as on the investigated strain. Interestingly, the isolate from smoked salmon tolerated this environment better than the reference and the type strain. The water content of the lubricant had no influence on the survival.

Based on the results of this study we conclude that the investigated strains cannot survive in H1-lubricants.

LMV02

Development of a real-time PCR based system for the detection of *Cronobacter sakazakii* in powdered milk products

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Cronobacter sakazakii is an emerging food-borne pathogen which has been involved in life-threatening infections such as meningitis, enterocolitis and septicemia in neonates and infants. Outbreaks are rare, but in several of these cases contaminated powdered infant formula was identified as a source of *C. sakazakii* infections.

The detection of *C. sakazakii* with the current ISO/TS standard method 22964:2006 requires five to six days to confirm the species identification. Therefore, a rapid, specific and sensitive method for the detection and identification of *C. sakazakii* in powdered infant formula is necessary. This study describes the development of a real-time PCR (RT-PCR) based assay for the detection of *C. sakazakii* in powdered milk products and infant formula.

The proposed method combines an unspecific enrichment, sample preparation and RT-PCR.

All bacterial strains were grown in brain heart infusion broth at 37°C with agitation. Genomic DNA was extracted from overnight cultures using an illustra™ bacteria genomic Prep Mini Spin Kit (GE-Healthcare). Two primers and a TaqMan probe were designed with the Beacon Designer software to target outer membrane protein A gene (*ompA*). The assay was validated with 40 *C. sakazakii* strains and 17 strains of closely related species and genera.

A TaqMan RT-PCR assay was successfully developed with a detection limit of 0,001 µg DNA/ml and a reaction efficiency of 96.5 %. All tested *C. sakazakii* strains were identified in 100 min with reproducible amplification curves and all non *C. sakazakii* strains were not detectable.

The developed TaqMan RT-PCR assay reliably detects and identifies *C. sakazakii* very fast. The analysis time of the complete assay including enrichment and sample preparation was approximately 22 h. The described method is a solid basis for the fast and reliable detection of *C. sakazakii* in powdered milk products.

LMV03

Differential proteomic expression of enterohemorrhagic *E. coli* O157:H7 EDL933 in vitro

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Enterohemorrhagic *Escherichia coli* (EHEC) are enteric pathogens causing severe illness in humans. EHEC food-borne infections may lead to non-bloody diarrhea, bloody diarrhea and the hemolytic uremic syndrome (HUS). The survival of EHEC in the food chain and in the human intestine is dependent of environmental conditions such as available nutrients and/or the exposure to stressful agents such as bile salts and/or pancreatic enzymes. Exposure to different environments may cause a shift in metabolic pathways. In EHEC, this may also lead to a shift in the expression of virulence determinants.
In order to test the behaviour of EHEC O157:H7 strain EDL933 grown in the gut of the human host, we used simulated ileal environment medium (SIEM) and simulated colon environment medium (SCEM) and compared it to tryptic soy broth (TSB), used as a standard rich medium.
With two-dimensional gel electrophoresis, we differentiated cytosolic proteins with at least 2-fold higher expression in SIEM and SCEM as compared to TSB. After growth under aerobic and anaerobic conditions, the proteins were detected and evaluated by a software and identified by MALDI-TOF-analysis. In TSB, we found a high amount of the flagellin protein FliC, suggesting that it is downregulated in SIEM and SCEM. In contrast, several proteins involved in biofilm synthesis were up-regulated in the latter media. We also found modulated "locus of enterocyte effacement"-associated proteins such as EspP and EspB as well as overexpressed phage-associated proteins. In cooperation with the group of Wolfgang Eisenreich, TU Munich, we tested the incorporation rates of $^{13}C_6$-labelled glucose with respect to the synthesised amino acids. Therefore, EDL933 was cultured in SIEM, SCEM and TSB media. The results of these analyses gave us a hint how environmental conditions affect the metabolism and virulence properties of EHEC O157:H7 strain EDL933.

LMV04

Antifungal activity of lactic acid bacteria *Lactobacillus plantarum* IBL-2 AND TSD-10 AGAINST *Penicillium* sp.

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The staple food such as rice, wheat, corn, peanuts and greenbeans often stored in warehouse as stockpiled supplies, but it is often damage in large numbers by fungi. One of fungi causes of the damage is *Penicillium* sp. Several species of *Penicillium* can grow in food that was given potassium sorbate. The using of synthetic preservatives antifungal often induce resistance. Food industry has been using lactic acid bacteria widely as biocontrol agent to improve food safety at minimum food processing. It's important to exploring lactic acid bacteria as biopreservative antifungal. The aim of this research is to know antifungal activity of lactic acid bacteria *Lactobacillus plantarum* 1BL-2 and TSD-10 against *Penicillium* sp. Colonies of *Lactobacillus plantarum* isolates 1BL-2 and TSD-10 cultured in GYP liquid medium and then incubated at 37 °C for two days, then cultures were centrifuged. Some supernatant was done ammonium sulfate precipitation and then centrifuged. The precipitate obtained resuspended with buffer. 50 µL of sample were added to paper disk and put into MRS agar containing 0.5% of *Penicillium* sp. Antifungal activity of *Lactobacillus plantarum* stains against *Penicillium* sp. was range from 15 to 18 mm. Samples that have been neutralized still produce clear zone. this suggests that the antifungal activity not only because of acidic conditions. *Lactobacillus plantarum* 1BL-2 and TSD-10 produces plantaricin that have antifungal activity against *Penilcillium* sp.

Keyword
Lactobacillus plantarum, antifungal, *Penicillium* sp.

LMV05

Antibacterial effects of probiotics isolated from yoghurts containing probiotics against some common bacterial pathogens

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Introduction and Objectives: According to the definition of Food and Agriculture Organization of the United Nations/World Health Organization, probiotics is defined as Live microorganisms, which when administered in adequate amounts, conferring a health benefit on the host. Microorganisms that are probiotics in humans include *Enterococci*, *Bifidobacteria* and lactic acid bacteria, such as *Lactobacilli*, *Lactococci* and *Streptococci*. This research was conducted to determine the presence of antibacterial effects among the probiotics isolated from different Bioyoghurts against some common bacterial pathogens.
Material and Methods: Probiotics such as *Lactobacillus* sp., *Streptococcus* sp. and *Bifidobacterium* sp. from yoghurts were isolated and examined for their antibacterial effects against *Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa*. The modified agar overlay method was used to determine the presence of antibacterial effects among the isolated probiotics.
Results: Results showed the presence of antibacterial effects among the probiotics that were isolated from Bioyoghurts. The spectrum of their antibacterial effects varied against the selected pathogens. *Lactobacillus rhamnosus* GG killed all the isolates tested against them. *Bifidobacterium* spp. probiotics killed the test organisms *E. coli* and *S. typhi.* They were only inhibitory for *S. aureus*.
Conclusions: Antibacterial effects are one of the most important selection criteria for probiotics and verified antibacterial activity of probiotics supports the development of these functional foods as a key to the improvement of health in the consuming public.

Keywords
Probiotics, Bioyoghurts, *Lactobacillus* sp., *Bifidobacterium* sp., Antibacterial effects

LMV06

Bacterial Identification for Environmental Monitoring Using MALDI-TOF Technology

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Most technologies and databases designed for rapid identification of microorganisms are designed with clinical isolates in mind. When adapted for use in the microorganism monitoring programs of pharmaceutical, nutraceutical, and sterile manufacturing environments, these technologies underperform due to the diversity of organisms found in these environments. Whole cell proteotypic analysis with matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF) spectroscopy is an emerging rapid and inexpensive method of identifying bacteria. This study tests the accuracy, robustness, and reproducibility of this technology when applied to an extensive panel of known culture collection strains and frequently seen species found in pharmaceutical, nutraceutical and sterile manufacturing environmental monitoring programs. The MALDI-TOF manufacturer's recommended methods for sample processing and data analysis are directly compared to methods optimized in our laboratory for environmental monitoring. Advantages and limitations of using MALDI-TOF technology for this application are discussed. This study showed that fewer than 70% of frequently seen environmental isolates could be accurately identified with the MALDI Biotyper™ system (Bruker Daltonics). The >30% that failed to be identified were largely due to limitations in the clinically focused Biotyper™ database. At Accugenix, the methods were optimized and additions made to the library such that the number of samples that were identified increased. While a custom database and method modifications are required to optimize this technology for environmental monitoring applications, the low cost and rapid turnaround time of MALDI-TOF analysis remain strong advantages for this technology.

MPP01

Analysis of the *Bartonella* adhesin A (BadA) stalk from *B. henselae* reveals domain-specific and domain-overlapping functions in the host cell infection process

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Human pathogenic *Bartonella henselae* cause cat scratch disease and vasculoproliferative disorders. An important pathogenicity factor of *B. henselae* is the trimeric autotransporter adhesin *Bartonella* adhesin A (BadA) which is modularly constructed and consists of a head, a long and repetitive neck-stalk module with 22 repetitive neck/stalk repeats and a membrane anchor. The BadA head is crucial for bacterial adherence to host cells, binding to several extracellular matrix proteins and for the induction of vascular endothelial growth factor (VEGF) secretion. Here, we analyzed the biological role of the BadA stalk in the infection process in greater detail. For this purpose, BadA head-bearing and headless deletion mutants with different lengths in the neck-stalk module were produced and functionally analyzed. *B. henselae* with head-bearing and partially truncated

BadA stalks (one or four neck/stalk elements) bound to fibronectin, collagen and endothelial cells and induced VEGF secretion. A strongly truncated headless BadA mutant (one neck/stalk element) was deficient for these biological functions whereas the expression of a longer headless BadA mutant (four neck/stalk repeats) restored fibronectin binding, adherence to host cells and the induction of VEGF secretion. Our data suggest that (i) the stalk is exclusively responsible for fibronectin binding and that (ii) both the head and stalk of BadA mediate adherence to collagen and host cells and the induction of VEGF secretion. This indicates overlapping functions of the BadA head and stalk.

MPP02

Biochemical and molecular characterization of isocitrate- and malate dehydrogenase of *Pseudomonas aeruginosa*: Importance of the TCA- and glyoxylate shunt in cystic fibrosis (CF) lung adaptation

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The gram-negative bacterium ***Pseudomonas aeruginosa*** (PA) is a frequent opportunistic human pathogen and the leading cause of morbidity and mortality among patients suffering from cystic fibrosis (CF). For long-term persistence in the dehydrated and highly viscous sputum of CF airways PA has to adapt its phenotype remarkably in response to prevailing requirements of the CF lung environment, including a loss of acute virulence determinants. In contrast, PA gains obviously metabolic adaptations. Previous studies of our group have shown that in end stage CF isolates the ***icd*** and ***idh*** genes are significantly up-regulated in comparison to early-stage CF isolates. Interestingly, PA possesses two isoenzymes of isocitrate dehydrogenase, namely dimeric Icd and the monomeric Idh that both catalyze the oxidative decarboxylation of isocitrate to ketoglutarate, CO_2 and NAD(P)H. Beside PA, only very few bacteria have two isoenzymes of isocitrate dehydrogenase suggesting a role during adaptation to environmental changes. Additionally, isocitrate dehydrogenase is a metabolic key enzyme that controls the metabolic flux between tricarboxic acid (TCA) cycle and glyoxylate shunt.
By use of ***icd*** and ***idh*** single transposon mutants and the construction of double mutant we tried to figure out the metabolic role of both enzymes. By Blue-Native-PAGE we were able to display the activities of each enzyme. Furthermore, the double mutant showed a significant growth defect and a modified growth behavior in minimal media supplemented with different TCA intermediates/C-sources. Further, we constructed a malate dehydrogenase mutant, which is also involved in the TCA-cycle. Moreover, there are many lines of evidence that $NAD(P)^+$-dependent isocitrate dehydrogenase and malate dehydrogenase are involved in the defense against oxidative stress. Therefore, we will investigate these metabolic enzymes with a view to oxidative stress as well.

MPP03

Characterization of new virulence factors of *Campylobacter jejuni*

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Campylobacter jejuni, an important food-borne bacterial pathogen in industrialized countries and in the developing world, is one of the major causes of bacterial diarrhoea. To identify new genes which are important for the invasion of host cells by the pathogen, we screened a transposon generated mutant library based on the clinical *C. jejuni* isolate B2. Thereby, we identified clones with a transposon insertion in genes *cj0952c* and *cj0005c*. The gene encoding *cj0952c* together with the adjacent gene *cj0951c* were supposed to act as a chemoreceptor. We could show that these genes are not translated as one protein, diminish the motility of the pathogen, alter the chemotactical behaviour of *C. jejuni* towards formic acid, but are not related to the utilization of formic acid by formate dehydrogenase. The second gene *cj0005c* represents one subunit of a sulphite: cytochrome c oxidoreductase. Further characterization showed the *cj0005c*-deficient genotype to possess a clearly reduced motility and a diminished adherence to host cells. Furthermore, the transcription of genes responsible for the synthesis of legionaminic acid (LegAm) were downregulated and the mutant possessed a reduced capacity to autoagglutinate. In contrast, neither the proliferation of the mutant, nor its intracellular ATP quantity was altered compared to the parental strain.

MPP04

InFiRe - a novel computational method for the identification of transposon insertion sites in bacterial genomes

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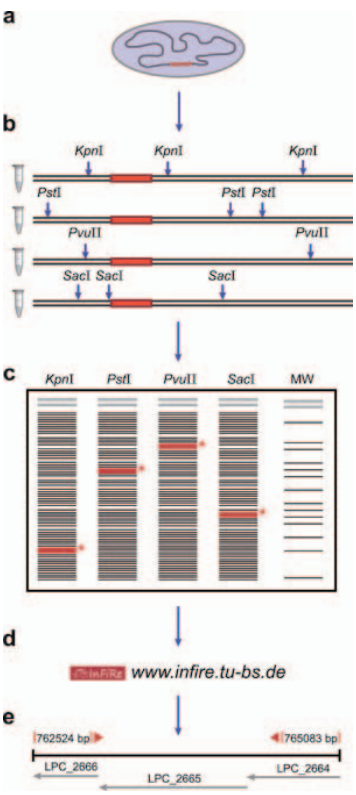
Transposons are mobile genetic elements capable of random insertion into their host genomes. This fundamental feature is employed since decades for the inactivation of genes, leaving a detectable signature at the locus of integration. Different modifications of the traditional transposon mutagenesis facilitated the development of new techniques, such as signature target mutagenesis, transposon mediated differential hybridization, genetic footprinting and gene expression analysis. The crucial step of all of these methods is the localization of the transposon insertion sites. This process is usually a time-consuming experimental procedure that requires intensive optimization.
InFiRe- Insertion Finder via Restriction digest - is a novel software tool which allows for the computational identification of transposon insertion sites in known bacterial genome sequences after transposon mutagenesis experiments.
The strategy of this procedure is outlined in **Fig. 1**. It is based on restriction digestions of genomic DNA in combination with Southern blot hybridization. In the first step, the genomic DNA is cleaved with different restriction endonucleases. Each restriction enzyme produces a unique pattern of DNA fragments with defined sizes. In the second step the sizes of the fragments with an inserted transposon are determined by Southern blot hybridization with a transposon-specific probe. The size of the fragment in the original genome equals the obtained fragment size minus the length of the transposon. Finally, using the derived fragment size pattern the most probable genomic position of the transposon can be calculated by the InFiRe software. Afterwards, confirmation of the accurate determination of insertion sites can be performed by PCR. The method is potentially applicable to any virus or sequenced organisms with known genomic sequence. The outlined method provides the solid basis for the establishment of a new high-throughput technology.
The software was implemented as a stand alone R-package. In addition, a web interface to the program is available at http://www.infire.tu-bs.de/.

Figure 1 Steps of the procedure for the identification of the transposon insertion in a bacterial genome. Chromosomal DNA from a transposon insertion mutant **(a)** is digested with a set of restriction enzymes **(b)**. Each restriction digest results in a unique pattern of DNA fragments. Separation of the DNA fragments by agarose gel electrophoresis followed by Southern blot hybridization with a transposon-specific probe allows determination of the approximate size of chromosomal fragments containing the transposon **(c)**. The pattern of the derived fragment sizes allows calculation of the most probable genomic position of the transposon via the InFiRe software **(d, e).**



MPP05

The alternative Sigma factor B modulates virulence gene expression in a murine *Staphylococcus aureus* infection model but does not influence kidney gene expression pattern of the host

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Staphylococcus aureus, persistent commensal of about 20 % of the human population, can be transmitted to the blood after body injury or by medical devices like catheters. An elementary model to mimic blood stream infection is the *intra-venous* infection of laboratory animals.
The influence of staphylococcal *i. v.* infection on murine kidney gene expression was analyzed in an *in vivo* model with BALB/c mice using the wild type strain RN1HG and its isogenic *sigB* mutant.
RT-qPCR of mixed eukaryotic and prokaryotic RNA from infected tissue resulted in the detection of SigB-dependent higher expression levels of *asp23* and *clfA* in the wild type strain *S. aureus* RN1HG, whereas, in agreement with *in vitro* data, expression of *hla* and *aur* was higher in kidney tissue from animals infected with the *sigB* mutant strain.
Although the virulence of *sigB* deficient strains is often reported to be similar to that of wild type strains, the pathomechanism of different infection settings might vary. Therefore, the rationale of this study was to investigate whether the deletion of *sigB* would lead to a different reaction of the infected host. Gene expression profiling indicated a highly reproducible host kidney response to infection with *S. aureus*. The comparison of infected with non-infected samples at 120 h post infection revealed a strong inflammatory reaction. This included e. g. TLR signaling, complement system, antigen presentation, IFN and IL-6 signaling, but also counter-regulatory IL-10 signaling. However, the results of this study did not provide any hints for differences in the pathomechanism of the *S. aureus* strains RN1HG and Δ*sigB* in the selected model of *i. v.* infection in mice, since the host response did not differ between infections with the two strains analyzed. If really existing, such differences might be transient and only apparent at earlier time points. Effects of SigB might also be compensated for in *in vivo* infection by the interlaced pattern of other regulators. Effects of missing activation and missing repression by SigB in the mutant might neutralize each other.
The study supports the conclusion that SigB might possess only to a lesser extent characteristics attributed to virulence factors and might act *in vivo* more like a virulence modulator and fine-tune bacterial reactions. SigB possibly might be important in special niches during infection.

MPP06

Modulation of cellular immune response by *Neisseria meningitidis* derived outer membrane vesicles (OMV)

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Outer membrane vesicles are released from the outer membrane of pathogenic *Neisseria* and previous works suggested a role of OMVs in invasive meningococcal disease (IMD). In more recent studies, OMVs are viewed as long distance delivery tools for bacterial pathogenicity factors. Because meningococcal OMV can be purified from plasma of IMD patients, we asked whether they impact polymorphonuclear neutrophils (PMN), which are a first line defence against IMD. By flow cytometry and fluorescence microscopy we detected fusion of *Neisseria meningitidis* (*Nm*) OMV to PMN, both in whole blood assays and in isolated cell fractions, and to differentiated HL-60 preparations, but not to T lymphycytes. As result of fusion of OMV, phagocytosis and ROS release by PMN was impaired. Live imaging revealed that PMN motility was also affected by OMVs. These results suggested reduced PMN mediated killing of *Nm* in the presence of meningococcal OMV. As a possible causal effector protein we identified a ribosyl transferase that proved to be present in OMVs and whose knock-out reduced the above mentioned OMV effects on neutrophils. Taken together, our data provide evidence that *Nm* is able to modulate cellular immune response mechanisms by release of OMV which fuse to host immune cells. This might aid the rapid spread of the bacteria once the blood vessels are invaded and thus support the rapid development of septic shock in many patients suffering from invasive meningococcal disease.

MPP07

Cell invasion by *N. meningitidis* requires as common interaction between focal adhesion kinase, c-Src and cortactin

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Entry of *Neisseria meningitidis* (the meningococcus) into human brain microvascular endothelial cells (HBMEC) is mediated by fibronectin or vitronectin bound to the surface protein Opc forming a bridge to the respective integrins. This interaction leads to cytoskeletal rearrangement and uptake of meningococci. Here we report that binding of pathogenic *N. meningitidis* triggered the recruitment of the focal contact associated proteins vinculin, paxillin and zyxin to the site of bacterial attachment. We determined that the focal adhesion kinase (FAK), which directly associates with integrins, is involved in integrin-mediated internalization of *N. meningitidis* in HBMEC.
Overexpression of the dominant-negative version of FAK (FRNK) blocked integrin-mediated internalization of *N. meningitidis*. Importantly, FAK-deficient fibroblasts were resistant to *N. meningitidis* invasion and showed reduced phosphorylation of the cytoskeleton proteins and decreased development of stress fibers. Furthermore *N. meningitidis* induced tyrosine phosphorylation of several host proteins including the c-Src /FAK complex substrate cortactin. Inhibition of cortactin expression by siRNA silencing and mutation of critical amino acid residues within cortactin significantly reduced meningococcal invasion into brain endothelial cells indicating a critical role for cortactin in meningococcal uptake. Moreover we demonstrate that the phosphorylation status of cortactin is enhanced during infection involving both FAK and Src kinase activity.
Together, these results indicate that *N. meningitidis* exploits the integrin signal pathway for its entry and that FAK is an important signalling molecule that mediates the transfer of signals from activated integrins to the cytoskeleton. A cooperative interplay between FAK, c-Src and cortactin then enables endocytosis of *N. meningitidis* into eukaryotic cells.

MPP08

***Neisseria meningitidis* induces platelet inhibition and increases vascular endothelial permeability via nitric oxide regulated pathways**

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Despite antibiotic therapy, infections with *Neisseria meningitidis* still demonstrate a high rate of morbidity and mortality even in developed countries. The fulminant septicemic course, named Waterhouse-Friderichsen syndrome, with massive hemorrhage into the adrenal glands and widespread petechial bleeding suggest pathophysiological inhibition of platelet function. Our data show that *N. meningitidis* produces the important physiological platelet inhibitor and cardiovascular signalling molecule nitric oxide (NO), also known as endothelium-derived relaxing factor (EDRF). *N. meningitidis*-derived NO inhibited ADP induced platelet aggregation through the activation of soluble guanylyl cyclase (sGC) followed by an increase in platelet cyclic nucleotide levels and subsequent activation of platelet cGMP- and cAMP- dependent protein kinases (PKG and PKA). Furthermore, direct measurement of horseradish peroxidase (HRP) passage through a vascular endothelial cell monolayer revealed that *N. meningitidis* significantly increased endothelial monolayer permeability. Immunfluorescence analysis demonstrated NO dependent disturbances in the structure of endothelial adherens junctions after coincubation with *N. meningitidis.* In contrast to platelet inhibition, the NO effects on HBMEC were not mediated by cyclic nucleotides. Our study provides evidence that NO plays an essential role in the pathophysiology of septicemic meningococcal infection.

MPP09

Interaction of *Legionella pneumophila* outer membrane vesicles with host cells and bacteria

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Legionella pneumophila is a Gram-negative intracellular pathogen that can cause a severe form of pneumonia. This bacterium naturally inhabits freshwaters and parasitizes protozoan hosts. After aerosol formation in man-made water systems, *L. pneumophila* can enter, colonize and destroy the human lung. During infection the pathogen employs sophisticated machineries to deliver proteins to host cells and tissues.
Besides the secretion of individual proteins, *L. pneumophila* sheds vesicles derived from its outer membrane. Outer membrane vesicles (OMVs) are

spherical bilayer structures and consist of characteristic outer membrane constituents including outer membrane proteins, phospholipids and lipopolysaccharides (LPS; Shevchuk *et al.,* 2011) as well as periplasmic components.
The group has described a comprehensive proteome reference map for OMVs of *L. pneumophila* (Galka *et al.,* 2008). A functional classification of the proteome showed that OMVs contain many virulence factors. Zymography and enzyme assays demonstrate that OMVs display proteolytic and lipolytic activities which may contribute to the destruction of the alveolar lining during infection.
Confocal laser scanning microscopy revealed a spatial association between *L. pneumophila* OMVs and the host cell surface. It remains unclear if this indicates adhesion, binding or fusion events between OMVs and host membranes. The role of *L. pneumophila* OMVs in interbacterial communication is also unknown. To address the question if OMV material is incorporated into target cell membranes, human macrophages and different bacteria were co-incubated with OMVs in PBS at 25 °C. Samples were taken and analysed at various time points. The presence of the *L. pneumophila* major outer membrane protein (MOMP) could not be detected in any of the target cells by Western blotting.
This finding hints towards a weak interaction with cell surfaces or rapid ingestion and degradation of OMV material. Ongoing studies address the effect of *L. pneumophila* OMVs on human macrophages in regard to metabolic activity and cytoskeleton rearrangements. Interbacterial effects of *L. pneumophila* OMVs are dissected by immunofluorescence microscopy and FACS analysis.

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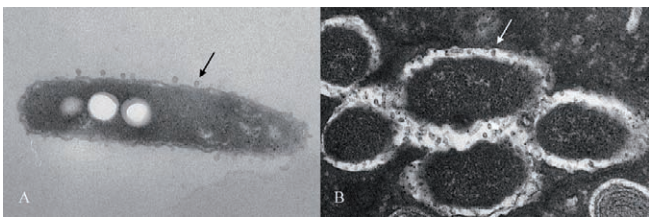
Figure 1
L. pneumophila sheds OMVs (arrows) from its surface during growth in liquid media (A) and within phagosomes of the amoeba *Dictyostelium discoideum* (B; Galka *et al.,* 2008)



MPP10
Borrelia valaisiana resist complement-mediated killing by a novel resistance mechanism

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Introduction: The ability of Lyme disease spirochetes to perpetuate their natural vertebrate-tick infectious cycle and to persistently infect their hosts requires numerous strategies to evade innate and adaptive immune responses. Concerning the immune evasion mechanisms of *B. valaisiana* only fragmentary information are available. Here we aim to elucidate the serum susceptibility of three *B. valaisiana* strains originated from ticks collected in Switzerland and at two distinct geographical regions in Germany.
Material and Methods: Complement resistance among *B. valaisiana* isolates was assessed by analyzing survival of cells in normal human serum (NHS) using a growth inhibition assay. Fluorescence microscopy was performed to investigate deposition of complement components C3, C6 and the membrane attack complex (MAC) on serum-treated cells. In addition, binding of purified complement regulators C4Bp (classical pathway), Factor H and Factor H like protein 1 (FHL1) (alternative pathway) as well as CFHR1 (Factor H-related protein 1) (terminal pathway) and their properties to inactivate complement components were analysed by Western blotting.
Results: Serum bactericidal assays revealed that growth of strains VS116 and Bv9 was inhibited in the presence of 50% NHS while growth of strain ZWU3-Ny3 remained unaffected. In addition, strains VS116 and Bv9 showed strong deposition of complement components C3, C6, and MAC on their cell surface. The destructive activity of complement leads to substantial morphological changes as exemplified by the generation of numerous blebs and cell ghosts. In contrast, a lower percentage of complement-positive cells and no aberrations in cell morphology have been detected when employing strain ZWU3-Ny3. While further investigating the protective role of bound complement regulators in mediating complement resistance as previously described for serum resistant borrelial genospecies including *B. burgdorferi*, *B. afzelii* and *B. spielmanii*, unexpectedly, none of the strains analyzed were able to bind the complement regulators C4Bp, Factor H, FHL1 or CFHR1. Degradation of C3b and C4b by an intrinsic *Borrelia*-mediated proteolytic activity as described for other human pathogens has also been excluded.
Discussion: Serum-resistant borrelial genospecies, in general, bind human complement regulators that allow spirochetes to control complement activation directly on their surface. Here we present data on the intra-genospecies heterogeneity on serum resistance of *B. valaisiana*. In contrast to other serum resistant isolates, *B. valaisiana* ZWU3-Ny3 did not bind complement regulators to their surface to inactivate complement. In addition, no intrinsic complement regulatory activity could be detected. Thus, elucidation of the molecular mechanism utilized by this particular strain to resist complement-mediated killing requires further investigations.

MPP11
Activity of the alternative sigma factor σ^H in *Staphylococcus aureus*

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S. aureus is an important human pathogen that expresses a plethora of virulence factors, which are controlled by a complex regulatory network. Alternative sigma factors have been shown in many bacteria to be involved in virulence and the ability to survive under extreme or changing conditions. *S. aureus* produces three alternative sigma factors, σ^B, σ^H, and σ^S. While σ^B of *S. aureus* has been studied extensively, only little is known about the latter two sigma subunits. σ^S belongs to the group of ECF-type sigma factors, and was recently shown to modulate the stress response and virulence of this pathogen. σ^H, on the other hand, shares homology to σ^H of *Bacillus subtilis* and ComX of *Streptococcus pneumoniae*, which are both involved in regulating genetic competence and stress response. In *S. aureus*, this alternative sigma factor seems to control a very small regulon, which includes the operons *SA1374-SA1371, SA1418-SA1416*, and the prophage-encoded integrase gene *int* only. However, nothing is known to date, whether and under which condition σ^H is active in this pathogen.
To address this question we analyzed the expression of *sigH* and the σ^H-depended genes by different approaches. We first constructed a *gfp/lux* based reporter system under the control of the *SA1418* promoter (pTF9), and a vector harboring *sigH* under the control of a cadmium-inducible promoter (pTF8). Co-transforming these plasmids into *S. aureus* strain RN4220 allowed confirming the σ^H-dependence of the *SA1418* promoter. Monitoring the luciferase activity of a *S. aureus* Newman strain harboring pTF9 during growth in TSB, however, failed to identify significant levels of luciferase activity or Gfp formation at nearly any growth stage analyzed, confirming previous findings indicating that σ^H is essentially not active during growth under in vitro conditions. Only at the early *lag* phase, immediately after the inoculation of the fresh medium with cells of an overnight culture, we detected a short but clear increase in luciferase activity. Real-time PCR experiments allowed confirming this finding by showing clear increases in *sigH, SA1374* and *SA1418* transcription during the early *lag* phase in strain Newman, while no such effect was visible in a Newman *sigH* mutant.
Interestingly, neither the overexpression of *sigH* in strain Newman did allow to enhance the transcription of the *int* gene, nor did the inoculation of Newman and its *sigH*-derivative into fresh medium reveal clear differences in *int* transcription between the wild-type and the mutant, strongly suggesting that the *int* gene is not a direct target of σ^H.

MPP12
Staphylococcus epidermidis and *Staphylococcus aureus* Quorum sensing system *agr* regulates Formyl Peptide receptor 2 Ligand secretion and thereby the activation of the innate immune system

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Highly pathogenic *Staphylococcus aureus* and the opportunistic pathogen S*taphylococcus epidermidis* secrete phenol-soluble modulin (PSM) peptides. Virulence of *S. epidermidis* depends mostly on the PSM peptides, which induce chemotaxis in neutrophils and cytokine induction in peripheral blood mononuclear cells (PBMCs). The regulation of PSM

secretion and production occurs through the *agr* regulator. While chemotaxis and cytokine induction are crucial for infections, the molecular basis of the recognition by leucocytes has remained unknown. Here we demonstrate that the human formyl peptide receptor 2 (FPR2) senses *S. epidermidis* PSMs at nanomolar concentrations. Specific blocking of FPR2 or the down regulation of the PSM genes in the *agr* mutant led to severely diminished capacities of neutrophils to detect *S. epidermidis* PSMs. Moreover, Staphylococci developed the quorum sensing system *agr* to control their detection via human FPR2. Thus, the innate immune system uses a global mechanism to detect bacterial pathogens. Targeting FPR2 may help to manage severe infections induced by different pathogens.

MPP13

Endothelial cytokine-expression in response to *Streptococcus gallolyticus* subsp. *gallolyticus*

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Introduction: Infective endocarditis (IE) is an infection of endocardium with a broad spectrum of causative microorganisms. *Streptococcus gallolyticus* subsp. *gallolyticus* (SGG) was identified in up to 20% of streptococcal-caused IE cases. In this study, the influences of different SGG strains on endothelial cell damage was analyzed on the basis of the expression profile of interleukin-1β (IL-1β), IL-6, IL-8, macrophage inflammatory protein-1β (MIP-1β) and tumor necrosis factor-α (TNF-α).
Material and Methods: The endothelial cell line EA.hy926 was inoculated with 6 isolates of SGG (10^5-10^6 CFU/ml). Cells were lysed after 2 h and 6 h of incubation, followed by RNA-isolation and cDNA-synthesis. Gene expression of inflammatory cytokines (IL-1β, IL-6, IL-8, MIP-1β) was quantified using real-time PCR. TNF-α gene expression of EA.hy926 cells was incapable of measurement by quantitative real-time PCR. Furthermore, 27 different SGG-isolates (10^5-10^6 CFU/ml) were compared concerning the potential for stimulation the TNF-α-synthesis in whole blood during 4 h of incubation. TNF-α measurement was conducted using the IMMULITE 1000 Immunoassay System.
Results: Overall, different SGG isolates showed significant differences in the stimulation-intensity for IL-6, IL-8 and MIP-1β (e.g. IL-6 relative gene expression after 6 h: isolate 10672: 1.43 vs. isolate 05950: 2.05). The relative gene expression of IL-8 was significant increased even after 2 h inoculation SGG with endothelial cells (relative gene expression: 2.31 up to 3.14). After 6 h incubation, the gene expression level was still increased (relative gene expression: 1.27 up to 2.16). In contrast, the gene expression of IL-6 was significant increased after 6 h, but not markedly after 2 h incubation. MIP-1β gene expression was stimulated only marginal after 2 h inoculation of endothelial cells with SGG. After 6 h inoculation, two isolates caused an increased gene expression of MIP-1β. SGG caused no increase of IL-1β gene expression in endothelial cells during incubation period. The comparison of the potential of different strains to stimulate TNF-α-synthesis in whole blood revealed significant differences (e.g. isolate ATCC9809 c (TNF-α) = 2800 pg/ml vs. isolate AC1181 c (TNF-α) = 640 pg/ml).
Conclusions: SGG isolates revealed differences in the potential to stimulate IL-6, IL-8, MIP-1β and TNF-α gene expression in both, endothelial cells and whole blood. These divergences could affect the dimension and progress of endothelial cell damage in IE. Further characterization of the inflammatory process will focus on orchestration of inflammatory parameters during interaction of SGG, endothelial cells and monocytes.

MPP14

Surface-exposed phosphoglycerate kinase of *Streptococcus pneumoniae* binds to human angiostatin

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Introduction: *Streptococcus pneumoniae* is colonizing the nasopharyngeal cavity of humans and is the causative agent of otitis media, meningitis, pneumonia and septicaemia. Pneumococci bind human plasminogen on the bacterial cell surface via surface exposed glycolytic enzymes (Bergmann *et al.,* 2001). The phosphoglycerate kinase (PGK) is present in the bacterial cytoplasm and converting 1,3-bisphoshoglycerate into 3-phosphoglycerate within glycolytic pathway thereby generating ATP. Plasminogen is a fibrinolytic proenzyme of the serine protease plasmin, which is a key enzyme of the fibrinolytic pathway (Miyashita *et al.,* 1988). The first four kringle domains of plasminogen act as inhibitory molecule for angiogenesis and are termed angiostatin.
Material and Methods:
The *pgk* gene was cloned using pQE30expression system (Qiagen) and the expressed recombinant His-tagged protein was purified by affinitychromatography. Plasminogen binding was analysed using Western blot overlay, dot spot analyses and spot membranes representing the amino acid sequence of PGK by overlapping peptides of 16 amino acids each immobilized on a membrane. Localization of PGK on the pneumococcal surface was performed by immune electron microscopy. Binding kinetic parameters of plasminogen-PGK interaction was determined by surface plasmon resonance.
Results: In plasminogen binding analyses, PGK of *Streptococcus pneumoniae* was identified as plasminogen-binding protein. Electron microscopic studies using PGK-specific antibodies visualized its localization on the pneumococcal surface of both encapsulated and non capsulated strains. Protein overlay analyses demonstrated that PGK binds to angiostatin. The dissociation constants for PGK-plasminogen interaction revealed a k_D within the nanomolar range. Peptide spot array indicated the presence of two plasminogen/angiostatin binding sites located within the N-terminal PGK fragment and detected lysines[14/15] and arginines[53] as crucial for plasminogen interactions. Protein structure of the pneumococcal PGK was determined by crystal structure analysis performed at the CSIC in Madrid and confirmed localization of these amino acids on the PGK molecule surface.
Discussion: Pneumococcal glycolytic enzymes provide a group of multifunctional proteins displayed on the bacterial surface and binding to plasminogen. Interestingly, the amino acid sequences, which are crucial for plasminogen binding seem to vary with respect to localization within the glycolytic proteins and with respect to charge properties required for specific interaction. Since PGK binds preferentially to the N-terminal kringle domains of plasminogen, the interaction between pneumococcal PGK and angiostatin displays a new mode of interaction.

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MPP15

Mechanisms of pneumococcal endocytosis into host cells

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Streptococcus pneumoniae (pneumococci) colonize the human mucosal respiratory epithelium and are able to cause severe local infections such as otitis media and life-threatening diseases including community-acquired pneumonia and meningitis. Pneumococci have evolved sophisticated strategies to encounter its host and produce a wide variety of virulence factors contributing to the pathogenicity. The pneumococcal surface protein C (PspC) is a major virulence factor and adhesin interacting with the complement regulator Factor H and the polymeric immunoglobulin receptor (pIgR) of respiratory mucosal epithelial cells. The human specific PspC-pIgR interaction mediates pneumococcal adherence to and invasion of host cells. However, the mechanism of pneumococcal endocytosis via the PspC-hpIgR interaction is not known. Here, we demonstrated that PspC-mediated endocytosis requires clathrin coated vesicle formation, which is regulated by dynamin, and visualized the subsequent endosomal trafficking of pneumococci upon ingestions by host cells. Pretreatment of epithelial cells with dynasore, which is a potent inhibitor of dynamin, reduced pneumococcal uptake up to 60% compared to untreated cells. Similar, the knock-down of dynamin by shRNA also impaired pneumococcal internalization suggesting that pneumococcal uptake requires functional dynamin. Pneumococcal internalization was also reduced significantly by inhibiting clathrin with chlorpromazine and clathrin shRNA, indicative of the important role of the clathrin machinery for pneumococcal internalization by pIgR expressing host epithelial cells. Furthermore, confocal laser scanning microscopy was performed to elucidate the endocytic pathway of pneumococci. Thirty minutes post infection pneumococci were co-localized with clathrin and 1 hour post infection pneumococcal trafficking was visualized to the various endosomal compartments including early, recycling and late endosomes by using GFP-labeled Rab proteins. In conclusion, the results suggest that pneumococci are internalized via the clathrin-mediated endocytic pathway and then sorted into different endosomal compartments of the epithelial host cells. However, the intracellular fate after reaching the phago-lysosomes is still a matter of debate.

MPP16

Sustained eIF2α-phosphorylation antagonizes cell autonomous defense against S. aureus α-toxin

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Previously we have shown that cells mount an autonomous defense against pore forming toxins, which allows them to survive membrane perforation. For *S. aureus* α-toxin we have demonstrated that clearance of pore complexes from the plasma membrane of HaCaT cells is but one requirement for cellular survival (1). Because α-toxin caused a state of energy crisis, starvation, and translational arrest, and because inhibition of PI3K prevented replenishment of cellular ATP, we further proposed that autophagy serves to maintain metabolic homeostasis in target cells (2). In keeping with this concept, autophagosome formation in α-toxin-treated cells depended on eIF2α-phosphorylation, like in starved cells. Because Huh7 cells fail to recover from attack by α-toxin, we investigated eIF2α-phosphorylation in these cells. Interestingly, eIF2α-phosphorylation was triggered by α-toxin with similar kinetics as in HaCaT cells, but the response was markedly sustained in Huh7 cells. Moreover, transmission electron microscopy analysis of toxin-treated Huh7 cells revealed stronger vesiculation in Huh7 cells. When eIF2α-dephosphorylation in toxin-treated HaCaT cells was pharmacologically inhibited, cells failed to recover from toxin attack. Since this effect was not due to a block of translation, sustained phosphorylation of eIF2α seems to promote killing of α-toxin-treated cells by an unusual mechanism.

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MPP17

Infection of human endothelial progenitor cells with *Bartonella henselae* induces vessel growth in vitro

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Endothelial progenitor cells (EPCs) are a heterogeneous mixture of adult stem cells that play an essential role in revascularization after vascular damage and have shown promising results in regenerative therapy for ischemic injury. In our work we investigated an unconventional method of improving the angiogenic potential of EPCs through bacterial infection. *Bartonella spp.* are facultative intracellular pathogens and the only known bacteria to induce angiogenesis in humans. Here we describe the course of a bacterial infection of EPCs with the vasculotropic bacterium *B. henselae*. Our data demonstrate that EPCs are highly susceptible to *B. henselae* and that the infection does not disturb their differentiation towards endothelial cells. Upon infection EPCs show a strong activation of hypoxia inducible factor-1 (HIF-1), the key transcription factor in angiogenesis. This is followed by the signature HIF-1-dependent pro-angiogenic cell response including production of cytokines such as vascular endothelial growth factor (VEGF) and adrenomedullin (ADM). Furthermore, *B. henselae* prevents apoptosis of EPCs and induces cell migration along a stromal cell-derived factor (SDF)-1 gradient, both essential functional components of the angiogenic response. Finally, when culture plates are coated with a basement membrane which simulates the extra-cellular matrix *(Matrigel™)*, infected EPCs proliferate and assemble into complex, lumen containing vessel-like structures *in vitro*. Experiments examining these structures reveal a strong up regulation of endothelial marker genes vascular endothelial growth factor receptor-2 (VEGF-2), endothelial nitric oxide synthase-3 (eNOS) and vascular endothelial cadherin (VE-Cadherin) indicating that the vessel-like structures are indeed be made up of endothelial cells. Cumulatively, our data demonstrate that infection with *B. henselae* can improve the angiogenic capacity of EPCs and even induce vessel-like growth *in vitro*.

MPP18

The role of β1-integrins for Yop translocation in *Yersinia enterocolitica*

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Yersinia enterocolitica injects effector proteins (Yops) into host cells with a Type Three Secretion System (TTSS). Yops target mainly immune cells and affect several cell functions what finally leads to immune evasion.
Former studies using cultured cells showed that an interaction of *Yersinia* via the adhesion factors YadA and Invasin with β1-integrins on the host cell site is a prerequisite for Yop translocation [1].
In this study we want to show how YadA, Invasin and β1-integrins contribute to Yop translocation in vitro. For this purpose a β-lactamase reporter system was used to detect and quantify Yop injection in infected cells. We will present evidence that (i) YadA and Invasin show striking differences how they contribute to Yop translocation. (ii) For Invasin mediated Yop translocation an intact β1-integrin cytoplasmic domain and its connection to the actin cytoskeleton seems to be important in terms of the integrity of the whole receptor and its ability to perform high affinity ligand binding. (iii) In contrast YadA does not require the β1-integrin cytoplasmic domain but might require additional factors beside integrins for effective Yop injection. (iiii) Depending on cell type YadA mediated Yop injection even occurs completely independent on the expression of β1-integrins.

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MPP19

Surface-exposed thioredoxin-like proteins of *Streptococcus pneumoniae* play a role in defense against oxidative stress

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Streptococcus pneumoniae (pneumococci) are a Gram-positive bacteria highly adapted to the human respiratory tract. *S. pneumoniae*, a microaerophilic pathogen and strictly fermentative, requires rapid and efficient response mechanisms against oxidative stress for the ability to grow and survive in the host and under reactive oxidant conditions like other facultative anaerobes. The protection from cellular macromolecules from reactive oxygen species (ROS) produced endogenously during metabolism of dioxygen (O_2) in the aerobic growth and exogenously by polymorphonuclear leukocytes (PMNs) or macrophages of host immune responses is essential for pneumococcal survival. Beside other protection mechanisms, thioredoxin family proteins play an important role in oxidative stress tolerance. This group of small ubiquitous proteins has a number of different important cellular functions in all living organisms. Thioredoxins possess a highly conserved secondary structure with a CXXC active site motif and a common 3-D architecture thioredoxin motif. Two putative surface-exposed lipoprotein genes were predicted in the pneumococcal genomes belonging to the family of the thioredoxin-like proteins. These proteins are responsible of keeping the cellular reducing environment accepting electrons from NADPH and transferring them to other acceptors. To investigate their role during pneumococcal growth, oxidative stress, phagocytosis and intracellular survival, the genes were mutated by allelic replacement in pneumococci with different genetic backgrounds. In conclusion, the data will show the individual impact of the thioredoxin-like proteins and their additive effect on resistance of pneumococci against oxidative stress, which is essential for invasive pneumococcal diseases.

MPP20

Pneumococcal lipoproteins and their impact on bacterial physiology and virulence

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The cell surface proteins of *Streptococcus pneumoniae* (pneumococci) play a key role during the infection process and are therefore considered to be important virulence factors of the pathogen. Surface proteins such as PspA and PspC are known to mediate bacterial adhesion to host cells, contribute to colonization and invasion and are involved in immune evasion. Different clusters of proteins decorate the cell surface of pneumococci. There are LPxTG cell wall-anchored proteins, choline-binding proteins such as PspC

and PspA, and lipoproteins. The surface-exposed lipoproteins of Gram-positive bacteria fulfill important functions in cell signaling, protein export, substrate transport and protein folding. Due to the pleiotropic effect upon inactivation of the lipoprotein components of ABC transporters they were initially identified as adhesins such as PsaA or virulence factors. A direct contribution to virulence has yet only been shown for the lipoprotein SlrA, which belongs to the family of peptidyl-prolyl cis/trans isomerases, and PpmA. The role of other lipoproteins for pneumococcal colonization, virulence and transmission has not been systematically investigated although functional genomic analysis points towards their implication in virulence. Bioinformatic analysis of pneumococcal genomes revealed the presence of approximately 40 genes encoding lipoproteins. Hypothetical lipoproteins and lipoproteins with unknown function were selected to explore their role for pneumococcal fitness and virulence. Strikingly, all candidates were identified by surface proteome analysis. Pneumococcal mutants of the selected ten lipoprotein encoding genes were generated by insertion deletion mutagenesis in pneumococci with different genetic backgrounds. The initial comprehensive analysis included the growth behavior and stability of the mutants, which were unaltered even in chemically-defined medium. To assess the effect of the individual lipoproteins on phagocytosis and intracellular survival, phagocytosis assays were conducted using the murine phagocytes J774. The role of lipoproteins in pneumococcal adherence to and invasion into A549 lung alveolar epithelial cells was elucidated by the antibiotic protection assay and immunofluorescence microscopic studies. The results revealed a various impact on intracellular survival and adherence. A more detailed functional analysis will be performed using *in vivo* infection models and binding experiments with the recombinant proteins.

MPP21

Doubleganger of *Staphylococcus aureus* Serogroup B phages use unique L-type wall teichoic acid as adsorption receptor

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The Gram positive bacterium *Staphylococcus aureus* is a human pathogen causing several diseases including nosocomial infections such as sepsis and endocarditis. One very important factor for colonising, resistance to antimicrobial peptides and antibiotics is a cell wall anchored glycopolymer known as wall teichoic acid (WTA). While most of the *S. aureus* strains produce a ribitolphosphate (RboP) WTA polymer decorated with N-acetylglucusamine or D-alanine (D-ala), *S. aureus* strain L produces an extraordinary kind of WTA consisting of repetitive units of polyglycerolphosphate (GroP) substituted with by D-ala or N-acetylgalatosamine (GalNAc). We renamed this WTA as L-type WTA. So far staphylococcal phages can be classified into 4 major sergroups A, B, D and F. They also differ in morphology e.g. in tail length, flexibility, contractibility, head shape and size. Interestingly electron micrograph images revealed that serogroup L phage Φ187, a phage only infecting strain L is a doubleganger of serogroup B phages. Although serogroup B phages have almost the same morphology they are not able to infect strain L. So it was our intention to analyse if the L-type WTA serves as adsorption receptor of phage Φ187. Therefore we created for the first time an L-type WTA deficient mutant in this strain background via deletion of the *tagO* gene responsible for the initial step of WTA biosynthesis. We confirmed complete loss of WTA in *S. aureus* strain L Δ*tagO* with a phosphate assay. Moreover *S. aureus* strain L Δ*tagO* is resistant to serogroup L and serogroup D phages shown in phage susceptibility and EOP assays. To show that phage resistance is due to the loss of WTA phage susceptibility could be restored by complementation using a vector expressing the *tagO* gene. Furthermore phage adsorption assays were performed to analyse if the observed phage resistance is due to the loss of the receptor or due to unknown post adsorption processes. Our data clearly demonstrate that L-type WTA serves as adsorption receptor of serogroup L phage Φ187. Beside phage adsorption studies we analysed the mutant in further detail. Growth kinetics demonstrates that loss of L-type WTA is dispensable for growth *in vitro*. In contrast L-type WTA is essential for growth at high temperatures. Furthermore the mutant produces lower amounts of biofilm so a possible role of L-type WTA in biofilm formation can not be excluded so far. Taken together and compared to known functions of RboP/GlcNAc WTA type our data suggesting a similar role of this unique L-type WTA.

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MPP22

Small membrane-active peptides (PSMα) are required for phagosomal escape by *Staphylococcus aureus* in non-professional phagocytes

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Background: *Staphylococcus aureus* is one of the most common bacterial pathogens in men. *S. aureus* is able to invade human host cells non-professional phagocytes (e.g. epithelial and endothelial cells). We and others have reported a phagosomal escape of *S. aureus*. However, there has been a controversy regarding the mechanism of phagosomal escape, which is only partially understood.
Methods: Phagosomal integrity in human host cells (293 and HeLa) was monitored by pH determination with FITC-labeled bacteria. Intra-phagosomal pH was determined using FACS analysis 2 h and 6 h after infection. As marker for acidification quenching of FITC fluorescence by low pH was used (Lâm *et al.*, 2010). Additionally, a microscopic reporter recruitment technique was employed to demonstrate phagosomal escape (Giese *et al.*, 2011). Isogenic mutants deficient in specific toxins were compared to wild type strains.
Results: In both cell lines a loss of the intra-phagosomal pH 6 h after infection demonstrated an escape of *S. aureus* strains 6850 (strong β-toxin producer), as well as the β-toxin-negative strains LAC (USA300) and MW2 (USA400). In all three strains, the PSMα-deficient mutants were unable to reduce the acidification signal. Complementation partially restored the wild type phenotype. By contrast, other mutants tested (PSMβ, δ-toxin, PVL) behaved identical to the parental wild type strains LAC and MW2. A β-toxin-deficient mutant in strain 6850 did not differ from wild type, as determined by pH-monitoring and YFP-Fc co-localization. Time course analysis using rifampicin inhibition demonstrated a susceptible window of roughly 3-4 hrs for strains LAC and 6850. Furthermore, we have identified two additional strains with a moderate phagosomal escape phenotype.
Discussion: Our data suggest that PSMα, but not PSMβ, δ-toxin, or PVL, are required for phagosomal escape of the staphylococcal strains 6850, LAC, and MW2 in 293 and HeLa cells. Surprisingly, β-toxin appears not to be required in this model. We were able to identify several escape-positive strains with different, distantly genetic backgrounds using a common mechanism.

MPP23

Role of CRASP-4 in complement resistance of *Borrelia burgdorferi*

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Introduction: *Borrelia burgdorferi* employs a number of strategies to escape the host innate immune response. One important mechanism is the binding of complement regulators to resist complement-mediated lysis. Interaction with complement regulators factor H (CFH), factor H-like protein 1 (FHL1) or other factor-H related proteins (CFHRs) is facilitated through five distinct borrelial outer surface proteins, collectively termed CRASPs or complement-regulator-acquiring surface proteins. Here we investigate the role of the CRASP-4 protein in mediating complement resistance of *Borrelia* and its interactions with human complement regulators.
Methods: To determine whether CRASP-4 interacts with different complement-regulators, recombinant CRASP-4 was immobilized on magnetic particles. After incubation with human serum, bound proteins were eluted, separated by SDS-PAGE and identified by Western blotting. In addition, binding of the complement regulators to CRASP-4 was also assessed using ELISA or Far Western blot.
The role of CRASP-4 in complement resistance was further elucidated by transformation of a CRASP lacking strain with a borrelial shuttle vector that harbors the entire CRASP-4 encoding *erpC* gene. Acquisition of different complement regulators by live transformed cells was assessed by serum adsorption assays. In addition, susceptibility of CRASP-4 producing spirochetes in the presence of native human serum was tested using a

growth inhibition assay and the deposition of activated complement components was analyzed by immunofluorescence microscopy.
Results: Binding of complement regulators CFHR1, CFHR2, and CFHR5 to immobilized CRASP-4 could be detected by Western blotting and ELISA using specific antibodies. Unexpectedly, CFH was not present in the eluate fraction as assessed by SDS-PAGE and Western blotting. However, binding of serum-purified CFH to recombinant CRASP-4 was demonstrated by ELISA and Far Western blot.
Serum adsorption assays revealed that CRASP-4 producing borrelial cells were capable of binding CFHR1 and CFHR2 and to some extent CFHR5 but not CFH. Functional analysis also shown that upon incubation with active human serum, CRASP-4 expressing spirochetes were killed by complement and large amounts of complement components C3, C6 and the membrane attack complex were deposited on their surface.
Discussion: Taken together, we identified complement proteins CFHR2 and CFHR5 as novel ligands for the infection-associated CRASP-4 protein of *B. burgdorferi*. Seemingly, binding of different CFHRs is not sufficient to protect cells from complement-mediated killing. Thus, our data strongly suggest that CRASP-4 plays a subordinate but supporting role in complement resistance of *B. burgdorferi.*

MPP24

Complete genome sequence of a *streptococcus dysgalactiae subsp. equisimilis* possessing Lancefield's Group A antigen and its comparative genomics

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Introduction: Information on the genomes of *S. dysgalactiae* subsp. *equisimilis* (SDSE) is highly desirable since this pathogen causes a spectrum of infections similar to those caused by *S. pyogenes*. Two genomes of SDSE (serological group G) were recently published. Here we report on the complete genome sequence of SDSE strain AC-2713 possessing a group A cell wall carbohydrate (Lancefield) antigen and compare it to other streptococcal genomes.
Material and methods: SDSE strain AC-2713 originated from a blood culture of an adult (without STSS). Pyrosequencing (shot-gun and an 8 kb paired-end library, combined 59-fold coverage - Roche/454 method) was applied. Gap closure was achieved by Sanger sequencing of PCR products. Assembly and finishing was obtained by applying different bioinformatics tools. Annotation was performed by combining results of the "GenDB", "RAST", and "BASys" servers and manual curation.
Results: The AC-2713 genome consists of one circular chromosome of 2,179,445 bp; 2,181 predicted protein coding sequences (CDSs) were identified; 15 rRNAs (5 rRNA loci) and 57 tRNAs were found. The G+C content is 39.5%. Three phage-related chromosomal islands (PRCIs) - but no complete prophages - were identified that did not contain any of the known phage-encoded virulence factors of *S. pyogenes*. Two "clustered regularly interspaced short palindromic repeats" (CRISPRs) were identified having up to 19 spacers, some with homologies to *S. pyogenes* phages; this may explain the lack of complete phages in the AC-2713 genome and the low degree of rearrangements in SDSE genomes, in contrast to *S. pyogenes*. Numerous orthologs of (non-phage-associated) virulence factor genes of *S. pyogenes* including determinants for an M-protein (emm stG485.0), pili, streptokinase, hemolysins (SLO, SLS), NADase, extracellular matrix-protein binding surface proteins, etc. were detected. *Spegg*4 was found as the only superantigen gene. Some unique features are found in the AC-2713 genome, including a salivaricin A gene cluster. The putative genes for group A cell wall polysaccharide synthesis were identified. Comparative analyses revealed a high degree of homology and colinearity with the published *S. dysgalactiae* genomes and closer relationships to *S. pyogenes* (based on available genomes) than to other beta-hemolytic streptococci including groups C and G streptococci of animal origin.
Conclusion/Discussion: AC-2713, an SDSE possessing Lancefield's group A antigen, is highly homologous to other *S. dysgalactiae* and more closely related to *S. pyogenes* than to other streptococcal species, including groups C and G streptococci of animal origin.

MPP25

Surface proteins of Gram-positive bacteria contribute to the interaction with human Thrombospondin-1

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Adherence of bacteria to host cells is a multifactorial process and proceeds bacterial infections. The versatile interplay between pathogenic bacteria and its host depend on numerous interactions of bacterial surface structures and host matrix proteins. The matricellular glycoprotein Thrombospondin-1 (TSP-1) is mainly secreted by thrombocytes but also by many other human cell types. TSP-1 is a multifunctional, multidomain 420 kDa homotrimer with a wide range of predicted functions in adherence and migration, cell morphology, proliferation and apoptosis as well as in interaction with extracellular proteases. Furthermore, TSP-1 is part of the extracellular matrix and shows binding to different matrix proteins, including fibronectin, fibrinogen, heparin and furthermore to the surface receptors CD36, CD47 and integrin $\alpha_5\beta_1$ (CD49e/CD29). A recent study revealed a new role of TSP-1 for the interplay of different Gram-positive pathogens with host cells. The TSP-1 was shown to act as a molecular bridge between host cells and Gram-positive bacteria, which facilitated adherence to and invasion into different human epithelial and endothelial cells. Nevertheless, the receptor on the bacterial side as well as on the host side is still unknown. In this work, TSP-1 was isolated from human buffy coats and purified via fast protein liquid chromatography. The bioactivity of purified TSP-1 was verified in binding assays with human epithelial cells (A549) and different Gram-positive pathogenic bacteria using flow cytometry. Surface plasmon resonance (SPR) studies with TSP-1 immobilized on CM5 biosensors and ligand overlay assays revealed a potential proteinous binding partner on the bacterial side in *Staphylococcus aureus* and *Streptococcus pneumoniae*. To identify the protein of interest, 2D-gelelectrophoresis of surface protein fractions from *S. aureus* was performed and peptides were analyzed by mass spectrometry. The results indicated binding candidates whose specific binding is under investigation. The interaction of TSP-1 with different Gram-positive bacteria via surface exposed proteins is most likely important during colonization and bacterial dissemination and could be beneficial in the development of severe infections like infective endocarditis or pneumonia.

MPP26

Discovery of novel lantibiotic producers by genomic data mining

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The discovery of antibiotics in 1929 was one of the most important milestones in medicine and in the fight against infectious diseases. Today, the rapid evolution and the emergence of multidrug-resistant bacteria necessitate a further search for new antibiotic substances and sources. Lantibiotics (lanthionine containing antibiotics) are ribosomally produced bacterial peptide antibiotics that show interesting antibacterial activities even in the nanomolar range against (multiresistant) human pathogens. The characteristic thioether amino acids lanthionine and methyllanthionine are introduced by extensive enzyme-mediated posttranslational modifications. These rare amino acids form intramolecular rings that are essential for the three-dimensional structure of lantibiotics, their enhanced stability against proteases and oxidation and convey the antimicrobial activity. These features make lantibiotics interesting candidates or lead structures for novel antimicrobial applications in medical and food industry (Ross and Vanderas, 2011).
Blast searches employing the characteristic lantibiotic modification enzymes (LanM,B,C) in the NCBI database showed that ORFs coding for proteins involved in lantibiotic production are widespread in bacteria of different phyla. Based on these genomic data, we identified putative lantibiotic gene clusters also in bacterial phyla for which production of lantibiotics had never been described before, like a cyanobacterial *Nostoc punctiforme* strain. The focus of our project is the homologous and/or heterologous expression of those, so far uncharacterized, lantibiotics. In this context, we were able to identify and characterize the novel two-peptide lantibiotic lichenicidin that is produced by *Bacillus licheniformis* DSM13. Additionally, we revealed the presence of a partial lantibiotic gene cluster coding for the proteins involved in the producer self-protection against the well-known lantibiotic mersacidin in *Bacillus amyloliquefaciens* FZB42. Transfer of the biosynthetic part of the mersacidin gene cluster into *B. amyloliquefaciens* FZB42 resulted in the successful expression of fully

modified and active mersacidin in this strain. Other putative lantibiotic producers, including an *Anaerococcus thermophilum* strain, were identified in this project and are still in the focus of the ongoing work.

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MPP27

Purification and activity testing of the full-length histidine-kinases YycG (WalK/VicK) and VraS of *Staphylococcus aureus*

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Gram-positive bacteria are characterized by a massive cell wall, formed by multiple layers of peptidoglycan. The structural integrity of the cell wall directly affects the viability of the bacterium itself and biosynthesis of peptidoglycan has to be tightly controlled. This also emphasizes the importance of cell wall targeting antibiotics in the clinical treatment of bacterial infections.
Despite that, much less is known in detail about how bacteria control the interplay between degradation and new synthesis of peptidoglycan to coordinate cell division and cell growth. Two-component regulatory systems (TCSs), composed of a sensor histidine-kinase and a cognate response regulator protein, play a predominant role in the way how bacteria respond and adapt to environmental and stress conditions. In *S. aureus* the essential YycFG (WalRK/VicRK) TCS plays a major role in the homeostasis of cell wall by controlling the expression of autolysins [1]. In context with antiinfective treatment the VraSR TCS is involved in resistance to vancomycin and β-lactam antibiotics [3, 4, 5], and moreover confers mechanisms to sense inhibition / damage of the bacterial cell wall [6].
In general, research on TC systems has been restricted to the use of truncated proteins, missing parts of the entire kinases. Here, we want to show the *in vitro* phosphorylation activities of the full-length VraS and YycG kinases, of mutated versions of YycG and phosphotransfer to their cognate response regulator proteins VraR and YycF.

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MPP28

Lipopolysaccharides of Gram-negative and teichoic acids and proteins of Gram-positive bacteria contribute to binding of platelet factor 4 to bacteria facilitating the creation of heparin-induced thrombocytopenia-eliciting antibodies.

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Gram-positive and Gram-negative bacteria are apart from being life-threatening infectious pathogens also the supposed etiologic agents for the development of heparin-induced thrombocytopenia (HIT). HIT is an IgG-antibody-mediated adverse drug reaction against complexes of the positively-charged chemokine platelet factor 4 (PF4) and the most frequently used anionic anticoagulant in clinical medicine, heparin. Interestingly, even heparin-naïve patients can generate IgG-antibodies specific for PF4/heparin complexes as soon as 4 days after exposure to heparin, presumably because these patients have encountered complexes similar to PF4/heparin before such as PF4 bound to anionic bacterial surfaces during infections. Likely candidates for negatively charged molecules on bacterial surfaces are lipopolysaccharides (LPS) and teichoic acids (TA) as well as proteins. In this study, we were able to pinpoint the bacterial binding partners of PF4 by showing that PF4 interacts with LPS of the Gram-negative model organisms *Escherichia coli* and *Salmonella typhimurium* and with teichoic acids of the Gram-positive *Staphylococcus aureus*. This was shown in ELISA and competitive inhibition binding experiments experiments. Surprisingly, *E. coli* and *S. typhimurium* mutants with successively shortened LPS-backbone displayed increasing PF4 binding capacity. The highest binding was detected in *E. coli* Δ*waaC* and *E. coli* Δ*waaA*. These mutants both lack the O-antigens and the core LPS. However, the *E. coli* mutant Δ*waaA* is additionally deficient in the 3-deoxy-D-manno-octulosonic acids (KDO) suggesting that Lipid A is the structure contributing to PF4-binding. Similarly, a *S. aureus* mutant with severely truncated wall teichoic acids (SA113 Δ*tagO*) showed increased PF4 recruitment compared to the wild-type strain. Additionally, pre-treatment of bacteria with pronase E and trypsin impaired PF4 binding to *S. aureus* but not to *E. coli*, indicating proteins as another major binding partner of PF4 on Gram-positive bacteria. The anti-PF4/heparin antibodies generated upon binding of PF4 to bacteria enhanced phagocytosis of PF4-coated bacteria. Thus, by acting as a bacteria-opsonizing protein, PF4 is able to induce a humoral immune response specific for a wide variety of bacterial species and might therefore be an ancient host defense mechanism at the interface of innate and adaptive immunity.

MPP29

Evidence for a role of hypoxia inducible factor 1 (HIF-1) regulated lysyl oxidase in *Staphylococcus aureus* abscess-formation

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HIF-1 is the key transcriptional factor involved in the adaption process of mammals to hypoxia and plays a crucial role in, e.g., cancer angiogenesis. Recent evidence suggests a leading role of HIF-1 in inflammatory and infectious diseases. We demonstrated previously that human pathogenic *S. aureus* leads to a HIF-1 activation *in vitro*, *ex vivo* and *in vivo* and that this HIF-1 activation is detrimental in a *S. aureus* peritonitis model. To analyze the role of HIF-1 in *S. aureus* infections in more detail, we investigated the HIF-1 dependent host cell response upon a *S. aureus* infection. For this purpose, control and HIF-1 $^{-/-}$ HepG2 cells were infected with *S. aureus* and gene expression was analyzed four hours later using Affymetrix WT 1.0 gene arrays. Data revealed that expression of in total 253 genes was influenced of which 22 turned out to be regulated via HIF-1. These 22 genes include genes involved in cell metabolism and genes known to affect tumour growth. One of the upregulated genes was lysyl oxidase, (*lox*), a copper-dependent amine oxidase, which catalyzes the cross linking of collagen and elastin molecules in the extracellular matrix and might be involved in the development of a fibrosis after *S. aureus* infection. Further experiments revealed that *lox* is upregulated *in vitro* and *in vivo* using HepG2 infection models, abscess-harbouring kidneys of intravenously infected NMRI-mice and tissue samples from patients suffering from microbiological proven *S. aureus* infections. The role of lox in abscess formation and, moreover, in chronic infections known to result in fibrous scarring of tissues needs to be further elucidated.

MPP30

Characterizing the anti-apoptotic activity of the *Coxiella burnetii* effector AnkG

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Coxiella burnetii is a Gram-negative, obligate intracellular pathogen that causes Q-fever, a worldwide zoonotic disease. Q-fever is a mild flu-like illness, but can be associated with chronic or even fatal outcomes. *C. burnetii* are typically transmitted to humans by inhalation of infectious material. For intracellular pathogens such as *C. burnetii*, manipulation of host cell pathways is important to ensure a productive infection and may play a role in establishing chronic disease. *C. burnetii* employs a type IV secretion system (T4SS) and several substrates of the T4SS have been identified. One of these substrate proteins, AnkG, inhibits pathogen-induced apoptosis, probably through binding to the host cell protein p32 (gC1qR). The mitochondrial matrix protein p32 seems to be a pro-apoptotic protein, however the mechanism is unknown. Studies with different truncation derivatives showed that the amino terminal $AnkG_{1-69}$ region is sufficient to interact with p32 and to inhibit host cell apoptosis whereas $AnkG_{70-339}$ did not interact with p32 nor inhibit host cell apoptosis. Ectopically expressed AnkG shows a vesicular pattern with some mitochondrial association. The p32 protein seems to be involved in bridging a signaling pathway that extends from the mitochondria to the cell nucleus.
Here, we aim to investigate how the interaction of AnkG with p32 determines the anti-apoptotic activity of AnkG. Therefore, we tested whether the interaction of AnkG with p32 is direct or indirect. Using GST- as well as His-pull-down experiments we demonstrated direct binding. As p32 is a mitochondrial protein we hypothesized that if the anti-apoptotic activity of AnkG is mediated by p32, AnkG only inhibits the intrinsic and

not the extrinsic apoptosis pathway. This hypothesis was confirmed using ectopic expression experiments. Furthermore, using localization studies we showed that AnkG shuttles from the cytosol to the nucleus after apoptosis induction. Interestingly, ectopic expression showed that the N-terminus ($AnkG_{1-69}$) is nuclear, while $AnkG_{70-339}$ is cytosolic. In both cases the localization did not change after apoptosis induction. These data suggest that nuclear localisation as well as binding to p32 is necessary for the anti-apoptotic activity of the *Coxiella burnetii* effector protein AnkG.

MPP31

D-serine-transporter in *Staphylococcus saprophyticus* identified

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Staphylococcus saprophyticus is an important cause of urinary tract infections in young women. The amino acid D-serine occurs in relatively high concentrations in human urine and has a bacteriostatic or toxic effect on many non-uropathogenic bacteria. *S. saprophyticus* harbors the cytoplasmatic enzyme D-serine deaminase (DsdA), which is found in many uropathogens. It is shown that DsdA enables *S. saprophyticus* to cleave D-serine into pyruvate and ammonia. This is probably a factor that enables colonization of the urinary tract. In contrast to *E. coli* the *dsd*A-operon of *S. saprophyticus* does not encode a specific D-serine-transporter, but there are three genes in the genome of *S. saprophyticus* that encode for putative D-serine-transporters. SSP0286, SSP1070 and SSP2171 show a identity of 50 % and a similarity of 72 % to CycA an unspecific D-serine transporter of the uropathogen *E.coli* CFT073. We created knock-out mutants of these genes in order to characterize the D-serine-transport of *S. saprophyticus* 7108. In a defined medium D-serine elongates the lag-Phase of *S. saprophyticus* but not of the transporter mutant SSP1070. We measured D-serine transport in an uptake essay by analyzing the remaining concentrations of D-serine in the supernatant of a bacterial suspension by NMR-spectroscopy. In keeping with the observation of growth in the defined medium, the D-serine uptake of ΔSSP1070 is lower in the NMR assay. The reduction of D-serine uptake has therefore a positive effect on growth in defined medium under this conditions. To take account of this results SSP1070 appears to be the major player in D-serine uptake in *S. saprophyticus* 7108. The knowledge of D-serine transport and metabolism will presumably offer us more insights in to the physiology of virulence of *S. saprophyticus*.

MPP32

Yersinia enterocolitica invasin - topology and biogenesis

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A large group of bacterial surface proteins is represented by the family of autotransporter proteins. Representatives belong to the type V secretion system (1) and are found in almost all Gram-negative bacteria. Autotransporter proteins frequently represent important virulence factors, e.g. the trimeric autotransporter adhesin YadA of the enteropathogenic *Yersinia enterocolitica.* Typically, a autotransporter consist of three functional domains: a N-terminal signal sequence, a C-terminal translocator domain and a passenger domain in between. After synthesis in the cytosol and Sec-mediated transport across the inner membrane into the periplasm, the translocator domain forms a beta-barrel pore in the outer membrane, presumably with the help of the Bam complex (2). Through this pore, the passenger domain is then translocated to the surface of the cell (3).
A second, unrelated family of outer membrane proteins that expose passenger domains on the bacterial outer surface are the intimins and invasins, nonfimbrial adhesins from pathogenic bacteria, which specifically interact with host cell surface receptors and mediate bacterial attachment or invasion. They are integrated into the bacterial outer membrane with the amino-terminal region, while the carboxy-terminal region of the polypeptide is exposed on the bacterial outer membrane. Whereas the surface-localized parts of the protein are functionally well described, the topology and insertion of the N-terminal membrane domain and the translocation process have not been described. To investigate the topology and the mechanism of translocation in more detail, we had a closer look at the amino acid sequence of invasin and intimin using SignalP, PsiBLAST and HHAlign. Herefrom we got a prediction of the signal peptide and twelve different β-strands, which might build up the β-barrel within the outer membrane. Out of these predictions, we developed topology models of the membrane anchor of invasin and intimin. By introduction of HA-Tags defining the orientation of the translocator domain within the outer membrane and extensive immunofluorescence studies, we were able to confirm our models. Moreover, we came to believe that Intimin (Int) and Invasin (Inv), two major pathogenicity factors of *E.coli* and *Yersinia*, are monomeric autotransporters, with the remarkable difference that their domain order is reversed.

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MPP33

The genetic locus of *Streptococcus anginosus* haemolysin production

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Streptococcus anginosus is a commensal of the oral cavity, the gastrointestinal and the female urogenital tract. It has clinical significance in abscess formation and has recently been suggested to play a pathogenic role in patients with cystic fibrosis. An interesting feature of these bacteria is the inconsistent phenotype regarding Lancefield antigens as well as haemolytic activity. While a considerable percentage of *S. anginosus* strains display a prominent ß-haemolytic phenotype, the corresponding genes have not been identified yet. In different streptococcal species the ß-haemolysin is a modified short peptide (SLS) that is related to Class I bacteriocins. It is encoded in the *sag* gene cluster including genes for the corresponding posttranslational modification and transport machinery.
By random chromosomal integration of the pGhost9:IS*S1* transposition vector we generated a plasmid-based mutant library of the haemolytic *Streptococcus anginosus* strain ATCC 12395. This library was screened for mutants showing a loss of the ß-haemolytic phenotype on blood agar plates and non-haemolytic mutants were selected for further investigation. By sequencing the insertion sites of these mutants we identified thus far 10 different mutations sites in a gene cluster of 9 kb harbouring 9 open reading frames (ORFs), with significant similarities to the *sag* (SLS associated gene) gene cluster of *Streptococcus pyogenes* that encodes the haemolysin Streptolysin S (SLS). ORFs corresponding to all of the 9 *sag* genes (*sagA* to *sagI*) could be identified. Similarities of the deduced amino acids of the putative *S. anginosus sag* gene cluster to the Sag-proteins of *S. pyogenes* range from 37 % (sagF) to 81 % (sagD). To further investigate the *S. anginosus* haemolysin, a functional haemolysin assay with culture supernatants and whole bacteria was carried out. Haemolytic activity was only observed with whole cells, but not in the supernatant, indicating that like SLS of *S. pyogenes,* the *S. anginosus* haemolysin is able to lyse erythrocytes only in cell-associated form. In summary we were able to successfully establish a pGhost9:IS*S1* mutant library in *S. anginosus* and identify an SLS-like gene cluster as the genetic basis of *S. anginosus* ß-haemolysin production.

MPP34

A magnetic isolation method to analyze differential *Chlamydia pneumoniae* interacting protein profiles in persistence infection

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Introduction: *Chlamydia pneumoniae* (*Cpn*) is an obligate intracellular bacterial pathogen and causes acute infections of the respiratory tract. Further, *Cpn* can also cause prolonged or chronic infections which may be due to persistent infections in respiratory and non-respiratory tissues and have been implicated in the development of a number of chronic diseases such as asthma, COPD and atherosclerosis. Persistent chlamydiae exhibit characteristic protein expression profiles and are refractory to killing by antibiotics. However, host-pathogen interacting proteins during persistent infection have not been investigated so far. Recently, we developed a novel magnetic isolation technique for *Chlamydiae* using chlamydial lipid biotinylation and magnetic nanobeads labeling. Key characteristics of this technique are gentle mechanical sonication of the plasma membrane, followed by a magnetic separation of labelled targets, which subsequently allows the biochemical detection of compartment-containing proteins. In this study, we applied this method to investigate persistent *Cpn* interacting proteins.
Material and methods: FACS, confocal microscopy and electron microscopy were used to check labelling efficacy of biotinylated lipid and magnetic nanobeads. Fractions containing magnetically labelled *Cpn* were

isolated by using a magnetic chamber. Interferon (IFN)-γ was used to induce persistent infections of *Cpn*. Persistent *Cpn* interacting protein profile was investigated by SDS-PAGE.
Results: Fractions containing magnetically labelled *Cpn* were efficiently separated from non-labeled cytoplasmic fractions by using a magnetic chamber. In early infection (1 h p.i.), not only *Cpn* proteins like heat shock protein (HSP) 60, inclusion membrane protein (Inc) A and macrophage infectivity potentiator (MIP) but also early endosome marker proteins such as Rab5 and Rab4 were significantly detected in the magnetic "chlamydial" fraction compared to mock sample. GAPDH served as a control and was strongly enriched in the non-magnetic "cytoplasmic" fraction. Expression of HSP60, IncA and MIP was reduced in the magnetic "chlamydial" fraction in IFN-γ treated cells in comparison to non-treated cells 24 h p.i. In contrast, 11 protein bands were enhanced detected in the magnetic "chlamydial" fraction of the IFN-γ treated cells by SDS-PAGE that needs further characterization.
Conclusion/Discussion: Our results indicate the suitability of the novel magnetic isolation method to identify pathogen-host interacting proteins in a targeted and untargeted approach.

MPP35

Evaluation of the distribution of mutations in the *bar*A/*uvr*Y two-component system of extraintestinal pathogenic *Escherichia coli*

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Escherichia coli strains are the most prevalent source of urinary tract infections (UTI), one of the most common bacterial infections in humans and a major cause of morbidity. Uropathogenic *E. coli* (UPEC) causing UTI can be divided into pathogenic strains causing symptomatic UTI and non-pathogenic strains causing an asyptomatic bacteriuria (ABU) without provoking a host response. The reason why ABU-associated *E. coli* strains do not evoke symptoms in the host is not completely understood yet. Many ABU *E. coli* isolates seem to lack virulence associated phenotypes, although they carry many typical UPEC virulence genes. It has been shown that such isolates express less virulence factors and that there is an evolution towards commensalism because of virulence attenuation and genome reduction. Alterations in essential virulence genes including single base mutations, deletions or DNA rearrangements might be a general mechanism for long-term colonizing mucosal pathogens to evolve towards commensalism, consistent with a low immune response of the host.
In this study, the importance of the two-component system (TCS) BarA/UvrY for urovirulence of *E. coli* should be analysed. The BarA/UvrY TCS is involved in bacterial adaption and survival in the urinary tract. It plays a significant role in controlling a common regulatory network affecting a multitude of cellular functions, e.g. regulation of carbon metabolism, biofilm formation or motility and adhesion, and thus contributes to urovirulence. In previous studies, genome sequence analysis revealed the frequent occurrence of single nucleotide polymorphisms in the *uvrY* gene upon prolonged colonization of the urinary tract. This may indicate that *uvrY* is (i) a mutation hot spot and (ii) under positive selection during bacterial adaption during long-term growth in the urinary tract. To correlate *uvrY* nucleotide sequence variability with prolonged colonization of the urinary tract, the *uvrY* gene was sequenced in 216 *E. coli* strains representing ABU and acute UTI isolates as well as a control group of commensal variants. The sequence data obtained was compared with DNA sequences of housekeeping genes in these strains. The results will be discussed in the light of genome plasticity and bacterial adaptation strategies to changing environmental conditions.

MPP36

sarA negatively regulates *Staphylococcus epidermidis* biofilm formation by modulating expression of 1 MDa extracellular matrix binding protein and autolysis dependent release of eDNA

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Despite being key for *Staphylococcus epidermidis* pathogenicity in implant-associated infections, large proportions of invasive *Staphylococcus epidermidis* isolates do not form biofilms under *in vitro* conditions. We here tested the hypothesis that this apparent paradox is related to the existence of superimposed regulatory systems suppressing a multi-cellular biofilm life style. Indeed, by transposon mutagenesis of clinical significant but biofilm negative *S. epidermidis* 1585, *sarA*, chief regulator of staphylococcal virulence, was identified as a negative regulator of *S. epidermidis* biofilm formation. Genetic analysis found that inactivation of *sarA* induced expression of giant 1 MDa extracellular matrix binding protein (Embp) promoting intercellular adhesion and biofilm accumulation. Furthermore, related to significant autolytic behaviour, *sarA* mutants extensively released eDNA into the biofilm matrix. Increased autolysis, eDNA release and biofilm formation were directly linked with boosted processing of major autolysin AtlE by metallo protease SepA being over-expressed in *sarA* mutants. Hence, our results provide first evidence that key regulator *sarA*, while promoting polysaccharide intercellular adhesin dependent biofilm formation on one hand, negatively controls *S. epidermidis* biofilm formation by suppressing Embp production and autolysis-related eDNA release on the other. Hence, this report for the first time identifies a regulator enabling *S. epidermidis* to switch between polysaccharide-dependent and independent biofilm formation. Furthermore, the novel function of *sarA* links SepA mediated escape from defensin dermicidine with biofilm related protection from phagocytosis and decreased proinflammatory macrophage responses. Apparently, *sarA* plays a central role as regulator ensuring *S. epidermidis* adaptation to a hostile environment.

MPP37

Pneumococcal surface protein C: a multifunctional pneumococcal virulence factor and vitronectin-binding protein

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Streptococcus pneumoniae has evolved versatile strategies to adhere directly to human host cells or indirectly by interacting with components of the extracellular matrix (ECM), which is a prerequisite for pneumococci to colonize the upper respiratory airways. Pneumococcal attachment to host components is mediated by bacterial surface molecules including cell wall components, choline-binding proteins, LPxTG proteins, lipoproteins and non-classical adhesins such as surface-associated glycolytic enzymes. The major adhesin of pneumococci is PspC, the Pneumococcal surface protein C, which binds to the ectodomain of the human polymeric Ig receptor (hpIgR). In addition, PspC recruits complement factor H. Consequently, PspC is directly involved in pneumococcal adhesion to human host cells via its binding to the SC of hpIgR, and indirectly via the factor H-mediated adhesion mechanism. Recently, we have shown that multimeric host-cell-bound vitronectin (Vn), an adhesive glycoprotein present in plasma and the ECM, is exploited by pneumococci as a molecular bridge facilitating their adherence to and invasion into host cells by inducing proteins of the host signal transduction cascades. Although the interaction of pneumococci with vitronectin and its cellular consequences were demonstrated, the pneumococcal adhesin for vitronectin remains unknown. Here we provide first evidences that the multifunctional PspC protein is capable to interact with human vitronectin. Surface plasmon resonance studies were conducted with vitronectin immobilized on a CM5 biosensor chip and different PspC derivatives as analytes. The sensorgrams demonstrated binding of N-terminal PspC derivatives to vitronectin. In addition, PspC and PspC-like Hic were expressed on the surface of non-pathogenic *Lactococcus lactis*. Similar to pneumococci, the heterologous *L. lactis* but not the control strain interacted with immobilized vitronectin. Remarkably, PspC-deficient pneumococci showed a lower capability to bind vitronectin as analyzed by flow cytometry. Currently, proteomic approaches are being employed to confirm the interaction of PspC with vitronectin. In order to localize the binding site for PspC in the vitronectin molecule different Vn derivatives were cloned into the pET28-TEV His_6-tag expression vector. The expressed and purified recombinant Vn fragments will be applied in biochemical and immunological assays. In conclusion, PspC possesses vitronectin-binding activity, however, its role in pneumococcal pathogenesis via vitronectin has to be assessed in more detail.

MPP38

Identification of protein-protein interactions of *Salmonella* sv. Typhimurium SPI-4 components using the HBH-tagging approach

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Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is a facultative intracellular pathogen able to cause acute gastroenteritis and diarrhea in humans and a typhoid fever-like disease in mice. Many genes encoding virulence factors of *S.* Typhimurium cluster together on *Salmonella* pathogenicity islands (SPI). *Salmonella* pathogenicity island 4 (SPI-4) encodes a type I secretion system (SiiCDF) which secretes SiiE, a giant non-fimbrial adhesin shown to mediate intimate contact of *Salmonella* to the apical membrane of polarized epithelial cells. SiiA and SiiB are two additional SPI-4-encoded inner membrane-associated proteins that might regulate SiiE function. However, expression of SPI-4 is controlled by regulators encoded outside the locus. Besides the central regulator HilA, the

transcriptional activator SirA acts as a global regulator of *Salmonella* enteropathogenesis, controlling expression of SPI-1, as well as SPI-4. SirA is part of the two-component regulatory system BarA/SirA sensing environmental signals and activating genes, among others important for motility and invasion. Both, deletion of the entire SPI-4 locus, as well as deletion of *sirA* were shown to result in the same dramatic reduction in adhesion to polarized epithelial cells.

To gain more insight into SPI-4 function we want to investigate protein-protein interactions of SPI-4 encoded proteins using a generic protein complex purification strategy. The method includes (i) addition of a histidine-biotin-histidine (HBH) tag to the bait proteins (e.g. SiiB or SirA) via recombinant DNA techniques, (ii) *in vivo* biotinylation and (iii) *in vivo* cross-linking with *para*-formaldehyde (PFA). The bacterially derived *in vivo* biotinylation signal peptide, inducing efficient biotin attachment to the HBH-tag, functions in eukaryotes like *Saccharomyces cerevisiae* as well as in bacteria. The membrane-permeable cross-linking reagent PFA is used to stabilize transient and weak protein interactions. Cross-linked complexes are enriched by tandem affinity purification (TAP) under fully denaturing conditions, followed by identification of proteins bound to the bait protein by liquid-chromatography in conjunction with tandem mass-spectrometry (LC-MS). To verify the efficiency of this purification approach, we used the membrane-associated protein SiiB and the cytosolic protein SirA as bait protein, respectively.

We could immunodetect distinct sets of protein complexes for C-terminal HBH-tagged SiiB and SirA using an antibody against the RGS-H6 peptide implying low cross-reactivity against non-tagged proteins. For MS analyses, we included two negative controls to eliminate the possibility of identifying background and non-specifically bound proteins as interacting partners. Our results will provide new insights into the function of SPI-4 for successful adhesion to and subsequent colonization of host cells.

MPP39

Prevalence of fibrinogen binding protein and hemolysin genes in *S. lugdunensis*

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Staphylococcus lugdunensis is a coagulase-negative staphylococcus (CNS) that can cause severe infections, like skin and soft tissue infections, sepsis and potentially fatal endocarditis, similar to diseases caused by *S. aureus*. In contrast to *S. aureus*, only very few virulence associated proteins have been described. A fibrinogen binding protein and von Willebrand binding adhesin, and synergistic hemolysins have been discussed as putative pathogenicity factors in *S. lugdunensis*.

In this study we designed primer for the detection of two annotated putative fibrinogen binding adhesins and three annotated hemolysins in a collection of more than 100 different *Staphylococcus lugdunensis* strains, to detect the occurrence of these genes. The prevalence of these fibrinogen binding protein and hemolysin genes were compared with their phenotypic correlates. All strains were tested for their binding to fibrinogen and also on Columbia blood agar plates for hemolysis.

Except for the SLUSH gene cluster all the other alleged hemolysins and adhesins had a very high prevalence. SLGD_00847 (slu_hemolysinIII) had a prevalence of 97 % and SLGD_00006 (slu_hemolysin) of 95 %. The SLUSH gene cluster was only found in 45 %. This indicates that the SLUSH gene cluster was absent or the gene cluster was different in these strains.

After 48 h of incubation 93.1 % of the *S. lugdunensis* strains showed hemolysis and 12 of them even had a strong hemolysis. As already shown in a previously report, hemolysis after 48 h is typical for *S. lugdunensis*. The high occurrence of the hemolysin genes also indicate that the hemolysis attribute is less likely determined by a single hemolysin gene but rather a supposed combined effect of several hemolysin genes or differences in their expression.

MPP40

Staphylococcus aureus Fibronectin-binding proteins (FnBPs) lead to an increased permeability of the endothelial barrier without affecting cell viability

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Background: *S. aureus* frequently causes severe intravascular infections (e.g. endocarditis). In a large proportion of patients this may occur without previously damaged endothelium. FnBPs are crucial in the pathogenesis of *S. aureus* endocarditis (Que *et al.*, 2005) and mediate invasion of endothelial cells via fibronectin to α5β1 integrins (Sinha *et al.*, 1999). In polarized cells α5β1 integrins are primarily located to the basolateral side, only a minor fraction is localized to the apical membrane. We hypothesize that initial binding of FnBPs to apically located receptors increases the permeability of the endothelial barrier and thus facilitates access to the basolateral pool of α5β1 integrins.

Methods: Human endothelial cells (EA.hy 926) were infected with different wild type and specific mutant / complemented staphylococcal strains on transwell filters. Permeability was measured by transendothelial flux of FITC-dextran at different time points. Inhibition experiments were performed with Src family protein-tyrosine kinase inhibitors genistein and PP2. PP3, an EGFR inhibitor, served as a control for specificity. Effects mediated by cytotoxicity were quantified by several viability tests. Changes in impedance which reflect changes in cell morphology of the endothelial layer have been determined by real-time impedance analysis.

Results: Infection with wild type *S. aureus* and *S. carnosus* heterologously expressing FnBPs, but not Clumping factor A (Clf A), induced an increase in permeability compared to non-infected cells. By contrast, the non-pathogenic *S. carnosus* wild type had an opposite effect. An FnBP double knock out mutant of wild type *S. aureus* failed to increase permeability. Genistein and PP2 reduced loss of integrity by roughly 50%, whereas PP3 had no protective effect. FnBP expression per se did not lead to substantial cell death, as determined by cell mass (crystal violet staining), mitochondrial respiratory activity (WST-1 conversion) and hypo-diploid DNA (modified cell cycle analysis). Real-time impedance analysis yielded similar results as permeability data demonstrating that impedance declines upon infection with FnBP-expressing strains.

Discussion: Our data show that FnBPs are required for an increase in permeability of the endothelial cell layer. Src family kinases are crucial components of the integrin signaling pathways. Partial protection by genistein and PP2 suggests that Src family kinases mediate an increased permeability of endothelial cells, supposedly by affecting tight junction proteins. This effect might be mediated by phosphorylation of either tight junction or regulatory proteins. Further studies will include scanning electron microscopy to determine changes in cell morphology, immuno-detection and localization of tight junction proteins and siRNA transfection to knock down Src family kinases.

MPP41

Characterization of the surface proteome of *Streptococcus pneumoniae* and its isogenic Lipoprotein maturation mutants

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Streptococcus pneumoniae cell surface proteins play a central role during the infection process and are considered to be important virulence factors of the pathogen. They are thought to be implicated in colonization, bacterial adhesion to and invasion into host cells, and immune evasion mechanisms as well. Different clusters of proteins such as LPxTG cell wall-anchored proteins, choline-binding proteins, and lipoproteins decorate the pneumococcal cell surface. Among them lipoproteins represent the largest group. They are anchored to the membrane via a lipid moiety which is covalently attached by the diacylglyceryl transferase Lgt. The signal peptide is cut off by the lipoprotein-specific signal peptidase Lsp. A global analysis of pneumococcal surface-associated and extracellular proteins was performed using a combination of biotinylation and GeLC-MS/MS. To study the fate of lipoproteins in pneumococci the surface- and exoproteome of non-encapsulated D39Δ*cps* was compared to the protein profiles of isogenic Δ*lgt*, Δ*lsp*, and Δ*lgt*Δ*lsp* mutants, which are deficient in lipoprotein maturation. Based on the genome data 37 lipoproteins, 16 LPxTG-anchored proteins, and 12 choline-binding proteins were predicted for strain D39. Out of them 33, 12, and 2, respectively, were identified. The abundance of lipoproteins found to be surface-associated is significantly decreased in the Δ*lgt* mutant. Accordingly, their abundance in the exoproteome is considerably increased. In contrast, the amount of surface-exposed lipoproteins in the Δ*lsp* mutant was unaffected for the majority of identified lipoproteins. Only a few lipoproteins are less abundant, however, the corresponding exoproteome of the Δ*lsp* mutant showed no significant changes. The double knockout Δ*lgt*Δ*lsp* showed for the majority of lipoproteins similar amounts on the surface, while only a minority is less abundant. Remarkably, the amount of lipoproteins in the exoproteome is significantly increased for D39Δ*cps*Δ*lgt*Δ*lsp*.

MPP42

Structure function relationship of the Laminin adhesin Lmb of *Streptococcus agalactiae*

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Streptococcus agalactiae colonizes the urogenital tract of 10-30% of healthy women. During delivery it can be transmitted from the mother to the newborn and cause sepsis, pneumonia and meningitis. Adhesion to extracellular matrix protein is an important pathogenicity mechanism of gram positive bacteria. The laminin receptor on brain endothelial cells has recently been shown to be crucial for the initiation of contact between meningeal pathogens and the blood brain barrier. In *S. agalactiae* the surface protein Lmb mediates the attachment to human placental laminin and is located on a pathogenicity islet that is absent in many bovine *S. agalactiae* isolates. The crystal structure of Lmb was recently solved and shows that it belongs to the cluster 9 family of ABC (ATP binding cassette) transport system. The structural organization of Lmb closely resembles ABC type solute binding proteins (SBPs) in which two structurally related globular domains interact with each other to form a metal binding cavity at the interface. The bound zinc in Lmb is tetrahedrally coordinated to residues H66, H142, H206 and E281 from both the domains. A highly disordered loop spanning residues 123-138 has been observed and it has been shown to play a role in metal uptake and release of the protein in homologous structures. To analyze the role of individual amino acid residues for the binding properties of Lmb to immobilized laminin targeted mutations of the Zn binding motif were introduced into an *lmb* vector construct that allows the expression of *lmb* as a histidine fusion protein. Binding of recombinant Lmb to laminin was investigated in ELISA plates coated with human placental laminin. Analysis of the mutated and wild type versions of Lmb showed that a deletion of the metal binding loop and the mutation of His66 led to a severe reduction in the binding to laminin. Being ubiquitous in all the serotypes of Group B Streptococcus (GBS), a detailed knowledge of the amino acid residues critical for laminin binding may allow the future development of targeted antimicrobial strategies interfering with the adhesion properties of *S. agalactiae.*

MPP43

Impact of hypoxia on persistent *Chlamydia pneumoniae* infection and its effect on airway remodelling

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Introduction: The immune effector molecule interferon (IFN)-γ arrests the intracellular growth of *Chlamydia pneumoniae* (*Cpn*) and induces persistent infection. This implies the induction of the JAK-STAT signaling pathway and the tryptophan catabolising enzyme, indoleamine 2,3-dioxygenase (IDO). Persistent infection is suggested to promote disease progression in chronic obstructive pulmonary disease (COPD) in which epithelial to mesenchymal cell transition (EMT) is known to be a key factor. The disease is characterized by regional declines of the available oxygen content that are fostered by the underlying inflammatory processes. *Chlamydiae* are well adapted to a low oxygen environment (<3%O_2). We therefore analyzed the effects of acute and persistent *Cpn* infections on EMT under different environmental oxygen concentrations.
Methods: HEp-2 and A549 cells were seeded in 6-well plates and treated with IFN-γ to induce chlamydial persistence. After 24h incubation under normoxic (20%O_2) or hypoxic conditions (2%O_2), cells were infected with *Cpn* (CWL029). Recovery assays were performed 72h p.i. and IFUs/µl were calculated by using immunofluorescence microscopy. p-STAT1 (Tyr701) and IDO expression were analyzed by western blot. α-smooth muscle actin (α-SMA) expression level was analyzed by immunofluorescence microscopy. Statistical analysis was performed with a one tailed, unpaired Student *t*-test.
Results: IFN-γ treatment was not effective to limit growth of *Cpn* at low oxygen concentrations (2% O_2, $3x10^5$±$1x10^5$ IFUs/µl) compared to normoxic conditions (20% O_2, 0±0.2 IFUs/µl). This was accompanied by diminished phosphorylation of STAT-1 (Tyr701) and subsequent reduced expression of IDO under hypoxic conditions. Interestingly, *Cpn* could be rescued from IFN-γ induced persistence under hypoxic conditions. The expression of the epithelial marker protein E-cadherin was significantly downregulated in acute and persistently infected A549 cells (p<0.05) whereas the mesenchymal marker protein α-SMA was upregulated under normoxic and hypoxic conditions. This effect was more pronounced when the cells were cultured under hypoxic conditions.
Conclusion: IFN-γ mediated immune control of *Cpn* depends on the environmental oxygen availability. Thus, *Cpn* escapes host immune control in a hypoxic atmosphere. Acute and persistent *Cpn* induce EMT in lung cells that is even more pronounced when oxygen is scarce.

MPP44

NWMN_0641, a putative LysR-type regulator that affects virulence determinant production in *Staphylococcus aureus*

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Carbon catabolite repression (CCR) in bacteria is a widespread, global regulatory phenomenon that allows modulation of the expression of genes and operons involved in carbon utilization and metabolization in the presence of preferred carbon source(s). In low-GC gram-positive bacteria, CCR is mediated mainly by the catabolite control protein A (CcpA), a member of the GalR-LacI repressor family. In *Staphylococcus aureus*, CcpA has been shown to modulate the expression of metabolic genes and virulence determinants in response to glucose. A second regulator that links carbon metabolism and virulence factor production in this organism is CodY, a sensor of carbon and nitrogen availability that responds to intracellular concentrations of branched-chain amino acids (BCAA) and GTP. We recently reported that *S. aureus* produces a third regulatory molecule, NWMN_0641 (a member of the LysR family of transcriptional regulators with homology to CcpC of *B. subtilis*) that affects the expression of TCA cycle genes.
Here we show that this regulatory molecule also links carbon metabolism with virulence in *S. aureus* by altering the expression of important virulence factors, and affecting pathogenicity of this organism. We found that inactivation of NWMN_0641 significantly decreased the transcription of the *cap* operon, and reduced the capsule formation of the mutant. However, transcription of *hla* (encoding a-hemolysin), and of *RNAIII*, the effector molecule of the *agr* locus, were significantly increased by the mutation, suggesting that NWMN_0641 acts as a repressor of *hla* and *RNAIII*, and as an activator of *cap* transcription. In a murine footpad infection model, the deletion mutant caused an increased footpad swelling, if compared to the wild-type and the complemented derivate, indicating a higher infectivity of the mutant in this systemic animal model.

MPP45

Importance of *Staphylococcus aureus* ClpB and ClpC in intracellular persistence

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The Hsp100/ClpATPases belong to a group of closely related proteins that operate as molecular chaperons. These chaperons are regarded as an important element of bacteria to respond to various stress conditions by refolding or degrading misfolded proteins. The major human pathogen *Staphylococcus aureus* expresses a number of such Hsp100/ClpATPases, namely ClpB, ClpC, ClpL, ClpX and ClpY. In *S. aureus,* ClpB and ClpC are both regulated by the repressor CtsR, with ClpC probably supporting the active conformation of this repressor. Under conditions of stress, ClpC shifts from CtsR to damaged proteins. As a consequence CtsR looses its active conformation which results in a transcriptional induction of CtsR repressed genes, such as *clpB*, *clpC* and *ctsR* itself. ClpB, and to a lesser extent, ClpC have been shown to promote the intracellular multiplication of *S. aureus* in epithelial cells. Additionally, ClpC was found to stimulate the expression of *citB* (encoding the tricarboxylic acid (TCA) cycle enzyme aconitase) and to support the metabolization of acetate, thereby affecting the post-stationary-phase recovery. Inactivation of *clpC*, on the other hand, led to an enhanced stationary-phase survival. Based on these findings, we wondered about the impact of ClpB and ClpC on the intracellular persistence of *S. aureus* within human cells.
Our in vitro viability studies confirmed previous findings by showing that *S. aureus* DSM20231 *clpC* and *clpBC* mutant cultures possessed an increased stationary phase survival, if compared to the wild-type and the *clpB* mutant cultures. In line with this, we detected an enhanced survival of the *clpC* and *clpBC* double mutants in keratinocytes 96 hours after the internalisation, while this was not the case with the *clpB* mutant, which showed a persistence rate comparable to that of the wild-type. In terms of invasiveness, however, the *clpB* and *clpBC* mutants possessed significantly decreased internalization capacities, probably due to lowered productions of

fibronectin binding proteins A and B. These findings suggest that ClpB contributes to the invasiveness of this pathogen, but does not influence the persistence capacity of this organism, while a functional ClpC seems to decrease the intracellular survival capacity of *S. aureus* but does not affect the invasiveness of this organism, irrespective of its impact on *clpB* expression.

MPP46

The role of NADPH oxidase in the control of *Aggregatibacter actinomycetemcomitans*-induced periodontitis in mice

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Aggregatibacter actinomycetemcomitans is a Gram-negative commensal bacterium of the oral cavity which has been associated with the pathogenesis of aggressive periodontitis. However, genetic mechanisms underlying host susceptibility to periodontal infection and to periodontitis progress are still ill-defined. This study aimed to identify genetic host factors which confer resistance against *A. actinomycetemcomitans*-induced periodontitis in a murine model. NADPH oxidase-deficient mice (gp91phox KO), inducible nitric oxide synthase-deficient mice (iNOS KO), and C57BL/6 wild-type mice were orally inoculated with *A. actinomycetemcomitans*. To analyse bacterial colonization in the different mouse strains at various time points after infection *A. actinomycetemcomitans* cultures of oral swabs were performed. Furthermore, linear measurements and three-dimensional micro-computed tomography were used to quantify the degree of alveolar bone destruction as well as alveolar bone mineral density. Persistence of *A. actinomycetemcomitans* in the murine oral cavity varied among the three mice strains showing the highest rate in infected gp91phox KO mice. In accordance with *A. actinomycetemcomitans* persistence, gp91phox KO mice developed more severe periodontitis compared to iNOS KO and C57BL/6 wild-type mice and uninfected controls, characterized by significant higher alveolar bone loss and inflammatory reaction. Additionally, a significantly lower alveolar bone mineral density was observed in all three groups of mice after infection with *A. actinomycetemcomitans* compared to uninfected controls. Our results indicate that NADPH oxidase is important for resistance of the murine oral cavity against *A. actinomycetemcomitans* colonization which is clearly associated with alveolar bone destruction and influx of inflammatory cells in NADPH oxidase-deficient mice. This model should give further insights into the molecular and genetic mechanisms of the *A. actinomycetemcomitans* induced periodontitis development.

MPP47

Functional analysis of the role of *Yersinia* adhesin YadA variants in regard to adherence and pathogenicity of *Yersinia enterocolitica*

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Yersinia enterocolitica and *Y. pseudotuberculosis* are enteropathogenic bacteria causing diseases ranging from self-limiting diarrhoea to sepsis. In the mouse model yersiniae pass the gut via M cells and invade Peyer´s patches with subsequent septic dissemination. The trimeric autotransporter Yersinia adhesin A (YadA) is an essential mouse virulence (MV) factor for *Y. enterocolitica*, but not for *Y. pseudotuberculosis*. It forms a lollipop-like structure with a head, connector, stalk and membrane anchor. YadA in different *Yersinia* species shows differences in its head and stalk regions. To compare the role of these differences in regard to autoagglutination, extracellular matrix or cell adherence, serum resistance and MV, different YadA variants were introduced into *Y. enterocolitica* strain WA-314. Our results show that even though in vitro differences in collagen or fibronectin autoagglutination exist between those variants, there is no difference in their capability to induce mouse virulence. These data show that all tested *Y. enterocolitica* and *Y. pseudotuberculosis* YadA variants are capable to mediate comparable MV in *Y. enterocolitica* WA-314.

MPP48

Minimal medium optimisation for Fluxome analysis: *Mycobacterium tuberculosis* eats what it gets

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Introduction: New technologies such as fluxome analysis, metabolomics and transcriptomics allow an insight into the metabolism of bacteria. These techniques often require a medium defined to a single carbon source. Media, however, used for culture of *Mycobacterium* (*M.*) *tuberculosis* are quite complex and contain several carbon and nitrogen sources. Here we show that *M. tuberculosis* growing in minimal medium with glucose as carbon source also metabolizes other supplemented ingredients like the fatty acids contamination of albumine and surprisingly also the detergent tween.
Methods: For this study *M. tuberculosis* was cultivated in defined minimal medium with U-^{13}C labelled glucose as sole carbon source supplemented with albumine and the detergents tween or tyloxapol, respectively. Methods used here involve isotopic tracer studies and GC-MS (gas chromatography mass spectrometry) for measurement of the labelling pattern of metabolites.
Results: Using minimal medium with fully ^{13}C-labelled glucose, we expected to measure 1 % at the most non labelled amino acids by GC-MS. However it turned out that 20 % of metabolites are not labelled. Thus it leads us to two suggestions: Either *M. tuberculosis* uses another carbon source beside the labelled glucose or the washing steps are insufficient. Improvement of the washing steps did not change the fraction of non labelled amino acids, however the substitution of albumine (contaminated with 1 % fatty acids) against highly purified albumine (contaminated with 0.02 % fatty acids) could decrease the fraction of non labelled amino acids to less than 10 %. When we replaced tween with tyloxapol we could further diminish the non labelled amino acids to about 5 %. Further improvement of the protocol for preparation of *M. tuberculosis* cell extracts by using cellulose nitrate filter instead of harvesting the cells by centrifugation could diminish the part of non labelled amino acids to almost 0 %.
Conclusion: In summary our studies showed that *M. tuberculosis* uses the minimal medium supplementations tween and fatty acids that adhere to albumine for their metabolism. Thus new challenging approaches like fluxome analysis require a defined medium with carefully selected ingredients and well established protocols for harvesting the cells.

MPP49

A new gateway-based approach for simultaneous generation of fusion proteins in bacteria.

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Introduction: Protein-protein interactions are important regulatory layers in all kingdoms of life. They are involved in a vast set of cellular processes including virulence-associated pathways. Identification and characterization of these interactions is one challenging task of the post-genomic era and crucial for understanding of the molecular bases of virulence. Several methods have been successfully employed in the past decades to identify protein-protein interactions in bacteria. These methods often include tedious and time-consuming manipulations of DNA. The Gateway system is a fast and convenient tool, for efficient transfer of DNA-fragments between plasmids. It uses a set of proprietary recombination sequences derived from the bacteriophage lambda site-specific recombination system. Using a combination of different *att*-sites, MultiSite Gateway allows for site directed cloning of up to four fragments into one construct.
Method: Here we developed a new set of Gateway vectors to facilitate the investigation of protein-protein interactions in bacteria. The set includes custom made entry vectors for cloning of a gene of interest in front of an optimized RBS and modular destination vectors. To test our approach, we designed a destination vector including two recombination loci in frame with the fragments of split *Gaussia* luciferase (Gluc), each under control of a tetracycline-inducible promoter.
Result: *Salmonella* pathogenicity Island 1 (SPI-1) encodes for a type three secretion system (TTSS) together with effectors and cognate secretion chaperones. The chaperone InvB binds the effector SipA via its N-terminal chaperone binding domain (CBD) comprising the first 47 amino acids. As proof of principle, we wanted to characterize the interaction between the effector SipA and its chaperone InvB using the split Gluc approach. A single Gateway recombination reaction resulted in the simultaneous integration of both genes in the customized destination vector. In the final expression vector, C- and N-terminal portions of Gluc were fused to the C-termini of InvB and SipA, respectively. After transfer of constructs in

Salmonella enterica sv. Typhimurium (*S*. Typhimurium), luminescence measurements revealed significant higher signal with full length SipA compared to its truncated version lacking the CBD.
Discussion: In conclusion our system provides a valuable tool for investigating protein-protein interactions in bacteria. The approach simplifies cloning steps and its modular nature enables fast adaption to all kinds of fusion proteins.

MPP50

Plasminogen binding in *Mycoplasma pneumoniae*

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Mycoplasma pneumoniae (M.p.) can cause infections of the human respiratory tract up to severe cases of atypical pneumonia. The reduced genome of mycoplasmas resulted in a remarkable limitation of pathogenicity factors and metabolic capacities. As the major pathway for ATP production these bacteria use the glycolysis in which enolase (Eno) represents the central glycolytic enzyme. In the last years, glycolytic enzymes are reported as occurring not only intracellularly but also at the surface of different microorganisms mediating interactions to human extracellular matrix proteins (ECM). For example, pyruvate dehydrogenase (PDH) and glyceraldehyde-3-phoshate dehydrogenase (GAPDH) of M.p. were characterized as binding partners for human fibronectin and fibrinogen. On the other hand, interactions between Eno and human plasminogen (plg) were described in different bacterial species like *Streptococcus pneumoniae* and *Mycoplasma fermentans*. Little is known about an interaction of plg with M.p. proteins. Western blot experiments with whole cell extracts of M.p. indicated that two proteins show plg binding. To prove this preliminary result, different glycolytic enzymes of M.p. were expressed in *E. coli* and polyclonal antisera were produced. Purified recombinant Eno and PDH bind human plg. Further Western blot, colony blot, ELISA and immunofluorescence experiments confirmed that Eno occurred primarily in the fraction of cytosolic proteins of M.p. whereas PDH could be detected in high concentrations both in the cytosol and at the surface of the bacteria. As expected, trypsin treatment of whole M.p. cells digested PDH but not Eno. In contrast, the data of a FACS-based adherence-inhibition assay demonstrated, that anti-PDH has no influence on the rate of mycoplasma adhesion to HeLa cells. The results of the study confirm an interaction of the glycolytic enzymes Eno and PDH of M.p. with human plg. Together with the previously described involvement of further glycolytic enzymes in binding to different proteins of the human ECM a complex network of interactions can be suggested playing a role in the colonization of the human respiratory tract with M.p..

MPP51

The ß-hemolysin and intracellular survival of *Streptococcus agalactiae* in monocytic and granulocytic cells

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Streptococcus agalactiae (Group B Streptococci, GBS) is an important cause of human invasive infections in newborns, pregnant women and immunocompromised adult patients. The ß-hemolysin of *S. agalactiae* is a surface associated toxin and regarded as a major virulence factor. It is regulated by the *cov* two-component regulatory system, which controls numerous virulence factors of GBS. Mutations of the *cov* gene have been associated with increased hemolytic activity, but impaired intracellular survival of GBS in granulocytes. To determine the role of the ß-hemolysin for intracellular survival and to rule out the effect of other virulence factors controlled by *cov*, we investigated hemolytic and nonhemolytic GBS mutants for intracellular survival in primary human granulocytes and THP-1 monocytic cells.
We examined the role of ß-hemolysin for interaction with the monocytic and granulocytic cells using a serotype Ia *S. agalactiae* wild type strain and an isogenic nonhemolytic deletion mutant of this strain. Both strains were fluorescently labeled with an EGFP expressing plasmid. Following infection of eukaryotic cells with GBS at a multiplicity of infection (MOI) ranging from 5 to 25 the intracellular bacteria were evaluated by FACS analysis and culturing of intracellular bacteria. Interestingly, the non-hemolytic mutants were able to survive in the intracellular environment in significantly higher numbers than the hemolytic strain. A finding that was observed for primary granulocytes as well as for THP-1 cells. To exclude the possibility that the observed differences in survival were due to host cell death induced by the hemolytic but not the non-hemolytic strain, Lactate Dehydrogenase (LDH) assays were carried out and confirmed the better survival capacity of the nonhemolytic strain. The ß-hemolysin of GBS has previously been described as a potent inducer of IL-8 in macrophages. To assess the induction of IL-8 following infection with GBS, ELISA determinations were performed. While a considerable release of IL-8 could be observed, however we could not find a significant difference in the ability of hemolytic or nonhemolytic strains to induce the chemokine. To determine the bacterial mediators of IL-8 release in this setting, cell wall preparations from both strains were incubated with THP-1 cells. Both preparations were found to exert a potent proinflammatory stimulus on THP-1 cells. In conclusion, our results indicate that the *S. agalactiae* ß-hemolysin has a strong influence on the intracellular survival of GBS and that a tightly controlled regulation of ß-hemolysin expression is required for the successful establishment of GBS in different host niches.

MPP52

Fitness analysis of rare meningococcal strains with mutation in the outer membrane receptor ZnuD involved in Zn uptake

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Neisseria meningitidis (Nm) has developed efficient mechanisms to compete for essential trace elements. An outer membrane receptor involved in zinc uptake (ZnuD) has been described. ZnuD was expressed in response to zinc starvation (Stork *et al*., PLoSPathogens, 2010). We analyzed ZnuD expression in a sample of 67 meningococcal strains (3 serogroups; 11 clonal complexes [cc]). Western blot revealed that the majority of isolates expressed ZnuD (56/67) upon zinc depletion with the chelator TPEN. In 7 out of 21 ST-23 cc *Nm*Y and in 4 out of 5 ST-8 cc *Nm*B strains, ZnuD could not be induced. Sequence analysis of six strains revealed premature stop codons at nt 562 in three *Nm*Y, at nt 2260 in one *Nm*Y, and at nt 76 in two *Nm*B isolates. We tested whether natural isolates from invasive disease with ZnuD mutation grow in the presence of TPEN. The *Nm*Y strain with C-terminal truncation of ZnuD at position 2260 was not affected in contrast to three *Nm*Y strains with mutation at position 562. Reduced fitness under Zn limited conditions in mutant strains argues against redundancy of zinc uptake systems. In conclusion, the ability to express ZnuD upon zinc starvation was well conserved among meningococci, which argues for an essential role of meningococcal ZnuD. It is intriguing that inactivating ZnuD mutation events seem to be restricted to only two clonal lineages. In ST-23 cc the mutations are inserted by slipped strand mispairing. *Ex vivo* events induced by culture must therefore be considered.

MPP53

Eap stimulates the internalisation of *Staphylococcus aureus* by interference with eukaryotic uptake mechanisms

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Background: *Staphylococcus aureus* (SA) is the leading cause of superficial and deep skin infections, and is frequently associated with non-healing wounds. Invasion of and persistence in eukaryotic cells such as endothelial cells, fibroblasts, or bone cells is considered of importance in infection. A multifunctional secreted SA protein, the extracellular adherence protein Eap, has been demonstrated to be of importance for attachment, internalisation, and modulation of host cell functions. The role of Eap in the interaction of SA with uptake mechanisms of epithelial cells, however, is not defined.
Results: Preincubation of HaCaT cells with Eap alone caused a significant, concentration-dependent increase in adhesion, and - even more pronounced - in internalisation of SA. Subsequent experiments focussed on the mechanisms of this Eap-mediated effect. Addition of genistein or Src-kinase-inhibitor (competitive tyrosin kinase inhibitors) reduced uptake of SA, yet, this reduction was partially relieved by Eap. Since treatment of Eap with acetylphosphate yielded a reduction of the by Eap stimulated bacterial internalisation, a role of phosphorylation-dependent manipulation of kinases by Eap, and consequently an interference with intracellular signal transduction can be hypothesized. As a prerequisite for such an interference, Eap was identified in the cytosol of keratinocytes (originating from internalised bacteria or from extracellular Eap sources).
Conclusion: Taking into account the increased Eap expression that was found in SA-infected wounds, we suspect that Eap might induce an increased internalisation of bacteria in wound tissue in order to protect the pathogen from the host immune system and to enable it to invade new ecological niches within its host.

MPP54

New real-time technology for investigatimg host cell response to meningococcal infection

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During bacterial infection, host cells undergo a change in cell morphology, proliferation and cell death. Corresponding measurements are performed as end-point assays that represent the cell response at a single time point. Therefore, it is difficult to receive a detailed picture of the wide-range effects of bacteria on eukaryotic cell function during the complete infection process. A new technology from Roche Applied Science and ACEA Biosciences (the xCELLigence system) based on impedance measurements allows continuous monitoring of cells in real-time. For this purpose, cells are seeded and infected in specialized cell culture microplates containing micro electrodes. The xCELLigence system enables quantification of cell adhesion, proliferation, spreading, cell death and detachment in real time. Furthermore, lag and log phases can be determined to estimate optimal times to infect cells.
In this study, we took advantage of this new technology to provide valuable insights into cell function in response to several virulence factors of the meningitis causing pathogen *Neisseria meningitidis*, including the lipopolysaccharide (LPS), the polysaccharide capsule and one outer membrane protein (Opc). The data acquired with this system corresponded to established assays based on crystal violet staining, microscopy and correlated with measurement of MMP-8 activity as described recently[1]. LPS and polysaccharide capsule caused increased cytopathological effects with high MMP-8 secretion followed by strong cell detachment. Furthermore, cell function in response to 10 strains of apathogenic *Neisseria* spp. (*N. lactamica* and *N. mucosa)* was analyzed. In contrast to infection with pathogenic *N. meningitidis* isolates, infection with apathogenic *N. lactamica* isolates did not change electrical impedance monitored for 48 hours. Together our data show that this system can be used as tool for rapid monitoring of cellular function in response to bacterial infection in real-time and combines high data acquisition rates with ease of handling.

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MPP55

1-Methyl-Tryptophan (1-MT), the inhibitor of IDO, serves as tryptophan replacement for human cells and microorganisms

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Indoleamine 2,3-dioxygenase (IDO) is an immunoregulatory enzyme that is establishes tolerance in several biological settings by regulating local tryptophan (Trp) concentrations. Unfortunately, IDO activity can also lead to deleterious alterations of the immune response by promoting tolerance to some types of tumors. To suppress this disadvantageous IDO activity the competitive inhibitor 1-Methyl-Tryptophan (1-MT) has been generated and is currently used in clinical trials. Nevertheless, it is still elusive which stereoisomer of 1-MT is the more effective inhibitor of IDO-mediated immunosuppression. While IDO enzyme activity is more efficiently inhibited by 1-L-MT in cell-free or *in vitro* settings, 1-D-MT is seemingly superior to 1-L-MT in the enhancement of anti-tumor responses in murine *in vivo* models. Here, we present a new mode of action of 1-L-MT that compensates IDO-mediated tryptophan depletion *in vitro*.
In our work, we firstly describe that 1-L-MT, but not 1-D-MT, can act as a nutritive substrate that replaces tryptophan in *in vitro* cultures. In initial experiments we showed that 1-L-MT, but not 1-D-MT, allows the growth of tryptophan-auxotroph bacteria and parasites (*Staphylococcus aureus* ; *Toxoplasma gondii*) in the complete absence of tryptophan. In line with that we describe that 1-L-MT in contrast to 1-D-MT counteracts IDO-dependent inhibition of human T-cell proliferation. A detailed analysis showed that 1-L-MT, in contrast to 1-D-MT, is incorporated in human T-cell proteins when tryptophan is lacking in the culture medium.
In summary our data indicate that 1-L-MT inhibits the IDO-mediated antiproliferative activities via two independent effects: On the one hand 1-L-MT acts as a competitive enzyme inhibitor and therefore reduces tryptophan degradation by IDO. On the other hand 1-L-MT can replace tryptophan in protein biosynthesis and therefore allows bacterial, parasitical or cellular growth.
Further mass spectroscopy analysis is necessary to analyse whether 1-L-MT is incorporated into proteins either directly or after a demethylation process.

MPP56

The hypothetical type VI secretion protein BPSS1504 is involved in virulence of Burkholderia pseudomallei

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Burkholderia pseudomallei comprises a facultative intracellular Gram-negative rod and is the causative agent of melioidosis, an emerging infectious disease of tropical and subtropical areas world wide. The pathogen harbours a remarkable number of various secretion systems, including three type III and six type VI secretion systems. Testing of numerous *B. pseudomallei* transposon mutants in a cell-based plaque assay screening approach led to the identification of mutants defective in their intracellular life style. One mutant harbouring a defect in the BPSS1504 locus, a hypothetical protein within cluster 1 of the type VI secretion systems, was selected for further analysis. *B. pseudomallei ΔBPSS1504* did not show any growth defects in LB or minimal medium compared to the wild type strain, and motility and biofilm formation was also not affected. However, *B. pseudomallei ΔBPSS1504* was highly attenuated in an intranasal infection model of melioidosis in BALB/c mice, whereas in vivo virulence of the complemented mutant strain *B. pseudomallei ΔBPSS1504:Tn7BPSS1504* was almost fully restored to wild type level. Intracellular replication of *B. pseudomalleiΔBPSS1504* was significantly impaired in various mammalian phagocytic and non-phagocitic cell lines as well as in primary murine bone marrow derived macrophages. In accordance with that, we found reduced induction of cell death in *B. pseudomallei ΔBPSS1504* infected cells as verified by the measurement of lactate dehydrogenase release. The formation of multi-nucleated giant cells (MNGC) in the murine macrophage cell line RAW 264 was also strongly reduced after infection with *B. pseudomallei ΔBPSS1504* compared to the wild type, but restored in the complemented mutant strain. Our data indicate that the BPSS1504 locus is a critical component for the intracellular life cycle of this versatile pathogen and contributes to in vivo virulence in *B. pseudomallei* infection.

MPP57

Coaction of influenza virus and *S. aureus* toxin Pantone-Valentine-leukocidin on human granulocytes and epithelial cells

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Background: Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains, especially the Panton-Valentine-leukocidin (PVL) producing strain USA300, have been associated with severe necrotizing infections, such as necrotizing pneumonia, which have high mortality rates. Epidemiological studies revealed that necrotizing pneumonia is often preceded by influenza-like symptoms or by an influenza infection. Nevertheless, the combined action of influenza virus and the pore-forming *S. aureus* toxin PVL is hardly investigated.
Methods: In our study, we examined the effect of PVL on different cell types (granulocytes and epithelial cell line A549) with or without anteceded infection of the cells with the influenza virus Puerto Rico/8/34 (PR8). The pro-inflammatory activation of the cells was measured by chemokine expression via RT-PCR and CD11b expression and cell death induction was determined by flow cytometry. The integrity of the cell monolayers was evaluated by light microscopy.
Results: We found that PVL induced cell activation and rapid cell death in granulocytes, but did not affect cell viability in non-professional phagocytes such as epithelial cells. Influenza virus PR8 infection alone did not enhanced granulocyte cell death induction, but a previous infection with PR8 significantly enhanced the pro-inflammatory and cytotoxic effect of PVL on human granulocytes. By contrast, cell viability of epithelial cells was not affected by single or combined stimulation with PVL and/or PR8, but the influenza virus had a highly activating impact on the chemokine expression (RANTES, IP-10, IL8) of A549 cells. However, when we treated the epithelial cell monolayer with supernatants of human granulocytes, which were lysed by PVL and PR8, we found a dose-dependent destruction of the epithelial cell monolayer, mainly caused by detachment of epithelial cells. Fetal bovine serum, human serum and a Protease Inhibitor Cocktail interfered with the destructive impact of the granulocytes supernatant on A549 and preserved the integrity of the monolayer.
Conclusion: Our findings show that PVL has a lysing effect on granulocytes. The influenza virus has an activating impact on epithelial

cells that could result in massive recruitment of granulocytes, which can be subsequently lysed by PVL. PVL and influenza virus together showed even an enhanced impact on neutrophils. The substances released by the lysed neutrophils, e.g. granule proteins and defensins, are able, in turn, to destruct the epithelial cell monolayer. These results from our cell culture models suggest that PVL and influenza virus can act together and are possible agents to cause necrotizing pneumonia.

MPP58

Metabolism of intracellular *Salmonella enterica:* One lifestyle in intracellular infections

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The intracellular lifestyle of *Salmonella enterica* relies on the adaption to nutritional conditions within the *Salmonella*-containing vacuole (SCV) in host cells. Based on previous results, metabolic requirements for *Salmonella* in the SCV shall be characterized further. This includes (i) prediction of intracellular phenotypes of mutant strains based on metabolic modeling and experimental tests, (ii) isotopologue profiling using 13C compounds in intracellular *Salmonella*, and (iii) complementation of metabolic defects for attenuated mutant strains. The collaborative work between the Dandekar and Hensel groups should lead to a comprehensive understanding of the metabolic requirements of the intracellular lifestyle of *Salmonella*. Further analyses of intracellular phenotypes will be based on improved metabolic predictions. Vice versa, based on analyses of mutant phenotypes and isotopologue analyses, iterative refinement of metabolic fluxes will be achieved. Our vision is a comprehensive understanding of the adaption of *Salmonella* to its lifestyle in a membrane-bound compartment in mammalian cells. Towards an integrated model and profiting from SPP1316, we will do comparisons of the *Salmonella* lifestyle with other pathogens and their metabolic requirements during infection such as cytoplasmic lifestyle found in *Listeria* or within a membrane compartment as for *Legionella*.

MPP59

Metabolism changes when *Staphylococcus saprophyticus* is adapted to utilization of D-serine

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S. saprophyticus is the only species of the staphylococci that is typically uropathogenic and the genome is the only one of all sequenced staphylococci which possesses a D-serine deaminase. This enzyme converts D-serine to pyruvate and ammonia. Interestingly, it is also present in other uropathogenic bacteria like *E. coli* (UPEC). The amino acid D-serine is present in relatively high concentrations in human urine and is toxic or bacteriostatic to several non-uropathogenic bacteria. Therefore the uncommon ability to degrade D-serine may play an important role for the virulence of uropathogens. In addition, the presence of D-serine may be used as a cue by uropathogens for the presence in the urinary tract. To analyze the metabolism and to understand the significance of D-serine catabolism of *S. saprophyticus* for virulence, we conducted 2D-gels. To this end *S. saprophyticus* was grown with either glucose or D-serine as sole carbon and energy source in a chemically defined medium, which we developed previously. Protein extracts of *S. saprophyticus* adapted to glucose or to D-serine were applied to 2D-gels and the protein patterns were analyzed. Proteins which were reproducibly more than 2-fold upregulated were excised for a following identification via MALDI-TOF. Most of the proteins upregulated under D-serine conditions affect the carbohydrate metabolism, the amino acid metabolism and the energy metabolism, but we also found the accessory protein of the urease, a virulence factor, to be upregulated. These results lead us to the hypothesis that D-serine indeed is used as a cue for the presence within the urinary tract and regulates pathways that are important for viability in this environment.

MPP60

Staphylococcus saprophyticus: fibronectin binding of SdrI versus UafA

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Staphylococcus saprophyticus is a gram-positive and coagulase-negative pathogenic staphylococcus causing urinary tract infections in young women. It is hydrophobic, able to bind fibronectin, laminin and collagen and hemagglutinates sheep erythrocytes. Some of its surface proteins have been characterised in the early past. The serine-aspartate repeat protein I (SdrI), contains the longest SD repeat region described so far (854 aa) and a LPXTG-motif for cell wall anchoring. It is a member of the MSCRAMM protein family and shows a typical ABB domain structure. Within the A domain a specific amino acid sequence (TYTFTNYVD) is found. This motif or it variants is also found in many other fibronectin or collagen binding proteins from staphylococci, even in another surface protein of *S. saprophyticus*: the UafA.
Previous experiments have indicated that a SdrI mutant showed decreased fibronectin binding *in vivo*. Following this hint isolated SdrI domains were tested for fibronectin binding *in vitro* using fibronectin coated microtiter plates. These tests showed that fibronectin binding was mediated by the A domain. To localise the area of binding within the A domain, it was divided into three parts: N1, N2 and N3. N2, N3 and N2+3 were cloned into the pQE30Xa vector for overexpression and purification using a N-terminal His-Tag. Binding assays were carried out with N2, N3, N2+3 and the whole A domain as a positive control in coated microtiter plates. The bound protein was detected by an ELISA. As expected the results showed a high binding activity for N2, a slightly reduced binding for N2+3 compared to N2 and N3 alone showed no binding at all. N2 was further subjected to x-ray structure analysis but data analysis is still ongoing.
Suprisingly, the UafA knock out mutant was also associated with decreased fibronectin binding. This protein has also been separated into its domains which will be used for the binding test mentioned before. We expect to gain new insights into the A domain conformation of SdrI, the role of UafA and their implications for the uro-adherence of *Staphylococcus saprophyticus*.

MPP61

Comparative *in vivo* proteome analysis of co-internalized *Staphylococcus aureus* wild type and Δ*agr* cells

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Staphylococcus aureus, the cause of a wide spectrum of severe community-acquired and nosocomial infections, is recently acknowledged as an intracellular pathogen, as it can be internalized and persist in non-professional phagocytic cells *in vitro* [1]. During the internalization process, *S. aureus* has to adapt to the new intracellular environment to survive or even persist within the host. Recently, we were able to analyze this adaptive process of internalized *S. aureus* RN1HG using an infection assay which combines a pulse-chase labeling approach (SILAC [2]), high capacity cell sorting, and gel-free proteomics [3, 4]. The aim of this study is to comparatively investigate the adaptive response of *S. aureus* RN1GH wild type and isogenic Δ*agr* mutant cells upon co-internalization by human bronchial epithelial cells (S9).
The accessory gene regulator (*agr*) is one of the major global regulators of *S. aureus* virulence, which positively influences the synthesis of δ-hemolysin and proteases. In contrast adhesions are negatively influenced by *agr*. There is striking evidence that this regulatory system plays an important role in the establishment of infection. For instance, expression of *agr* is initially increased in the acute phase of infection in non-professional phagocytic cells [5]. Furthermore, *agr* mutants are attenuated in a variety of animal models and it was also shown that the escape from the endosome requires *agr*-dependent factors [6]. The striking advantage of a co-infection assay would be that both strains are being internalized simultaneously and adapt to the host under exactly the same conditions. This allows us to study the competitive intracellular behavior of the two strains on the proteome level in a time-resolved fashion.

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MPP62

Intracellular signaling after reactivation of persistent *Chlamydia trachomatis* under hypoxia

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Introduction: Genital tract infections with *Chlamydia trachomatis (C. trachomatis)* are the most frequent transmitted sexually disease in women that may result in severe clinical sequelae like pelvic inflammatory disease (PID), tubal occlusion and infertility. It is suggested that once the acute infection is not efficiently cleared by the immune system intracellular persistence of chlamydiae eventually occur, leading to chronic inflammatory processes within infected tissues. We could recently show that IFN-γ induced persistence of *C. trachomatis* in human fallopian tube cells can be overcome by decreasing the environmental oxygen concentration (<3%O_2). Under hypoxic conditions, chlamydiae start to replicate and re- differentiate to infectious elementary bodies. The aim of our study was to characterize hypoxia induced reactivation of intracellular chlamydial growth with respect to pro- inflammatory immune responses and MAPkinase signaling.
Material and Methods: Persistence was induced by treating *C. trachomatis* serovar D infected HeLa-229 cells with IFN-γ for 24h under normoxic conditions. Persistent *C. trachomatis* were subsequently incubated under normoxic (20%O_2) or hypoxic (2%O_2) conditions. Phosphorylation of ERK1/2 and p38 MAPkinases were detected by western blot analysis. Expression levels of IL-1ß, IL-8, MCP-1, and TNF-α mRNA were measured by real-time PCR.
Results: Reactivation of persistent *C. trachomatis* induced by IFN-γ was observed under hypoxic but not normoxic conditions. Preliminary experiments suggest that ERK1/2 and p38 MAPkinases are activated under normoxic but not hypoxic conditions. In contrast, mRNA expression levels of the pro- inflammatory and chemotactic cytokines IL-1ß, IL-8, MCP-1, and TNF-α were enhanced under hypoxic but not under normoxic conditions.
Discussions: Our data indicate for the first time that persistent chlamydiae are recovered in a hypoxic environment that is found under physiological conditions in the female urogenital tract. Intracellular reactivation of formerly persistent chlamydiae might perpetuate inflammatory processes that result in chronic sequelea in diseased tissues.

MPP63

In *Mycoplasma hominis* adhesion of OppA to HeLa cells and its binding to extracellular matrix molecules is mediated by different protein structures

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Introduction: Adherence to host tissue is one of the first steps of bacterial colonization. In progress of infection, extracellular matrix (ECM) components became accessible at the host's surface as receptors for bacterial adhesion and bridges for invasion. The cytoadhesion of *Mycoplasma hominis*, a facultative-pathogen of the human urogenital tract, depends on its ectoATPase activity which is uniquely mediated by OppA, the substrate-binding domain of an oligopeptide permease.
Materials and Methods: To characterize the binding of OppA to host cell structures, OppA mutants were generated with mutations of the Walker A and B motif of the ATPase domain and regions, which are conserved in OppA proteins of other species. The attachment of the histidine-tagged mutants to extracellular matrix (ECM) components was analyzed in microplate format and compared to their adherence to HeLa cells in the presence of increasing amounts of ECM components as competitors.
Results: Wild type OppA was shown to bind to sulfated components, like heparin, fucoidan and dextran sulfate, as well as to plasminogen, plasmin and laminin. No binding was observed to mucin. By the addition of plasminogen (1.2 µM) adhesion of OppA to HeLa cells was reduced to 45 %. In the presence of fucoidan (5 µM) or dextran sulfate (0.2µM) adhesion was nearly abolished (6%). Interestingly, mutation of the Walker A motif as well as deletion of the entire ATPase domain lead to a twofold enhanced binding to sulfated ECM structures, like heparin and dextran sulfate. Deletion of CS1, an N-terminal region which is conserved in bacterial OppA proteins, resulted in a reduced binding of this OppA mutant to dextran sulfate (to 38 %) and heparin (17%) demonstrating that this region is involved in binding to sulfated components which are abundant in human endometrium.
Discussion: The findings that the ecto-ATPase activity of OppA is essential for cytoadhesion of *M. hominis* whereas the CS1 region is more involved in ECM-binding suggest that these two functional regions play a different role in patho-physiological processes of host-pathogen interactions.

MPP64

Characterisation of a novel 18.4 kb genomic island in endemic monophasic *Salmonella* Typhimurium strains

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A monophasic variant of *Salmonella* Typhimurium not expressing the second phase flagellar antigene has become one of the predominant agents causing foodborne infections in humans in Germany. In 2010 the monophasic variant represented 48% of all human *S.* Typhimurium isolates sent to the National Reference Centre and was involved in 13 outbreaks. In spite of variations in phage type, PFGE and resistance pattern within the emerging monophasic strains one type characterised by phage type DT193, PFGE pattern STYMXB.0131 and tetra-drugresistance towards antibiotics including ampicillin, streptomycin, sulfamerazine and tetracycline clearly dominates in Germany. Meanwhile the same kind of strains is of increasing concern in various European countries. Genome-based analyses displayed an 18.4 kb fragment adjacent to the *thrW* tRNA locus in this dominant strain type. Sequencing of the detected insertion revealed 27 open reading frames and a significantly lower G+C content compared to the closely related *S.* Typhimurium LT2 genome (47.4% vs. 52.2%), indicating its acquisition via horizontal gene transfer. Transcripts have been shown for 24 ORFs. On nucleotide level, homologous sequences for most of the open reading frames have been found in *E. coli* and *Shigella* genomes. Protein BLAST analyses revealed several phage-related genes, indicating that the insertion might be of phage-origin. ORF 10 had been predicted to code for a T3SS effector. Our experiments suggest that its product is secreted but probably not via T3SS. Infection experiments showed a reduced replication capacity of the island-deleted mutant in the mouse macrophage cell line RAW. Further, it had been shown that the island is able to form a circular intermediate and therefore seems to be mobilised under certain conditions. Aim of our studies is to uncover the function of the different gene products and the role of the whole island in terms of bacterial fitness or pathogenicity.

MPP65

Yersinia pyruvate kinase isoenzymes PykA and PykF

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In *Yersinia* two pyruvate kinase (PK) isoenzymes (PykA and PykF) exist. This glycolytic enzyme catalyses the irreversible conversion of phosphoenolpyruvate (PEP) into pyruvate coupled with the synthesis of ATP, and requires monovalent K^+ and divalent Mg^{2+}. Most bacterial PKs are allosterically regulated via fructose-1,6-bishosphate (FBP), ATP/AMP and/or monophosphorylated sugars. We recombinantly produced *Y.enterocolitica* PykA and PykF in milligram amounts to characterise their structural and enzymatic properties. PykA and PykF were overproduced in *E. coli* without affinity tags and combined anion exchange chromatography and size exclusion chromatography to yield highly homogeneous proteins. We compared their enzymatic activities and allosteric regulation. Further, we studied the conformational state of both isoenzymes in the absence and presence of the allosteric effector FBP applying small angle X-ray scattering (SAXS) and native polyacrylamide gel electrophoresis techniques. Next, the spatial and temporal distribution of the two isoenzymes under various conditions shall be studied.

MPP66

Deciphering *Y. enterocolitica* subsp. *palearctica* O:3/4 genome.

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Moderate pathogenic *Y. enterocolitica* subsp. *palearctica* serobiotype O:3/4 is responsible for most yersiniosis cases worldwide. To address its dissemination and host adaptation we have deciphered the genomes of three *Y. enterocolitica* subsp. *palearctica* serobiotype O:3/4 human isolates. *Y. enterocolitica* subsp. *palearctica* Y11, a type strain from German DSMZ, was completely sequenced and two other strains, Y8265 and Y5307, were used for high coverage draft genome sequencing. All three genomes were compared to the available high-pathogenic *Y. enterocolitica* subsp. *enterocolitica* 8081 O:8/1B to address O:3/4 peculiarities.
Results
As expected, most high-pathogenicity-associated determinants of *Y. enterocolitica* subsp. *enterocolitica* (e.g. the High-Pathogenicity Island

(HPI), Type 2 and Type 3 secretion systems) are absent from the O:3/4 genomes analysed. However, the latter acquired putative virulence and fitness associated factors, which may substitute the classical yersinial virulence traits. Gene clusters encoding a large 350 kDa RTxA-like toxin, an alternative chromosomal *ysp* T3SS, an insecticidal toxin, a pertactin, β-fimbriae, a putative hemolysin, an invasin, several toxin-antitoxin systems and a large 21 kbp flagellar cluster, similar to the *flag-2* gene cluster of O:9/2, are evident O:3/4 acqusitions. A filamentous PhiYep-1 prophage, which is highly homologous to CUS-1 of *E. coli* and Ypf prophage of *Y. pestis,* is present in the Y11 genome. However, it has suffered successive deletions in strains Y8265 and Y5307.
A P2-like prophage designated PhiYep-3 is integrated in tRNA-Leu in the Y11 genome, but it is absent from Y8265 and Y5307. We have demonstrated the precise excision of PhiYep-3 and the restoration of its attachment site. The tRNA-Asn gene is occupied by HPI in O:8/1B and by an unidentified 14.9 kbp GIYep-01 genomic island in O:3/4. The island has a functional integrase and can leave its tRNA-Asn integration site.
Conclusions
Y. enterocolitica subsp. *palearctica* O:3/4 has an open genome that carries the traces of ancient and fresh acquisitions. The acquired putative virulence and fitness-associated determinants may play an important role in host pathoadaptation and support dissemination of this group of pathogens.

MPP67

The role of *Staphylococcus aureus* adhesin Emp in staphylococcal infections: Investigation of Emp binding sites to host structures and to the bacterial cell wall

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Staphylococcus aureus adherence to host structures, which is a critical first step in *S. aureus* pathogenesis, is mediated by multiple adhesins with overlapping functions. The role of some adhesins, particularly the MSCRAMs (microbial surface component recognizing adhesive matrix molecule) is already well established, whereas the function of the secreted and rebinding SERAMs (secretable expanded repertoire adhesive molecule), such as Emp (extracellular matrix binding protein) is less clear.
In this study, we focussed on the binding mechanism of Emp by elucidating the rebinding to the bacterial cell wall and by exploring binding sites to host structures. For this, the *Emp* gene was devided into different subdomains (*Emp1*, *Emp2*, *Emp3*, *Emp1+2*, *Emp2+3)*, which were cloned and overexpressed. To investigate Emp rebinding to the bacterial surface, adhesion assays were performed by adding bacterial suspensions of *S. aureus* strains Newman, Cowan I or 6850 to microtiter plates coated with Emp or its subdomains. The C-terminus of the Emp protein (Emp2, Emp3) appears to be responsible for the binding to *S. aureus*. Regarding the interaction between Emp and different extracellular matrix proteins (vitronectin, fibronectin, fibrinogen, collagen) as well as extracellular suprastructures from human skin and cartilage we found, that the Emp N-terminal part (Emp1, Emp2) is more important to mediate *S. aureus* adherence to host structures. This could also be confirmed by Emp binding assays to different eukaryotic cell types, such as endothelial and epithelial cells. As an alternative method, a peptide library comprised of 15 amino acids with 3 amino acids overlap, spanning full length Emp was used to identify clusters of amino acids showing binding activity to ligands. However, Emp binding could not be broken down to short amino acid sequences, in fact, binding is mediated by the whole fragments. Structural analysis (CD and ^{1}H-NMR) of Emp revealed that the protein is mainly forming random coils. This is in line with the calculated values using the self-optimized prediction method (SOPMA). We hypothesize, that the contact to the binding partner is essential for the protein structure.
Taken together, our results indicate that the N-terminal part of Emp mediates the binding to host structures, whereas the C-terminal part of Emp is responsible for the rebinding to the bacterial cell wall.

MPP68

Study of interaction between *Staphylococcus aureus and Pseudomonas aeruginosa* strains isolated from patients with cystic fibrosis.

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Introduction: Cystic fibrosis (CF) is one of the most common lethal autosomal recessive disorders in white individuals. The disease is caused by a mutation in the gene for the protein cystic fibrosis transmembrane conductance regulator (CFTR). The most common death in CF is respiratory failure secondary to pulmonary infection. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are often co-isolated from CF respiratory cultures in 20-50% of all patients.
Methods: CF patients were surveyed for colonization with *S. aureus* and *P. aeruginosa* during the last 7 years. Sputum cultures were collected whereas their routine clinical visits or admission to the hospital. Pulsed -field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were used to determine the clonal relationship between the strains. Microarray experiments allowed comparison of expression profiles of *S. aureus* isolates.
Results and Discussion: In our study we characterised the phenotypical and genotypical features of *S. aureus* isolates in terms of antibiotic resistance, biofilm formation and mutation frequency. PFGE revealed that some patients were infected with various *S. aureus* strains including MRSA or with the same strain during the whole period. Ongoing studies are aimed to identify the gene expression profiles of *S. aureus* strains persistent over a period of several years in the presence of *P. aeruginosa* isolates.

MPP69

Role of motor proteins in the intracellular life of *Salmonella* and the dynamics of *Salmonella*-induced filaments

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Salmonella, an intracellular pathogen, lives and replicates in a membrane bound compartment called *Salmonella* containing vacuole (SCV). While the entry of the pathogen into host cells is facilitated by the *Salmonella* pathogenicity island 1 (SPI1) encoded type three secretion system (T3SS), successful intracellular lifestyle requires the SPI2-T3SS. A hallmark of intracellular *Salmonella* is the formation of extended tubular network from the SCV called as *Salmonella*-induced filaments (SIFs). A recent study by our group has identified SIFs to be highly dynamic structures that extend, retract and branch. Although SIFs were identified long back, their role in the intracellular life of *Salmonella* and the molecular mechanism underlying the dynamics is not yet known. However, work by others and our group has identified that SIFs lie next to the microtubule tracks and are often associated or linked to motor proteins. Because of these observations we hypothesized that the SIFs move along the microtubule tracks and require the activities of the motor proteins kinesin and dynein. The identification of the roles of motor proteins in this context requires inhibiting their activities either by siRNA-mediated silencing or expression of dominant negative alleles. However, these approaches render the cells compromised even before the bacterial infection. Hence, in the present investigation, initial attempt was made to microinject specific antibodies against motor proteins at a specific time after the infection and characterize the roles of motor proteins in the intracellular life of *Salmonella* and dynamics of SIFs.

MPP70

Whole genome analysis and distribution of virulence genes in *Streptococcus gallolyticus* subsp. *gallolyticus,* an emerging pathogen of infective endocarditis

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Background: Infectious endocarditis (IE) is a disease with rising incidence and a high degree of mortality and morbidity. 16s rDNA PCR analysis of explanted human heart valves revealed a significant relevance of *Streptococcus gallolyticus* subsp. *gallolyticus* (Sgg) (former *S. bovis* biotype I) as a causative agent. Furthermore, the knowledge of virulence factors that contribute to the pathogenesis in Sgg IE is still limited.
Material and Methods: To elucidate potential virulence genes of this bacterium, we performed ultra-high-throughput DNA sequencing using the 454 sequencing platform. The selected strain was isolated in 2003 at the Herz- und Diabeteszentrum Nordrhein-Westfalen from an endocarditis-derived blood culture of a 68- year- old woman with aortic heart valve endocarditis. The GenDB and EDGAR software were used for genomic

analysis and comparison. Detection of microbial surface component recognizing matrix molecules (MSCRAMMs) in 41 isolates from different origins (clinical isolates IE patients, animal isolates, reference strains) was performed by PCR analysis. Nick translation DIG labeling of plasmid DNA and Southern blotting was used for plasmid screening.

Results: The genome of Sgg strain BAA-2069 contains a 2,356,444 bp circular DNA molecule with a G+C-content of 37.65% and a 20,765 bp plasmid designated as pSGG1. Bioinformatic analysis predicted 2309 ORFs and the presence of 80 tRNAs and 21 rRNAs. Additionally, 21 ORFs were detected on the plasmid pSGG1, including tetracycline resistance genes *telL* and *tetM*. Screening of 41 Sgg isolates revealed one plasmid (pSGG2) homologous to pSGG1.

Furthermore, 21 adhesion proteins containing the cell wall-sorting motif LPxTG, which were shown to play a functional role in the pathogenesis, were identified. Screening of 41 Sgg strains for these proteins revealed a heterogeneous distribution, correlating with binding characteristics to ECM-molecules. The whole genome comparison to the recently sequenced Sgg strain UCN34 revealed significant differences, including habitant and host-pathogen interacting characteristics. In order to detect a larger amount of putative virulence affecting genes in different strains, we designed a DNA microarray experiment.

Conclusions: The analysis of the whole genome sequence of Sgg advanced the understanding of genetic factors concerning pathogenesis and adhesion to the ECM of this pathogen. Furthermore we showed for the first time the occurrence of a resistance plasmid (pSGG1) with characteristics as a vector for lateral genetic transfer. The alignment of microarray data and already evaluated phenotypic data, including ECM-binding, adhesion and invasion to host cells and biofilm formation capability of 41 strains, will substantiate the underlying genomic based pathomechanisms.

MPP71

Characterization of the large Pmp adhesin family of *Chlamydia pneumoniae*

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Chlamydiae are obligate intracellular bacteria that are responsible for a wide range of diseases of significant importance to clinical and public health. *C. trachomatis* is a leading cause of sexually transmitted disease and blinding trachoma. *C. pneumoniae* is an important respiratory pathogen recognized as a common cause of community acquired pneumonia.

Bioinformatical studies have led to the identification of the large family of putatively autotransported polymorphic membrane proteins (Pmps) with 9 members in *C. trachomatis* and 21 in *C. pneumoniae*, which are divided into 6 subtypes. In previous studies, we characterized the cell surface localized protein Pmp21 as a bacterial adhesin mediating attachment of *C. pneumoniae* to human cells. Furthermore, domain and mutational analysis identified the two Pmp-characteristic repetitive motifs GGA(I,L,V) and FxxN to be essential for the adhesion process (Mölleken et al, 2010).

We have now characterized the other subtypes of the Pmp protein family in the adhesion and infection process by selecting representative candidates of each Pmp subtype from *C. pneumoniae* and 2 orthologues from *C. trachomatis*. Using the yeast display system as well as latex beads coated with recombinant protein, all tested Pmps were found to act as adhesins. The Pmp adhesins are important for the *C. pneumoniae* infection process, as preincubation of human cells with the different recombinant proteins reduced subsequent infection. Interestingly, using far western experiments we identified an interaction of Pmp21 with itself as with all other Pmps tested possibly suggesting that homo- and/or heteromeric Pmp adhesion complexes might be formed on the chlamydial cell surface. Moreover, we examined the expression and post-translational processing of the proteins Pmp6, Pmp20, and Pmp21 during the infection cycle of *C. pneumoniae*. The processing was not uniform but a Pmp-specific set of cleaved forms was detected in agreement with previous findings about Pmps being autotransporter proteins. Cell fractionation experiments revealed that cleaved Pmp-fragments are partially released from the chlamydial surface and may exist in the inclusion lumen and possibly in the host cytosol perhaps serving an additional function as effector proteins. Most interestingly, using immunofluorescence microscopy we detected that individual Pmp proteins were expressed only in a fraction of the inclusions analyzed possibly indicating a form of antigenic variation similar to what recently has been described for Pmp proteins from *C. trachomatis*.

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MPP72

The human pathogen *Chlamydia pneumoniae* nucleates and stabilizes F-actin via the secreted Cpn0572 protein to support host cell entry

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Chlamydiae are gram-negative obligate intracellular bacterial pathogens of humans and animals. Among *Chlamydiae* species, *C. pneumoniae* is a common respiratory pathogen. Like other pathogenic bacteria, It is believed that *Chlamydiae* deliver effector proteins to recruit F-actin at the site of entry to trigger their own entry into the host cell. Among the few chlamydial effector proteins identified thus far, the *C. trachomatis* Tarp is translocated into the host cell where it colocalizes with F-actin underneath the attached bacteria and nucleates actin polymerization (Clifton et al. PNAS 2004, Jewett et al. PNAS 2006). In this study, we characterized the *C. pneumoniae* homologous protein Cpn0572. Cpn0572 is present in the infectious form of the bacteria. About 20 minutes after infection Cpn0572 is secreted and could be found associated with the host F-actin. To analyze the role of Cpn0572 in modulating the actin cytoskeleton, we used the yeast *Saccaromyces cerevisiae* as a model system. Cpn0572-expressing yeast cells exhibited a strongly reduced growth phenotype. The analysis revealed that Cpn0572 transformed the yeast actin cytoskeleton into 1-2 actin clumps per cell. The GFP-Cpn0572 fusion protein exclusively colocalized with these F-actin clumps. Similar observations were obtained in Cpn0572 transfected HEK-239 cells. This actin phenotype is reminiscent of actin clumps formed in yeast and human cells treated with Jasplakinolide, a drug that induces actin polymerization and inhibits actin depolymerization. Indeed, we could show that in yeast Cpn0572 stabilizes F-actin against the actin destabilizing drug Latrunculin A implying that Cpn0572 inhibits actin depolymerization. Furthermore, we found evidence in vivo that Cpn0572 competes with and/or displaces the actin depolymerizing factor cofilin from binding F-actin in yeast. These results were extended to in vitro experiments using mammalian F-actin and cofilin proteins, in which we could show that presence of Cpn0572 abrogates cofilin binding to F-actin. In line with these data the actin clumps in Cpn0572 transfected HEK-293 cells were resistant to the actin depolymerizing drug cytochalasin D suggesting that Cpn0572 is indeed able to inhibit actin depolymerization in human cells. Additionally, we showed also that recombinant Cpn0572 alone induces actin nucleation in vitro independent from other cellular proteins. Finally, a deletion analysis identified a central domain within Cpn0572 (DUF1547) which is crucial for interaction with F-actin. Conserved hydrophobic amino acids within this domain were identified as being essential for this interaction. To our knowledge this is the first bacterial protein that exerts a dual function by inhibiting actin depolymerization and nucleating F-actin with the overall goal to maximize F-actin accumulation at the site of bacterial entry.

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MPP73

Auto- regulation of the *agr* peptide sensing system in *Listeria monocytogenes*

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Listeria monocytogenes is the causative agent of listeriosis, a common and highly infectious disease. Clinical manifestations are gastroenteritis, diarrhoea, and meningitis which in immune compromised, elderly or pregnant people frequently results in fatal outcomes. During the first step of infection the intracellular pathogen *Listeria monocytogenes* is located in phagosomes. Before maturing to fully acidified phagolysosomes, *L. monocytogenes* is able to actively lyse the membrane of this compartment and escapes to the cytosol where it replicates.

L. monocytogenes is equipped with a peptide sensing system which is homologous to the *Staphylococcus aureus agr* system and has been shown to be involved in the regulation of biofilm formation, *in vitro* and *in vivo* virulence and global gene expression. In *S. aureus*, expression of the *agr* system consists of four genes encoding for an export- and processing protein (AgrB), a putative auto-inducing peptide (AgrD), a response regulator (AgrA), and a receptor kinase (AgrC) and its expression is controlled by the P_{II} promoter.

Preliminary experiments with a P_{II}-*lux*-fusion showed a lack of measurable luciferase activity in a deletion mutant of *L. monocytogenes* EGDe which lacks *agrD*, the gene encoding the putative auto inducing peptide. To test, if the listerial AgrA acts as a transcription factor which, like the homolog in *S.aureus*, binds to the P_{II} promoter a 6xHis-AgrA fusion was expressed in *E.coli* BL21 (DE3), isolated and purified. The recombinant protein was tested for its binding capacity by electrophoretic mobility shift assay (EMSA). 6xHis-AgrA was able to shift the full length P_{II} promoter

sequence. This strongly suggests that the *agr* system of *L. monocytogenes* is subject to an auto-regulatory circuit.
A consensus AgrA-binding motif was established by in silico analysis of promoter sequences of the available peptide sensing systems from a variety of Gram-positive bacteria. Using this consensus motif a number of putative binding sites were identified in the listerial P_{II} promoter sequence. These putative binding sites were analysed by EMSA using different fragments of the P_{II} promoter and mutational alteration of the binding motifs.
Interestingly, a putative AgrA-binding motif was identifies in promoter of -*prfA*, the major virulence regulator of *L. monocytogenes* which could explain the strong regulation of this gene in the *agrD*-deletion mutant observed previously. Indeed, 6xHis-AgrA was able to shift a PCR-amplified prfA promoter. This further confirms the involvement of *agr* peptide sensing in virulence regulation of *L. monocytogene*s and is the first indication of a direct regulation of virulence genes in *L. monocytogenes* by the *agr* system.

MPP74

The antibiotic ADEP inhibits cell division in *Staphylococcus aureus* and other Gram-positive bacteria

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A novel class of antibacterial acyldepsipeptides (ADEPs) exerts prominent antibacterial activity against Gram-positive bacteria including multi-resistant *Staphylococcus aureus in vitro* and *in vivo* [1]. ADEPs act via a yet unprecedented mechanism by dysregulating the caseinolytic protease ClpP. Usually, the activity of ClpP is tightly controlled by ATP-dependent Clp-ATPases and accessory proteins. ADEPs overcome these tight control mechanisms, switching ClpP from a regulated to an uncontrolled protease that predominantly targets unfolded or flexible proteins as well as nascent polypeptides at the ribosome in the absence of Clp-ATPases [1, 2]. Although the activity of ADEPs can be explained on the molecular level of its target ClpP, the specific events that finally lead to bacterial cell death still remained unknown.
In our study, we investigated the effect of ADEP treatment on different Gram-positive species using high-resolution microscopy. In the presence of low inhibitory ADEP concentrations, the coccoid cells of *S. aureus* and *Streptococcus pneumoniae* swelled to more than 3-fold the volume of wild type cells, and the rod-shaped cells of *B. subtilis* grew into very long filaments, which reached 60- to 100-fold the length of untreated cells, clearly indicating stalled bacterial cell division. To gain further insights into the underlying molecular mechanism, we followed the events that led to the inhibition of cell division. Cell division in Gram-positive bacteria is concerted by the divisome, which is highly dynamic and is characterized by a time-dependent two-step assembly of specific cell division proteins like FtsZ, FtsA, ZapA and EzrA as well as PBP2B, FtsW, DivIVA, etc. [3]. We observed that ADEP treatment led to the inhibition of septum formation in *S. aureus* and *B. subtilis,* while chromosome segregation was rather unaffected. Localization studies with GFP-labeled cell division proteins revealed that the ADEP-ClpP complex interferes with key components of early cell division and therefore perturbs normal divisome formation. By analyzing cell extracts of ADEP-treated *S. aureus* and *B. subtilis* cells, immunoblotting revealed that the ADEP-ClpP proteolytic complex degrades essential components of the cell division machinery and, hence, destines the bacteria to death. ADEPs demonstrate that beside their interesting antibacterial potency they are excellent tools to examine central mechanism of bacterial physiology, like cell division and regulated proteolysis.

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MPP75

Genome sequence and morphological characterization of a *Staphylococcus aureus* mutant with reduced susceptibility to vancomycin and daptomycin

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Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are commonly treated with the glycopeptide vancomycin. However, MRSA strains with reduced susceptibility to vancomycin have emerged during the last decade. Due to increased antibiotic treatment failure, there is an obvious need to understand how bacteria develop resistance. In an approach to study the impact of an elevated mutation frequency on vancomycin resistance development, we had previously isolated the highly vancomycin resistant *S. aureus* strain VC40 (MIC: 64 µg/ml) after serial passage of strain RN4220Δ*mutS* (MIC: 2 µg/ml), a *mutS* gene deletion mutant of the laboratory strain RN4220, in the presence of increasing concentrations of vancomycin (1).
In the current study, antimicrobial susceptibility testing of strain VC40 additionally revealed cross resistance against the lipopeptide antibiotic daptomycin, which has recently been introduced for the treatment of complicated skin and skin structure infections (cSSSI) caused by MRSA. To gain further insights into the resistance phenotype of *S. aureus* VC40, the full genome sequences of this strain and its parent strain *S. aureus* RN4220Δ*mutS* were determined using 454 sequencing technology. In strain VC40, a total of 79 mutations in genes related to cell wall metabolism, transport and gene regulation, including the two-component regulatory systems VraSR and WalKR, were detected. Since the latter regulatory proteins significantly affect the biosynthesis and turnover rates of the bacterial cell wall (2,3), we performed further morphological studies using transmission electron microscopy. Here, strain VC40 was characterized by an abnormal cell envelope morphology including an increased cell wall thickness that may result from deregulated VraRS or WalKR systems. Cell wall thickening has previously been linked to vancomycin resistance and even has been correlated with daptomycin cross resistance (4). However, these findings alone cannot explain the high-level resistance phenotype of strain VC40. Further characterization of strain VC40 is necessary and will provide important information on how the accumulation of mutations resulted in the autonomous development of vancomycin and daptomycin high-level resistance in *S. aureus*.

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MPP76

Chlamydia trachomatis actively modifies its host-cell-niche during both acute and persistent infection

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Chlamydia trachomatis is specialized for survival and growth within the human epithelial host cell. *In vivo*, the bacteria very likely face adverse replication conditions, in particular when the immune response is activated and cytokines are secreted. Such conditions can be mimicked in a number of ways, for instance by the addition of β-lactam antibiotics to cultures *in vitro*. In these conditions Chlamydiae enter a so-called “persistent” state. The persistent phenotype is characterized by enlarged, aberrant reticulate bodies (RBs; the replicative form of *Chlamydia*), which are neither able to divide nor to re-differentiate into infectious elementary bodies (EBs) but which can be re-activated upon withdrawal of the persistence-inducing reagent.
As persistent infections are thought to be relevant for the outcome of chlamydial disease, especially in chronic infections, we are investigating the development of the chlamydial inclusion during this persistent state. Infection of human cervical epithelial cells (HeLas), mouse oviduct epithelial cells (C57epi.1) and mouse embryonic fibroblasts (MEFs) with *C. trachomatis* L2 in the presence of penicillin G resulted in the development of aberrant, non-proliferating RBs. Although there were only few of these RBs per inclusion, the vacuole grew substantially in size. We have proposed that growth of the inclusion during productive infection requires the secreted chlamydial protease CPAF. During persistent infection, expression and activity of CPAF was also detectable over a time course of 64 hours. Maturation of *C. trachomatis* is dependent on host cell lipids, which are acquired upon CPAF-dependent fragmentation of the Golgi

apparatus. During penicillin-induced persistence Golgi fragmentation likewise occurred, resulting in the formation of Golgi ministacks around the inclusion. These results suggest that even upon entry into the 'persistent' state during treatment with penicillin G, *C. trachomatis* can maintain the machinery for lipid acquisition and inclusion growth, very likely by maintaining production and secretion of CPAF. Understanding this chlamydial activity may help understanding persistent infection *in vivo*.

MPP77

Differences in protein A expression correlate with functional differences and reflect intra-strain diversity in *S. aureus* small colony variants from a single patient

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Introduction: *Staphylococcus aureus* small colony variants (SCV) are frequently isolated from cystic fibrosis patients. Defective airway clearance and prolonged antibiotic treatment predisposes for SCV formation.
Objectives: To assess the relevance of intra-strain diversity on the host-pathogen interaction in the present study we analyzed consecutive *S. aureus* SCV isolates from the same patient.
Methods and Results: Pulsed-field gel electrophoresis and MALDI biotyping demonstrated strain relatedness of all SCV isolates. Nevertheless, SCV isolates could be distinguished by *spa*-typing, e.g. one isolate displayed a truncated *spa*-type. Interestingly, Western blot analysis of protein A expression revealed that the truncated *spa* gene was associated with a protein of decreased size, and that two SCV isolates could be distinguished by increased protein A expression. For functional comparison of SCV isolates we compared TLR2 activity and biofilm formation. The results obtained indicated that increased SpA expression correlated with increased TLR2 activity and biofilm forming capacity. An enhancing role for protein A was subsequently confirmed by comparison of a *spa*-deficient *S. aureus* mutant SA113 D*spa* to the isogenic wild type strain SA113 WT in TLR2 and biofilm assays.
Conclusions: We conclude that quantitative differences in protein A expression reflect intra-strain variability and translate to functional differences modulating the host-pathogen interaction. However, we can only speculate that concomitant changes observed in the microbial environment (e.g. infection and colonization with other microbial species), in the pulmonary and clinical status of the patient and in the antibiotic regimes may be responsible for the functional and genetic changes in the SCV phenotype.

MPP78

Surface-associated motility of *Acinetobacter baumannii* depends on 1,3-diaminopropane

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While surface-associated motility has long been described in *Acinetobacter* lacking flagella, the mechanism of motility has never been elucidated. *Acinetobacter baumannii*, an often multidrug-resistant nosocomial pathogen, may profit from motility during infection or while persisting in the hospital environment. To identify determinants of *Acinetobacter* motility, a library of transposon mutants of strain ATCC 17978 was generated and screened for mutants with motility defects. We could identify two akinetic mutants carrying transposon insertions in the *dat* and *ddc* gene, respectively. These neighbouring genes encode L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase and L-2,4-diaminobutyrate decarboxylase, respectively, and contribute to synthesis of 1,3-diaminopropane (DAP), a polyamine ubiquitously produced in *Acinetobacter*. The motility defect of both mutants could be cured by supplementing semi-solid media with DAP. Making use of naturally competent clinical *Acinetobacter* isolates, we verified by transformation of the mutants' DNA that *ddc* and *dat* were required for motility in different isolates. Collectively, surface-associated motility of *Acinetobacter baumannii* requires DAP. Current work aims at elucidating how DAP contributes to motility.

MPP79

Substrate screening of the serine protease EspP from enterohemorrhagic Escherichia coli (EHEC)

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Enterohemorrhagic Escherichia coli (EHEC) naturally occur in the intestinal tract of cattle and other ruminants. Humans are mainly infected via contaminated animal food. In addition, infections via vegetables are possible, as shown by the current outbreak in Germany. The clinical outcome of EHEC infection range from intestinal dysfunctions to the life-threatening hemolytic-uremic syndrome. To date, systematic therapy is hampered by the fact that the detailed mechanism of pathogenicity is still not fully understood. EHEC express a number of factors that might contribute to virulence. One of them is the plasmid-encoded extracellular serine protease EspP. It has been shown that EspP might interact with hemostasis and immune response (1,2) but a systematic screening of relevant substrates is still lacking. We therefore screened for novel substrates of EspP using human plasma and platelets. Plasma was first depleted from albumins and IgG and incubated with purified recombinant EspP or the inactive mutant S263A. Fragmentation of plasma proteins was assessed by SDS-PAGE or 2D gel electrophoresis and compared to the non-incubated proteins. Analysis of EspP substrates in platelets was performed with platelet lysates to obtain whole cell extracts or collagen-activated platelets to analyze the secreted proteins. Following incubation with EspP or S263A protein pattern were investigated by aforementioned electrophoresis techniques. To indentify novel substrates, protein spots that shifted in gel electrophoretic mobility compared to the negative control were digested in-gel and subjected to mass spectrometrical analysis using MALDI-TOF-MS as well as nano-ESI-MS². Altogether, it has been shown that EspP cleaves several physiologically relevant proteins in plasma as well as in platelets including protease inhibitors and surface adhesion molecules. Further studies will elucidate the contribution of the newly identified substrates to the role of EspP as a possible virulence factor of EHEC.

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MPP80

HlyA from *E. coli* is secreted in an unfolded state and requires the C-terminal located amino acids for its lytic function.

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Escherichia coli haemolysin A (HlyA), an RTX toxin, is secreted probably as an unfolded intermediate, by the type I (ABC transporter-dependent) pathway, utilizing a C-terminal secretion signal. However, the mechanism of translocation and post-translocation folding is not understood.
We identified a mutation (hlyA99) at the extreme C terminus, which is dominant in competition experiments, blocking secretion of the wild-type toxin co-expressed in the same cell. This suggests that unlike recessive mutations which affect recognition of the translocation machinery, the hlyA99 mutation interferes with some later step in secretion. Indeed, the mutation reduced haemolytic activity of the toxin and the activity of beta-lactamase when the latter was fused to a C-terminal 23 kDa fragment of HlyA carrying the hlyA99 mutation. A second mutant (hlyAdel6), lacking the six C-terminal residues of HlyA, also showed reduced haemolytic activity and neither mutant protein regained normal haemolytic activity in in vitro unfolding/refolding experiments. Tryptophan fluorescence spectroscopy indicated differences in structure between the secreted forms of wild-type HlyA and the HlyA Del6 mutant. These results suggested that the mutations affected the correct folding of both HlyA and the beta-lactamase fusion. Thus, we propose a dual function for the HlyA C terminus involving an important role in post-translocation folding as well as targeting HlyA for secretion.
The Hly translocator complex exports a variety of passenger proteins when fused N-terminal to this secretion sequence. However, not all fusions are secreted efficiently. Here, we demonstrate that the maltose binding protein (MalE) lacking its natural export signal and fused to the HlyA secretion signal is poorly secreted by the Hly system. Anticipating that folding kinetics might be limiting, we introduced the 'folding' mutation Y283D into MalE and indeed the fusion protein was now secreted at a much higher level. This level was further enhanced by the introduction of a second MalE folding mutation (V8G or A276G). Folding analysis revealed that all mutations reduced the refolding rate of the substrate, whereas the unfolding

rate was unaffected. Thus, HlyA is secreted in a unfolded state and folds into its lytic conformation upon binding of calcium ion in the medium.

MPP81

Lipase A *of Pseudomonas aeruginosa* is involved in pyoverdine production

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One of the most essential elements for living of organism is iron. Iron acts as a cofactor for a range of redox enzymes which are involved in many central cellular processes like electron transport or DNA synthesis, it is also essential for amino acid and pyrimidine biosynthesis [1]. One characteristic feature of fluorescent *pseudomonads* is their ability to produce the yellow-green fluorescent pigment pyoverdine as their major siderophore [2]. The best studied pyoverdine is that of the opportunistic human pathogen *Pseudomonas aeruginosa*, where it is required for growth and plays a major role in virulence [3]. Among other factors, pyoverdine biosynthesis in *P. aeruginosa* is regulated by the iron starvation (IS) sigma factor PvdS [4].
We have applied a proteomic approach using the 2D DIGE system and transcriptional analysis by qPCR to study the role *of P. aeruginosa* lipase LipA for pyoverdine production. We clearly demonstrated that the lipase has a regulatory effect on the *pvdS* transcription.
A *lipA* negative mutant of *P. aeruginosa* (*ΔlipA*) showed a significant lower pyoverdine concentration in culture supernatant compared to the wild type. In addition, the secretome of *P. aeruginosa* Δ*lipA* revealed differences in PvdS regulated proteins including the proteases PrpL and AprA. These proteins are only present in the *lipA* deficient strain when lipase expression is complemented in trans. Results obtained by qPCR revealed a significantly decreased level of *pvdS* m-RNA in *P. aeruginosa ΔlipA* and, as a consequence, the transcription of several other PvdS regulated genes was also down-regulated.
These results indicate that the LipA plays an important role in the regulation of the iron starvation sigma factor PvdS in *P. aeruginosa* thereby influencing the synthesis of pyoverdine and other virulence factors.

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MPP82

Two novel subtilases influence virulence associated phenotypes of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is ubiquitously distributed, living in wet or humid surroundings ranging from soil to the human body and it produces a huge variety of extracellular proteins. An important part of the arsenal of bacterial virulence factors are proteases. Proteases are enzymes that hydrolyze peptide bonds and can therefore degrade proteins and peptides. As proteins are one of the basic building blocks in nature, proteases can influence a broad range of biological functions including the infection process. The opportunistic human pathogen *P. aeruginosa* has an arsenal of efficient proteases that helps establishing and maintaining an infection and thereby controlling and modifying the environment according to the needs of the bacterium within the host tissue. Some of these proteases, like Elastase and Protease IV, are well characterized but others exist, of which nothing is known (Hoge *et al*., 2010). We focused on serine proteases that belong to the peptidase S8 family (subtilases). These subtilases are encoded as preproenzyme. They carry a signal peptide that drives their translocation through the cytoplasmic membrane and a propeptide that acts as a folding mediator required to give the protease its final native conformation. By homology, we have identified the open reading frames *PA1242 (sprP)* and *PA3535 (sprS)* in the genome sequence of *P. aeruginosa* PAO1.
The gene *sprP* encodes for a so far hypothetical protein as a putative member of the subtilisin-like serine protease family S8. The gene product of *sprP* contains a predicted signal sequence and a peptidase S8 domain. Sequence analysis revealed the presence of an additional element in the domain organization of the protease. SprP carries, in addition to its signal peptide and the S8 domain, a domain of unknown function (DUF) between both elements. After the protease activity of SprP was demonstrated, we characterized different virulence associated phenotypes. We were able to illustrate an eminent role of SprP for the virulence of *P. aeruginosa* because the deletion of *sprP* resulted in a loss of motility, an increased biofilm formation, and the accumulation of cell aggregates during growth.
The second subtilase SprS belongs to the autotransporter protein family (type V secretion). In the case of SprS, the N-terminal passenger domain contains the protease function. Similar to the *sprP* deletion strain, the *P. aeruginosa* D*sprS* showed a loss of motility and an increased biofilm formation. Additionally the secretome showed that the *sprS*-negative strain produces less virulence associated extracellular proteases compared to the wild type. Initial results suggest a regulatory relationship in between these two proteases.
Finally, these results indicate that the two novel proteases, SprP and SprS, play a role in *P. aeruginosa* virulence.

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MPP83

Role of Iha in the virulence of enterohemorrhagic *Escherichia coli* O157:H7

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The iron-regulated gene A (IrgA) homologue adhesin (Iha) is encoded in O island (OI) 43 and 48 in the genome of prototypic enterohemorrhagic *E. coli* (EHEC) O157:H7 strain EDL933. The role of this molecule in the virulence of EHEC O157 is incompletely understood. In uropathogenic *E. coli* Iha functions as a siderophore receptor. We identified several EHEC O157:H7 strains that underwent spontaneous internal or complete deletions of OI-43 and/or OI-48 leading to *iha* loss. The *iha*-negative derivatives displayed, under iron-limited conditions that promote *iha* transcription, significantly lower adherence to human intestinal epithelial cells than did their *iha*-positive parental strains. Moreover, *iha* loss reduced the ability of strains to grow in liquid as well as on solid media under iron limitation. Isogenic *iha* deletion mutant of the parental strain displayed adherence capacity and growth ability comparable to that of the spontaneous *iha*-negative derivative, whereas *iha* complementation of the *iha* deletion mutant and the *iha*-negative spontaneous derivative restored the adherence and the growth speed to the level of the parental strain. These data suggest that Iha plays dual roles in the virulence and/or fitness of EHEC O157:H7, as an adhesin and a siderophore receptor.

MPP84

The *S. epidermidis* accumulation associated protein Aap: proteolytic processing and consequences for biofilm formation

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Implant associated Staphylococcus epidermidis infections essentially depend on biofilm formation. S. epidermidis biofilm formation depends on polysaccharide PIA and accumulation associated protein (Aap). 160 kDa Aap is essentially consists of an N-terminal domain A (aa 1 - 609) and a repetitive C-terminal domain B. Importantly, Aap mediates biofilm formation only after proteolytic processing, during which the A domain is removed. In turn, the repetitive B domain then induces cell cluster formation. Therefore, proteolytic processing is of fundamental importance for Aap function, however, the underlying mechanisms and proteases involved have not been characterized. Here we used specific S. epidermidis mutants, recombinant Aap sub-domains and N-terminal sequencing to characterize dynamic Aap processing. Aap is cleaved at least at three positions. Two cleavage sites were identified within the A domain at position aa 283 and 335. A third cleavage site is located at the junction between domain A and B at position of aa 596. By using supernantants from protease overexpressing S. epidermidis mutant 1457-M10sarA and mutant 1457-M10sarAsepA processing of Aap was assigned to metalloprotease SepA. Experiments using protease inhibitors suggest that Aap cleavage could also occur through additional proteases. Our results indicate that S. epidermidis uses specific proteases for the controlled and coordinated processing and functional activation of Aap. Given the importance of Aap-mediated S. epidermidis biofilm formation for pathogenesis of foreign-material associated infections our results could open new ways for the future development of therapeutic approaches, e.g.

by interference with protease dependent dynamics of pro-aggregative S. epidermidis cell surface properties.

MPP85

Indoleamine 2,3-dioxygenase (IDO) antimicrobial function is abrogated under hypoxia

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Tryptophan is an essential amino acid for human beings as well as for microorganisms. Therefore, the reduction of local tryptophan concentrations in tissues has an influence on the growth of microorganisms that have invaded the body. In human cells the IFN-γ inducible enzyme indoleamine 2,3-dioxygenase (IDO) degrades tryptophan and is therefore able to mediate an antimicrobial effect. It could be shown, that in *in vitro* systems an IDO activity inhibits the growth of bacteria, parasites or viruses. Additionally, it was observed that IDO has immunoregulatory functions, as it controls the activation and survival of T-cells.
IDO is a dioxygenase that catalyses the oxidative cleavage of tryptophan. Up to now, however, it remained unclear under which oxygen conditions IDO promotes its antimicrobial and immunoregulatory effects. Therefore, the enzymatic activity of IDO in different tissues with variable oxygen supply needs to be clarified.
In recent studies we could show that the activity of IDO is inhibited under hypoxic conditions *in vitro* (1% O_2). In comparison to normoxic conditions, IDO produces a reduced amount of kynurenine, the downstream product of tryptophan. In line with this observation, the antibacterial effect is abrogated under hypoxia. We could find that the IDO-mediated inhibition of *Staphylococcus aureus* as well as group B *Streptococcus* growth was abrogated in IFN-γ stimulated human glioblastoma cells or HeLa-cells, respectively.
Hypoxia not only impaired IDO enzyme activity but also resulted in reduced IFN- mediated signaling via the JAK-Stat pathway. IFN- induced IDO enzyme activity and JAK-Stat signaling were not inhibited when oxygen concentrations were >5% O_2.
These findings indicate that the influence of the oxygen concentration on IDO function could be important for the defense against several pathogens that affect organs with different oxygen supply.

MPP86

A novel phospholipase B from *Pseudomonas aeruginosa*

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Phospholipases A (PLA) represent a group of bacterial virulence factors with the ability to interact with host membranes and lipid signalling. Only a few PLA are proven virulence factors[1,2] but the total number of known PLA is still limited and the details about host-pathogen interaction mechanisms involving PLA remain to be elucidated. The opportunistic human pathogen *Pseudomonas aeruginosa* produces a variety of virulence factors; however, a PLA has not been described so far.
A comprehensive *in silico* analyses of the *P. aeruginosa* PA01 genome revealed number of genes encoding putative lipolytic enzymes. Here, we describe the identification and characterization of **PlbF**, a novel phospholipase B produced by *P. aeruginosa*. PlbF displaying sequence homology to prokaryotic and eukaryotic PLA, lipases and esterases involved in lipid metabolism and lipid signalling. We succeeded to purify this enzyme and to demonstrate its esterase and phospholipase B activities. Subcellular localisation experiments revealed its association with the membrane fraction of *P. aeruginosa*. More detailed localisation studies suggested that PlbF is most likely anchored to the bacterial inner membrane, as an integral membrane protein. Our experimental data are in agreement with *in silico* analyses which have predicted the presence of hydrophobic transmembrane helices in the N-terminal sequence of PlbF. Additionally, we recognised within the PlbF amino acid sequence a signature motif present in integral membrane proteins. This motif is required for helix-helix interactions of inner membrane proteins, suggesting that PlbF may exist as a dimer. This assumption was experimentally confirmed by cross linking experiments.
The contribution of PlbF to *P. aeruginosa* virulence was investigated in a *D. melanogaster* virulence modelby using a *plbF* negative *P. aeruginosa* mutant which was compared to the wild type strain *P. aeruginosa* PA01. Significant attenuation of the *plbF* negative mutant strain in killing of flies was observed confirming PlbF may represent a novel virulence factor produced by *P. aeruginosa*.
Further investigations of the cellular function of PlbF, particularly the relation of PlbF dimerisation and virulence of *P. aeruginosa* PAO1 might elucidate novel mechanisms of PLA-based pathogenicity mechanisms in bacteria.

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MPP87

Characterisation of a T-box regulatory RNA element governing methionine biosynthesis gene transcription in *Staphylococcus aureus* and *Staphylococcus epidermidis*

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Non-coding small RNAs (ncRNAs) have been recognised as important players in regulatory networks controlling gene expression in bacteria, and recent research has led to profound insights into their way of action. Thus, while *trans*-encoded ncRNAs often act through direct base pair interaction with target-mRNAs, *cis*-encoded ncRNAs directly regulate the transcript within which they are encoded. Many cis-encoded ncRNAs are located in the 5' untranslated region (5' UTR) of genes and operons and control downstream gene transcription by acting as riboswitches through the binding of various metabolites.
Here we describe the detection and characterisation of a novel *cis*-encoded ncRNA in the 5' UTR preceding the methionine biosynthesis genes in *S. aureus*, *S. epidermidis* and other staphylococci. Bioinformatic analyses of the staphylococcal *met* leader RNA revealed homologies and predicted structural elements associated with *Bacillus* T-box systems which represent terminator/antiterminator transcription control elements of Gram-positive bacteria that undergo conformational change upon specific binding of charged or uncharged tRNAs.
In the staphylococcal *met* leader RNA conserved sequence stretches were identified such as a T-box motif and a transcription terminator, as well as, unexpectedly, a CodY binding site. *In vitro* binding assays with the *met* leader RNA and $tRNA^{Met}$ demonstrated specific binding interaction between the two RNA species, and mutational studies of the T-box motif revealed the importance of single nucleotides for tRNA binding. Transcript levels of the *met* leader RNA and the downstream methionine biosynthesis genes were shown to vary between different staphylococcal strains, but to be generally induced under methionine starvation. The pleiotropic repressor CodY seems to regulate the overall transcription of the T-box system, but does not interfere with the tight methionine-dependent regulation of the biosynthesis genes by the *met* leader RNA itself. Various *S. aureus* RNase mutants were investigated for their influence on *met* leader RNA and *met*-operon transcription and stability, and these experiments demonstrated the involvement of RNase Y, RNase J2 and RNase III in *met* leader RNA/ mRNA processing and degradation.
The combined data demonstrate that in staphylococci *de novo* methionine biosynthesis is obviously under the control of a T-box regulatory element. This is unusual and in contrast to other Gram-positive bacteria which regulate this pathway mostly by S-box systems which represent S-adenosyl methionine (SAM)-binding riboswitches.

MPP88

Simkania negevensis replicates in the host endoplasmic reticulum

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Simkania negevensis is a Chlamydia-like emerging pathogen implicated in pulmonary diseases. However, knowledge regarding its intracellular accommodation remains sparse. Here, we demonstrated that expansion of the S. negevensis vacuole within epithelial cells is accompanied by massive spatial reorganization of host mitochondria and endoplasmic reticulum (ER). Spatial reorganization was mitochondria- and ER- specific as the Golgi apparatus appeared intact and was positioned between the nucleus and the S. negevensis vacuole. Ultrastructural analysis and 3D reconstruction revealed that S. negevensis forms one large vacuole located within the ER lumen. Location of the vacuole within the ER led to the formation of a so far not described pathogen-containing triple membrane structure.

MPP89

Expression of the non-fimbrial large adhesin BapA of *Salmonella enterica sv.* Typhimurium

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Two adhesins were recently described at the locus of the *Salmonella* Pathogenicity Island 4 (SPI4) and SPI9 to be secreted by type 1 secretion systems (T1SSs) encoded at the same loci. SiiE of the SPI4 is necessary for adhesion to polarized epithelial cells whereas BapA was associated with biofilm formation. To overexpress BapA, its putative promoter was substituted with the SPI4-promoter by λ-red recombination in a ΔSPI4 background strain. Mutant and luciferase reporter strains of the *bap*-operon as well as csgDBA mutant strains were also created by λ-red recombination. All strains used were derived from NCTC 12023 (WT) strain. Western Blot (WB) and immunostaining were performed by using α-BapA kindly provided by Dr. Lasa, Spain. P*sii*-promoter exchange clones, ΔSPI4, *bapA* and *bapD* and WT strains were incubated either with rigorous shaking or in static conditions for 72 h at 37° or 30° C. Bacterial cells and supernatant aliquots were taken from 2 to 72 h at several time intervals for WB analysis and expression kinetics, which were performed using the luciferase reporter gen. Static cultures of BapA-overexpressing strains incubated at 37° C developed biofilm after 72 h. Further results from WB analyses revealed a band with the theoretical molecular weight of BapA in bacterial pellets after the first 24 h; then, as in bacterial pellets as in supernatants at 48 h; and finally, only supernatant at 72 h. Expression kinetics were contrary to the observed phenotypes obtained in WB with the WT strain, since expression of the *bapA* or *bapD* in static conditions was around 10-fold higher at 30° C than at 37° C after 8 h in WT background. One could conclude that BapA-transcript underground degradation so that no signal can be observed using WB. Finally, images from CLSM from bacterial biofilm revealed several cluster patterns. In comparison to WT strain, *bapA* strains formed very large clusters but BapA-overexpressing strains were found as small groups of bacteria containing 3-7 cells. On the other hand, single isolated bacterial cells were only observed using the *csgD* strain. Altogether, these results showed that BapA may play a role in the surface of the bacteria. Its secretion pattern depending on the time and medium conditions could suggest a protective function to the bacterial cell. Further analysis of regulation of the expression and its possible role of invasion/adhesion is currently in progress to understand the role of BapA in host cells.

MPV01

Helicobacter pylori HtrA is a new secreted virulence factor that cleaves E-cadherin to disrupt intercellular adhesion

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Helicobacter pylori (Hp) is a gram-negative bacterium that colonizes the gastric epithelium of approximately 50% of world`s population, leading to the development of inflammatory diseases and even gastric cancer. To promote full virulence, *Hp* disrupts the gastric epithelial barrier to enter the intercellular space. However, the underlying mechanism of *Hp*-induced epithelial barrier subversion is currently not well understood. Here, we report an entirely new function of the serine protease and chaperone HtrA as a new secreted virulence factor from *Hp*, which cleaves the ectodomain of E-cadherin, an important molecule in cell-cell adhesions and tumor suppressor.
Upon infection, we detected the extracellular E-cadherin domain as a soluble fragment in the supernatants of *Hp*-colonized gastric epithelial cells. Correspondingly the amount of full length E-cadherin in cellular lysates was drastically reduced. Using pharmacological inhibitors and RNAi we excluded the involvement of host cell proteases in E-cadherin degradation. However, we identified HtrA as a secreted bacterial factor exhibiting proteolytic activity. *In vitro* studies using recombinant E-cadherin revealed that active HtrA specifically cleaved the N-terminal ectodomain of E-Cadherin, whereas an inactive, point-mutated HtrA variant did not. Most importantly, using a computer assisted homology model of the *E. coli* HtrA homologue DegP we designed and synthesized the small-molecule *Hp*HtrA inhibitor HHI. HHI efficiently blocked HtrA activity in E-cadherin cleavage experiments and on *Hp*-colonized epithelial cells, thereby significantly reducing intercellular invasion of *Hp* through epithelial monolayers and without affecting bacterial viability. Taken together, these data imply, that HtrA mediated E-Cadherin shedding during *Hp* infection disrupts epithelial barrier functions thus allowing the bacteria to access the intercellular space. We assume that blocking of HtrA activity might help combat *Hp* persistence in the early phases of bacterial infection.

MPV02

Methionine sulfoxide reductases protect *Salmonella* Typhimurium from oxidative stress and are important for bacterial pathogenesis

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Introduction: Oxidative stress converts methionine to a mixture of methionine-*S*-sulfoxide (Met-*S*-SO) and methionine-*R*-sulfoxide (Met-*R*-SO). Methionine sulfoxide reductases protect bacteria by repairing oxidized methionine. MsrA and MsrB reduce MetSO, the former being specific for the *S*-form and the latter being specific for the *R*-form. In this study we characterize MsrA, MsrB and a third methionine sulfoxide reductase, fRMsr, in *S.* Typhimurium. fRMsr has been previously shown to be specific for free Met-*R*-SO in *E. coli.*
Methods: Methods used for this study are the generation of deletion mutants in *S.* Typhimurium by one-step inactivation via homologous recombination and the transduction with the bacteriophage P22*int*, H_2O_2 susceptibility assays, infection experiments in the macrophage cell line RAW264.7, competitive infections of Balb/cJ mice with *S.* Typhimurium, growth experiments of *Salmonella* on methionine and methionine sulfoxide, overexpression and purification of *S.* Typhimurium His-tagged MsrA and MsrB, NADPH linked methionine reductase activity assay.
Results: Here we show that deletion of *msrA* in *S.* Typhimurium increased susceptibility to exogenous H_2O_2 and reduced bacterial replication inside activated macrophages and in mice. In contrast, a Δ*msrB* mutant showed the wild type phenotype. We constructed Δ*fRMsr* and Δ*msrB* mutant strains in a methionine auxotrophic background of *S.* Typhimurium. The Δ*fRMsr* mutant but not the Δ*msrB* mutant failed to utilize free oxidized methionine. Recombinant MsrA was active against both free and peptidyl Met-*S*-SO, whereas recombinant MsrB was only weakly active and specific for peptidyl Met-*R*-SO. To examine the role of *fRMsr* in oxidative stress response we compared a Δ*fRMsr* single mutant and a Δ*fRMsr/msrB* double mutant, and found that fRMsr affects survival of *S.* Typhimurium following exposure to H_2O_2, growth in macrophages and in mice.
Conclusion: Thus in summary, we showed that mutants of *S.* Typhimurium lacking components of the methionine sulfoxide reductase pathway are attenuated *in vitro* when exposed to H_2O_2, inside activated macrophages and in mice. Previously, MsrA and MsrB were considered to be the principle enzymes of the *msr*-system that play a role in oxidative stress response. Here we showed that in addition fRMsr contributes significantly to antagonize the damage caused by oxidative stress in *S.* Typhimurium.

MPV03

Complex c-di-GMP signaling networks mediate the transition between biofilm formation and virulence properties in *Salmonella enterica* serovar Typhimurium

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Upon *Salmonella enterica* serovar Typhimurium infection of the gut, an early line of defense is the gastrointestinal epithelium which senses the pathogen and intrusion along the epithelial barrier is one of the first events towards disease. Recently, we showed that high intracellular amounts of the secondary messenger c-di-GMP in *S. typhimurium* abolished stimulation of a pro-inflammatory immune response and inhibition of invasion of the gastrointestinal epithelial cell line HT-29 suggesting regulation of transition between biofilm formation and virulence by c-di-GMP in the intestine. Here we show that highly complex c-di-GMP signaling networks consisting of distinct groups of c-di-GMP synthesizing and degrading proteins modulate the virulence phenotypes IL-8 production, invasion and *in vivo* colonization in the streptomycin-treated mouse model implying a spatial and timely modulation of virulence properties in *S. typhimurium* by c-di-GMP signaling. Inhibition of the invasion phenotype by c-di-GMP is associated with inhibition of secretion of the type three secretion system effector protein SipA. Inhibition of the invasion and IL-8 phenotype by c-di-GMP (partially) requires the major biofilm activator CsgD and/or BcsA the synthase for the extracellular matrix component cellulose. Our findings

show that c-di-GMP signaling is at least equally important in the regulation of *Salmonella*-host interaction as in the regulation of biofilm formation at ambient temperature.

MPV04

Microevolution of a nonfilamentous *Candida albicans* mutant during co-incubation with macrophages

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Candida albicans is both a successful commensal and a pathogen of humans that can infect a broad range of body sites. During the infection process, *C. albicans* has the potential to adapt to different host niches and to survive the attack of phagocytes, such as macrophages. Previous evidence suggests that microevolution plays a central role in this process. For example, Forche *et al.* [1] found that *C. albicans* rapidly reacts to its environment by undergoing chromosome-level genetic variations, which are sufficient to generate new variants of *C. albicans*. In this study, we used an *in vitro* experimental microevolution approach to identify factors and activities necessary for survival within, and escape from, macrophages. Using this system, we also investigated whether *C. albicans* can utilise alternative signal transduction pathways under selective pressure. First, we analysed a range of mutants with defects in hyphal-formation for capacity to escape from macrophages. Normally, four hours post-infection, more than 50 % of wild type cells escaped from macrophages. Although none of the tested mutants achieved such high escape rates, many were capable of lower levels of escape. An *efg1*/*cph1* double mutant, on the other hand, was entirely unable to escape. We therefore chose this strain for further investigation and monitored its adaptation to macrophages during a series of co-culture passages. After 23 passages, the *efg1*/*cph1* mutant began to exhibit dramatic phenotypic changes and formed filaments. These filaments enabled the evolved mutant to escape from macrophages. Surprisingly, the strain was also now able to form filaments under other conditions and, in contrast to the original *efg1*/*cph1* mutant, has regained the ability to invade epithelial cells. Furthermore, it has significantly higher virulence in an alternative model of infection. The phenotype was stable in the absence of selective pressure, indicating that microevolutionary events have bypassed the reliance on Cph1 and Efg1 for filamentation. QRT-PCR, PFGE and high-throughput sequencing did not indicate major chromosomal rearrangements. These and further investigations of the genetic cause of the adaptation will yield insights into the genomic and genetic plasticity of this important fungal pathogen during adaptation to specific host niches.

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MPV05

Identification of a novel plasminogen-binding protein of *Borrelia burgdorferi*

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Introduction: *Borrelia burgdorferi*, the etiological agent of Lyme disease exploits an array of strategies in order to overcome the host immune response and establish an infection. Those strategies involve interactions of spirochetes with diverse host proteins, including plasminogen, extracellular matrix proteins and complement regulators. A number of genetically unrelated outer surface proteins, i.e. OspA, OspC, CspA, ErpA, ErpC, and ErpP have previously been identified as ligands for host-derived plasminogen. Here we report the identification of a novel *Borrelia* plasminogen binding protein, BopA.
Material and Methods: Binding of plasminogen to BopA was first assessed using ELISA. To verify whether bound plasminogen/plasmin is proteolytically active, we performed substrate degradation assays utilizing both a chromogenic substrate, D-valyl-leucyl-lysine-*p*-nitroanilidedihydrocholride as well as the natural substrate fibrinogen. For further characterization of the BopA-plasminogen interacting region we created a number of truncated versions of BopA and investigated binding with various plasminogen constructs. Finally, the *bopA* gene expression was assessed using RT-PCR analysis.
Results: When compared to other borrelial proteins, BopA displayed the strongest binding capacity for plasminogen. Bound plasminogen was converted to proteolytically active plasmin in the presence of urokinase type plasminogen activator and subsequently cleaved the chromogenic substrate D-valyl-leucyl-lysine-*p*-nitroanilidedihydrochloride as well as the natural substrate fibrinogen. Employing diverse truncated borrelial proteins, the plasminogen interacting site has been mapped within the C-terminus of BopA. Lysine residues located within this particular α-helical C-terminal domain appeared to be crucial for binding plasminogen, as the lysine analog tranexamic acid significantly inhibited these interactions. By utilizing several plasminogen constructs containing single or multiple Kringle domains, it was determined that the Kringle domain 4 most likely represents the major BopA interaction site. Finally, RT-PCR analysis revealed that *in vitro* cultivated spirochetes expressed the BopA encoding gene.
Discussion: Here we present data on the identification and characterization of a novel plasminogen binding protein, BopA of *B. burgdorferi*. BopA binds host plasminogen and upon activation surface-bound plasmin proteolytically cleaved host fibrinogen. Binding of plasminogen/plasmin might endow *B. burgdorferi* with the capability to resist opsonization and to degrade components of the extracellular matrix. Furthermore, interaction with the plasminogen activation system represents an important virulence factor and enables spirochaetes to successfully establish an infection and disseminate more efficiently in the human host.

MPV06

Growth behavior and interaction of *Staphylococcus aureus* with neutrophils in a three-dimensional collagen gel as a tissue-like infection model

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In vitro infection studies are usually based on cell culture monolayers (two dimensional systems), which are infected with the respective pathogenic microorganisms. A limitation of this model is the restricted dimensionality which contrasts three dimensional (3D) host tissue environment. Using a 3D collagen gel setup (3D-CoG), it has been shown that *Yersinia enterocolitica* as a prototype of an extracellularly replicating pathogen exhibits unusual growth behaviour in this system, resembling *in vivo* microcolony formation in infected tissues (Freund *et al.*, 2008). Here we present first results of the growth behaviour of *Staphylococcus aureus* and its interaction with neutrophils in this model using confocal microscopy. *S. aureus* is known to multiply extracellularly in tissue, terminating in abscess formation due to neutrophil infiltration. In a first approach we compared the growth behavior of strain Newman and the derived mutants *saeRS* and *ica*. Surprisingly, we found that strain Newman and both mutants formed bacterial clusters similar to those observed in cell culture medium without collagen. In a second approach we supplemented the 3D-CoG with defined plasma proteins to create tissue-like conditions. Strikingly, strain Newman and the *ica* mutant formed microcolonies of uniform size which were surrounded by concentric structures of different optical density. In contrast, the *saeRS* mutant formed microcolonies devoid of these structures. Finally, we studied the interaction of murine neutrophils with *S. aureus* grown in 3D-CoG. In 3D-CoG alone neutrophils migrated towards *S. aureus* microcolonies and phagocytosed the microbes. In contrast, the concentric structures formed in 3D-CoG supplemented with plasma proteins were able to prevent neutrophils from infiltration and phagocytosis of *S. aureus* microcolonies. By including further mutants in our study we were able to identify several *S. aureus* pathogenicity factors which are involved in *S. aureus* protection against neutrophil attack.

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MPV07

Pathogen vacuole proteomics of *Legionella pneumophila*-infected phagocytes

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Legionella pneumophila is an opportunistic human pathogen and the causative agent of the severe pneumonia Legionnaires´ disease. Upon internalization of the Gram-negative bacterium into phagocytic cells, a unique "*Legionella*-containing vacuole" (LCV) is formed, the prerequisite for intracellular growth. Fusion with lysosomes is avoided, but LCVs acquire endosomal markers and associate with mitochondria, early secretory vesicles and the endoplasmic reticulum. Internalization, LCV formation and intracellular growth of *L. pneumophila* are dependent on the bacterial Icm/Dot type IV secretion system (T4SS), which translocates more than 250 different "effector" proteins into the host cell. The identification of the biochemical and cellular function of some of these Icm/Dot substrates is challenging, as the deletion of single or multiple effectors in many cases does not show an intracellular growth phenotype.
We established a simple and fast method to isolate and purify intact LCVs from the social soil amoeba *Dictyostelium discoideum* (*1*). The straight-forward two-step protocol includes enrichment of LCVs by immuno-magnetic separation using an antibody against an effector that selectively binds to phosphatidylinositol-4-phosphate exclusively on LCVs, followed by density centrifugation through a Histodenz gradient cushion. The proteome of the isolated intact LCVs was analysed by LC-MS/MS and revealed more than 500 host cell proteins. Recently, we successfully adapted this protocol to isolate intact LCVs from macrophages, allowing comparative proteomic studies of different host cells. First results indicate a high overlap of identified host proteins, yet also reveal a number of small GTPases and other host proteins that previously have not been identified as LCV markers. We currently confirm the relevance for LCV formation of novel host cell proteins on the LCV by fluorescence microscopy, pulldown assays and functional tests.

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MPV08

Modulation of phosphoinositide metabolism by *Legionella* spp.

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The opportunistic pathogens *Legionella pneumophila* and *Legionella longbeachae* employ a conserved mechanism to replicate in amoebae and macrophages within a unique compartment called the "*Legionella*-containing vacuole" (LCV). Formation of LCVs requires the bacterial Icm/Dot type IV secretion system which, for *L. pneumophila*, translocates more than 250 "effector proteins" into the target host cell. *L. pneumophila* SidM and SidC are effector proteins that anchor to the phosphoinositide (PI) lipid PI(4)*P* on the cytosolic face of LCVs, where they interfere with host cell vesicle trafficking and signal transduction (*1*). Pulldown experiments with PI lipids coupled to agarose beads revealed that *L. longbeachae* SidC also specifically binds to PI(4)*P*.
L. pneumophila modifies the PI pattern of LCVs in an Icm/Dot dependent manner, yet the mechanism remained elusive. We recently discovered a bacterial PI phosphatase, which is translocated into the host via the Icm/Dot T4SS and preferentially hydrolyses poly-phosphorylated PIs yielding PI(4)*P*. This PI phosphatase, termed LppA, might shape the LCV PI pattern to promote binding of *L. pneumophila* effector proteins.
The genetically tractable social amoeba *Dictyostelium discoideum* has been used in a number of studies to analyze LCV formation of *L. pneumophila*. The PI 5-phosphatase OCRL1 and its *Dictyostelium* homologue Dd5P4 localize to LCVs, restrict intracellular bacterial growth and are implicated in retrograde trafficking (*1*). Similarly, intracellular replication of *L. longbeachae* was found to be enhanced in *D. discoideum* lacking Dd5P4. Current efforts aim at a detailed characterization of LCV formation and intracellular replication of *L. longbeachae* using *D. discoideum.*

MPV09

The epidermal growth factor receptor is a cellular target for the *C. pneumoniae* adhesion Pmp21

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Chlamydiae are significant human pathogens. *C. pneumoniae* is an important respiratory pathogen associated with 5 to 10 % of community-acquired cases of pneumonia worldwide and a seroprevalence of up to 70%. Our group is concentrating on the first and initial step of the infection, the adhesion to and subsequent internalization of *chlamydiae* by the human cell. Recently we characterized the first chlamydial adhesin - human receptor pair. We could demonstrate that glycosaminoglycans (GAGs) on host cell surfaces are involved in attachment and subsequent infection by *C. pneumoniae* and that the bacterial surface-associated OmcB protein is the GAG-binding adhesin (Moelleken and Hegemann 2008). Using our Yeast Surface Display system in combination with a functional adhesion test procedure we subsequently identified as a second *C. pneumoniae* adhesin Pmp21. In adhesion studies, using purified recombinant Pmp21 protein, we were able to show that it could bind to human cells. By generating deletion variants and single amino acid mutations within the protein, we mapped the adhesion properties to short repetitive amino acid motives GGA(I, V) and FXXN. Inhibition of the interaction between Pmp21 and the host cell by an anti-Pmp21 antibody or soluble recombinant Pmp21 protein let to a reduced infection as well as a reduced binding of the bacteria to the human cell. In a biochemical approach we tried to identify a human cellular interaction partner for the chlamydial Pmp21 protein. We generated a recombinant Pmp21 protein fragment that is analogous to a natural processed fragment found on EBs and used this protein to isolate interaction partners from the human cell surface. Through mass spectrometry we identified the epidermal growth factor receptor (EGFR1) as a potential target for Pmp21. Depletion of the EGF receptor by siRNA knock down showed a 60% decreased infection with *C. pneumoniae* in comparison to non-transfected cells or cells transfected with a non targeting siRNA. Most importantly a knock-down of EGFR resulted in a diminished binding of fluorescently labeled bacteria, indicating that EGFR indeed serves as a receptor for *C. pneumoniae*. Vice versa we could enhance the infection approximately by the factor two, when we overexpressed EGFR in cells deficient for this receptor tyrosine kinase. Deletion of the ligand-binding domain within the receptor significantly reduced the infection due to a reduced internalization of the bacteria. These findings imply that *C. pneumoniae* uses EGFR as a receptor for infection with Pmp21 on the bacterial surface as interaction partner.

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MPV10

Molecular mechanisms behind Chlamydia-induced reactive oxygen species production and inflammasome activation

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Reactive oxygen species (ROS) are conventionally considered to be part of the immune response to kill pathogens. The obligate intracellular bacteria, *Chlamydia trachomatis* (*C. trachomatis*), however evolved mechanisms to use ROS for its own advantage. *C. trachomatis*-induced, increased ROS levels activate caspase-1 by an inflammasome protein, NLRP3 dependent mechanism. Proteolytic activity of capase-1 is required for chlamydial inclusion formation in epithelial cells.
Despite the positive effect of ROS on chlamydial development, it is incompletely understood how different cellular conditions that lead to altered ROS production affect chlamydial growth. Therefore we aimed to decipher the molecular mechanisms underlying the beneficial effect of ROS on chlamydial inclusion formation and inflammasome activation.
Host cell mitochondrial and cytoplasmic ROS formations were modified by different approaches. Hypoxia and inhibition of mitochondrial complex III by Antimycin A that increased the concentrations of mitochondrial ROS supported and decreased *C. trachomatis* development respectively. Induction of cytoplasmic ROS by addition of small amount of exogenous tert-butyl hydrogen peroxide or cobalt-chloride increased *C. trachomatis* development. The role of cytoplasmic NADPH that is essential for

regeneration of host cell oxidative defense system was studied in cells permanently overexpressing the NAD-kinase enzyme. Consequent host cell signaling changes were followed by detection of hypoxia inducible factor, HIF-1α stabilization and phosphorylation of the acetyl-coenzym A-carboxylase (ACC) enzyme that might mediate the host cell metabolic changes that support chlamydial growth. Fluorescence lifetime imaging of the metabolic coenzyme, NAD(P)H by two-photon microscopy was used to precisely characterize the effect of different sources of ROS on the metabolic activity of *C. trachomatis*. Changes in chlamydial metabolism were detected by measuring the relative amount of protein-bound NAD(P)H and its fluorescence lifetime inside the inclusion. We also observed the trafficking of Golgi and lysosomes into close proximity to the nucleus in *C. trachomatis*-infected cells by live cell imaging. This organelle trafficking might escort the perinuclear clustering of inflammasomes that is required for their ROS-induced activation.
Our studies about the role of ROS in chlamydial development contribute to the understanding of the fundamentals of host-pathogen interactions. Chlamydial growth and progeny formation showed different regulation depending on the concentrations and origin of ROS generated in the host cell and on the consequent HIF-1α stabilization and metabolic changes.

MPV11

In vitro co-infection of HHV6 and *Chlamydia trachomatis* modulate each other's infectivity and survival

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Introduction: Both human herpes virus 6 (HHV6) and Chlamydia (*C. trachomatis* or *C. pneumoniae*) are detected together in different human disorders including chronic fatigues syndrome (CFS) and autoimmune disorders like multiple sclerosis (MS). The possible interaction between these two types of pathogens and its influence on their fate inside host cell is still unknown.
Material and methods: We used an *in vitro* epithelial cell culture model to study the cross talk between HHV6 and *C. trachomatis* during their co-infection.
Results: Under *in vitro* growth conditions acute HHV6 infection reduced the Chlamydial infectivity. On contrary, the bacterial replication and infectivity was strongly up regulated in latent HHV6 containing HeLa229 cells. Interestingly, *C. trachomatis* favored enhanced entry and survival of HHV6. In a co-infection model, both pathogens affected each other's entry, survival as well as infectivity and influenced the replication and transcription pattern of each other.
Conclusion: Our findings strongly argue for an increased biological significance of co-infection of both these pathogens.

MPV12

Transposon mutants of *Mycoplasma agalactiae*: exploiting a modified QTD PCR methodology as a novel strategy for sequencing Tn insertion sites and its optimization for selective quantitative screening

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As mycoplasmas are poorly understood pathogens, they are difficult to eradicate. Despite considerable advances in the area of mycoplasma molecular genetics in the past decade, progress towards understanding their pathogenicity mechanisms is hindered by the paucity of available genetic tools (1). *Mycoplasma agalactiae* (MA) is an important ruminant pathogen for which the pathogenicity mechanisms are largely unknown, mainly because of the previous incapability to genetically manipulate it until the year 2005 (2). We initiated studies in this direction (2) and have now constructed a transposon (Tn) mutant library with insertions in almost all important categories including hypothetical, lipoprotein, transporter and metabolism related genes. For the functional analysis of MA genes, the Tn mutants were subjected to several *in vitro* screening protocols, especially to those which could have a direct role in its pathogenicity, such as biofilm formation, growth, adherence and antibiotic sensitivity, in order to shortlist them for an experimental sheep infection study. This resulted in the identification of many important mutants in known and hypothetical genes displaying significant phenotypes, such as *pdhB, uhpT* and MAG0110 in growth and many lipoprotein genes in adhesion assays.
Although reported for some mycoplasmas, direct genomic sequencing and other methods to sequence the Tn insertion sites are not always successful, or are rather costly or laborious. We have exploited and developed a unique PCR strategy that is an integral part of the QTD method (3) for sequencing mutants. This involves selective and specific amplification of the Tn-insertion sites using the same pair of primers for all mutants in a ligation-mediated PCR, and offers an easy and fast alternative sequencing strategy. Since QTD is a novel, efficient, high-throughput method capable of also identifying quantitative phenotypes during mixed population screening (3), we have adapted it to MA and optimized various parameters to successfully reproduce specific amplification of individual as well as a pool of selected Tn-mutants. The study not only highlights the biological significance of some specific mutated genes but also paves the way for the successful application of a novel QTD method in mycoplasmas to facilitate a better understanding of their biology and pathogenicity.

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MPV13

S. aureus adhesion to endothelial cells under shear stress via released ultra-large von Willebrand factor fibers is independent of staphylococcal protein A

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Background: Inflammatory activation of the endothelium leads to a release of von Willebrand factor (VWF). Exposed to blood flow, VWF gets activated by a conformational change from a globular protein to stretched mulitmerized ultra large VWF (ULVWF) fibers. These ULVWF fibers bind platelets at sites of vascular injury even under high shear rate conditions. Moreover, ULVWF is considered as a potential binding partner for pathogenic bacteria. It is worth to emphasize that ULVWF fibers can be formed on an intact endothelial cell layer, independent of vascular damage. Previous studies indicate that *Staphylococcus aureus* binds to VWF via staphylococcal protein A (SpA). However, the influence of ULVWF fibers is not known so far. Therefore, the present study focused on the impact of ULVWF for the adhesion of *S. aureus* to an intact endothelial layer.
Methods: To simulate blood flow conditions at different shear rates we used various microfluidic devices. Human umbilical vein endothelial cells (HUVECs) were perfused with fluorescence-labelled *S. aureus* (Cowan I) enabling quantification of bacterial adhesion by fluorescence microscopy. To investigate the role of SpA, we compared the adhesion of *S. aureus* wild type to SpA-deficient isogenic mutant and to the non pathogenic *S. carnosus* (TM300). To prove whether the binding of *S. aureus* to the endothelium is specifically mediated by ULVWF we used in some experiments the specific VWF degrading protease ADAMTS-13 (a disintegrin and metalloproteinase with thrombospondin motifs 13).
Results: The overall capacity of ULVWF to bind *S. aureus* was dramatically higher in comparison to globular VWF. SpA supported the binding of *S. aureus* mainly to the globular form of VWF which is in agreement with previous studies. By contrast, binding of *S. aureus* to ULVWF fibers was largely independent of SpA. Adhesion of *S. carnosus* to ULVWF was comparable to *S. aureus* indicating an adhesion mechanism independent of *S. aureus*-specific surface structures. In addition, fixed and live staphylococci adhered to a similar extent to ULVWF. In experiments using ADAMTS-13 an instantaneously cleavage of ULVWF was documented and thereby an inhibition of bacterial adhesion.
Discussion: Within the context of clinical studies a significant contribution of VWF and ADAMTS-13 to vascular disease progression has been shown previously. Here, we show for the first time that *S. aureus* is able to bind to an intact endothelial cell layer under physiological blood flow conditions. Binding was mainly due to ULVWF fibers and independent of SpA but dependent of ADAMTS-13. Our data suggest an adhesion mechanism independent of secreted factors or surface associated adhesins. However, the acting molecular mechanism that triggers strong binding of *S. aureus* to ULVWF is currently unknown and requires further research.

MPV14

Streptococcus pneumoniae induces release of von Willebrand factor from primary human endothelial cells

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Introduction: *Streptococcus pneumoniae* is colonizing the human nasopharyngeal cavity. Invasive pneumococcal infections cause meningitis, pneumonia and septic dissemination within the vascular system (Cartwright, 2002). The pore-forming cytotoxin pneumolysin is described as a major virulence factor of pneumococci (Mitchell and Mitchell, 2010). Pulmonary microvascular endothelial cells (HPMEC) form the inner lining of blood microvessels and produce specialized storage granules named Weibel-Palade Bodies (WPB). WPB contain procoagulant factors like von Willebrand factor (vWF) and proinflammatory interleukin 8 (IL-8) and are released in response to inflammatory or stress-related stimuli (Weibel and Palade 1964; Rondaij *et al.,* 2004).
Material and Methods: Confluently grown primary HPMEC were cultured on glass cover slips or in two chamber transwell systems and were infected with serotype 35A and serotype 2 pneumococci and with pneumolysin-deficient mutants. Bacterial adherence and WPB exocytosis was visualized by immune fluorescence microscopy. Cell confluency was controlled by measurement of biological impedance using the CellZscope (Nanoanalytics). Quantification of vWF and IL-8 within cell culture supernatant was performed by ELISA.
Results: Microscopic quantification demonstrated that pneumococcal adherence to confluent HPMEC correlates with a decreasing amount of intracellular WPB. Following time course of infection with pneumococci, increasing amounts of vWF and IL-8 were determined in cell culture supernatants. Release of vWF and of IL8 was slightly lower after infection with pneumolysin-deficient pneumococcal mutants compared to wild-type bacteria, but sublytical amounts of recombinant pneumolysin significantly stimulated WPB exocytosis in similar levels as physiological agonists like histamine and thrombin. Moreover, the vWF release was determined in cell culture supernatant after infection of HPMEC from apical and basolateral cell surface in equal amounts.
Discussion: This report describes for the first time an induction of WPB exocytosis by pneumococcal adherence and by the cytotoxin pneumolysin using primary lung endothelial cells. The stimulation of vWF and IL8 release after apical and basolateral infection implies a stimulation of WPB exocytosis in both directions: from inside the vasculature and also following invasive pneumococcal transmigration from pulmonary tissue into the bloodstream. These data describe a new endothelial-specific response to severe pneumococcal infection and provide new insights into the pathophysiological consequences.

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MPV15

Adherence of *Staphylococcus epidermidis* to surface immobilized fibronectin depends on interactions of extracellular matrix binding protein (Embp) to Fn type III domains 12-13

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Staphylococcus epidermidis is a leading cause of foreign body associated infections. Pathogenicity of the species is mainly related to its biofilm forming capability. Biofilm formation crucially depends on bacterial interactions with surface organized host extracellular matrix components. Here we analyzed the role of giant 1 MDa extracellular matrix binding protein Embp in *S. epidermidis* adherence to surface bound fibronectin. Analysis of fibronectin interactions with recombinant Embp sub-domains, either comprising Found In Various Architectures- (FIVAR-) domains alone or in combination with G-related albumin binding- (GA-) domains, in ELSA-based assays and by surface plasmon resonance produced evidence that FIVAR domains are sufficient for Embp-Fn interactions. In addition, Far Western blotting experiments mapped Embp fibronectin interactions to FnIII 12 - 13 domains By making use of Embp expressing *S. epidermidis* strain 1585v and isogenic, Embp-negative mutant 1585v-M135 the functional relevance of Embp-Fn interactions for *S. epidermidis* surface colonization was then tested in semi-quantitative adherence assays, showing that Embp-expression is sufficient and necessary for *S. epidermidis* binding to surface immobilized Fn. The importance of Embp for bacterial adherence to fibronectin was further validated by testing surrogated host *S. carnosus* TM300 carrying a plasmid allowing for expression of a fusion protein comprising FIVAR domains, an N-terminal YSIRK-containing export signal and the putative C-terminal cell wall binding region. Whereas wild type *S. carnosus* exhibited almost no Fn-adherence capacity, *in trans* expression of this construct lead to a significant increase in bacterial binding. These results establish the role of Embp as a *bona fide* bacterial fibronectin adhesin. To test if *S. epidermidis* adherence to fibronectin also involves interactions with FnIII domains 12 - 13, adherence of *S. epidermidis* 1585v to immobilized Fn was tested in the presence of heparin. Here, a dose dependent inhibition of *S. epidermidis* adherence was recorded. Therefore, as heparin specifically binds to FnIII 12, this particular domain is obviously crucial for Embp mediated *S. epidermidis* adherence to fibronectin. Our findings establish the role of Embp as an important *S. epidermidis* adhesin mediating adherence to immobilized fibronectin, thereby contributing to the first phase of biofilm formation. The underlying molecular basis of Embp - fibronectin interactions involves a novel, so far undescribed mechanism.

MPV16

Beta 1 integrin clustered in ceramide enriched domains in airway epithelium of Cystic Fibrosis mice offers a potential binding mechanism via its RGD sequence receptor for Pseudomonas aeruginosa

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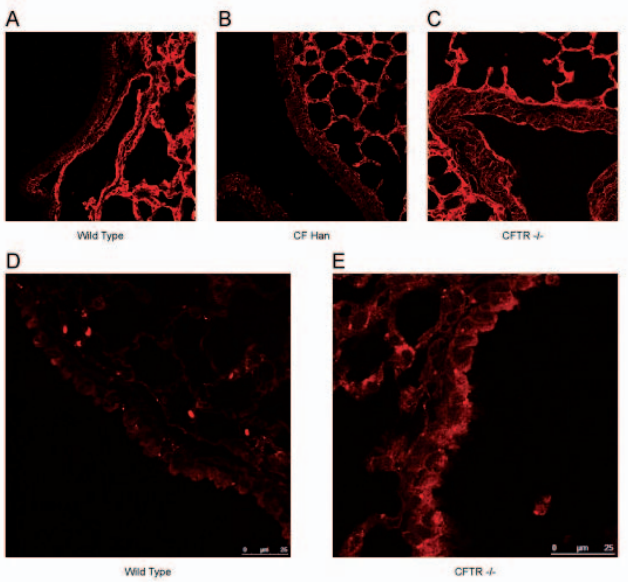
Pseudomonas aeruginosa is the leading respiratory pathogen and eventual cause of death for patients with Cystic Fibrosis (CF). Ceramide, a bioactive sphingolipid involved in cell signalling, has been shown to assemble along the cell membrane of the airway epithelium. Patients with CF have a relative abundance of ceramide enriched domains, termed platforms if of a larger size. Accumulation of domains has been directly correlated with patient maturation and vulnerability to infection. Increased assemblages of ceramide are a molecular outlier of patients with CF and act as potential stages for aberrant signalling. In this study we show that although commonly found along the basal lateral membrane, Beta 1 integrin maintains a permanent position on ceramide enriched domains on the luminal side of airway epithelial cells in CF mice. Visualization of ceramide, Beta 1 integrin and *P. aeruginosa* via immunohistochemistry indicates co-localization at the point of infection on trachea and bronchi epithelial tissue. Beta 1 integrin is known to have a RGD binding domain which facilitates binding of various other microbes. *P. aeruginosa's* adherence via its RGD sequence was tested both in vitro by treating Chang cells with and in vivo by inhalation of GRGDN peptide prior to infection; both treatments diminished the virulence of the bacteria as measured by bacterial adherence and mucocilliary clearance. Our data indicates that ceramide enriched domains, and the Beta 1 integrin molecules they host, play an important role in the virulence of *P. aeruginosa* within the airways of patients with Cystic Fibrosis. Additionally, we observed that competitive inhibition of an RGD sequence receptor decreased the pathogenicity of the bacteria in vivo. These two findings suggest the RGD binding domain of Beta 1 integrin may act as an adherence mechanism for *P. aeruginosa* and that increased clustering of this receptor in patients with CF may play an important role in the infectious manifestations of the disease. Preliminary data indicates inhalation of RGD peptides may be a novel treatment option for patients.
Figure 1: Confocal microscopy of immunofluorescence staining for Beta 1 Integrin in the bronchial epithelium. A shift is observed from basal lateral to apical side of the tissue in CFTR-/- mice. Paraffin embeded tissue (A,B,C) and in situ stained paraffin embedded tissue (D,E)

Figure 1



Confocal Microscopy of immunofluorescence staining for Beta 1 Integrin in the bronchial epithelium. A shift is observed from basal lateral to apical side of the tissue in CFTR-/- mice. Paraffin embeded tissue **(A,B,C)** and in situ stained paraffin embedded tissue **(D,E)**

MPV17

Yop injection into leukocyte populations during mouse infection depends on the presence of YadA and can occur in a β1 integrin independent manner

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The type three secretion system (TTSS) of *Yersinia enterocolitica* (Ye) is crucial to evade the host immune response. After adhesion of the bacteria via YadA or Invasin (Inv) to β1 integrins, the TTSS injects *Yersinia* outer proteins (Yops) into host cells [1]. To dissect the role of YadA, Invasin and β1 integrins for Yop injection a β-lactamase reporter system was used to measure Yop injection into leukocytes by flow cytometry [2]. *In vitro* infection of splenic leukocytes with a low infection dose of a Ye ΔYadAΔInv strain, revealed that Yop injection into DCs, macrophages and PMNs occurs to some extent and to a much lower extent in B and T cells in a YadA- and Inv-dependent manner. Yop injection after infection with a ΔYadA mutant occurred with similar frequency in all tested cell populations. After infection of a ΔInv strain PMNs were targeted for Yop injection with the highest and T cell with the lowest frequency. Detection of Yop injection was performed in mouse infection models which resulted in similar bacterial load in the spleen when wildtype and mutant *Yersinia* were compared. After infection with a ΔYadA mutant no Yop injection could be detected while a ΔInv mutant showed nearly similar Yop injection as a wildtype strain, indicating that YadA but not Inv is crucial for Yop injection into leukocytes during mouse infection. To investigate the role of β1 integrins Yop injection into PMNs, macrophages and B cells was investigated using β1 depleted splenocytes derived from conditional knockout mice. β1 integrin depletion had no impact on Yop injection by a ΔInv mutant but reduced significantly Yop injection by a ΔYadA mutant after infection of B cells, PMNs or macrophages. Our data provide evidence that during systemic mouse infection YadA but not Inv is essential for Yop injection. Inv mediated Yop injection into leukocytes in contrast to YadA mediated Yop injection can occur in a β1 integrin independent manner.

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MPV18

Trimeric autotransporter adhesin-dependent adherence of Bartonella henselae, Bartonella quintana and Yersinia enterocolitica to matrixcomponents and endothelial cells under static and dynamic flow conditions

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Trimeric autotransporter adhesins (TAAs) are important virulence factors of Gram-negative
bacteria responsible for adherence to extracellular matrix (ECM) and host cells. Here, we analyzed three different TAAs [Bartonella adhesin A (BadA) of *Bartonella henselae*, variably expressed outer membrane proteins (Vomps) of *Bartonella quintana*, Yersinia adhesin A (YadA) of *Yersinia enterocolitica*] for mediating bacterial adherence to ECM and endothelial cells. Using static (cell culture vials) and dynamic (capillary flow chambers) experimental settings, adherence of wildtype bacteria and the respective TAA-negative strains was analyzed. Under static conditions, ECM adherence of *B. henselae*, *B. quintana* and *Y. enterocolitica* was strongly dependent on the expression of their particular TAAs. YadA of *Y. enterocolitica* did not mediate bacterial binding to plasma or cellular fibronectin neither under static nor dynamic conditions. TAA-dependent host cell adherence appeared more significant under dynamic conditions although the total number of bound bacteria was diminished compared to static conditions. Dynamic models expand the methodology to perform bacterial adherence experiments under more realistic blood-stream like conditions and allow to dissect the biological role of TAAs in ECM and host cell adherence under static and dynamic conditions.

MPV19

Actin re-organization of polarized epithelial cells by *Salmonella enterica* sv. Typhimurium

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In order to adhere and invade polarized epithelial cells at the intestine, *Salmonella* injects virulence proteins by the type 3 secretion system-1 (T3SS-1) into the host cell which re-organize the actin cytoskeleton allowing the bacterial internalization. Recently, the microvilli effacement of polarized epithelial cells caused by *Salmonella* was described as consequence of cooperation of the adhesin SiiE and the T3SS-1. To investigate the disruption of the brush border of polarized epithelial cells, SL1344 wild-type (WT), *sipA*, *sopA*, *sopB*, *sopE*, *sopE2*mutant strains, M712 strain, which lacks the five mentioned effector proteins, and their complemented derivates were used for invasion experiments in MDCK Pf and C2*bbe1* cell lines, the later were also used to evaluate the disruption of the trans-epithelial electrical resistance (TEER) by the *Salmonella* infection. Results compared to the WT-strain showed that whereas the single *sopE*-mutant strain was two third less invasive, one third of the invasiveness was recovered by the single introduction of *sopE* in M712. On the other hand, the M712 + *sopE* abrogated the TEER similarly to the WT strain. No microvilli effacement and small ruffles were observed in actin-stained MDCK cells infected by the *sopE*-single mutant strain. Microvilli in these infected cells seemed to be fused to the *Salmonella* invasion point. Further results demonstrated that cells infected by the WT strain presented reticular actin around the entry point at the apical side of the host cell. Additionally, M712 + *sipA* strain intimately anchored to the microvilli; M712 + *sopA* or + *sopB* enhanced the adherence compared to the background mutant strain; M712 + *sopE2* induced small accumulation of actin; and finally, M712 + *sopE* effaced the microvilli and also induced reticular actin as observed with the WT strain. On the other hand, chain-like structures at the former tight-junction were observed in ZO-1 stained C2*bbe1* cells infected by M712 + *sopE*. To follow the invasion process, time lapse imaging using EGFP-LifeAct MDCK cells revealed that bacteria intimately adhered to the microvillus during the first 5 min.; thereafter, microvilli effacement simultaneously occurred with the membrane ruffling from 10 to 15 min.; finally, after 20 min. ruffles were fused promoting bacterial clustering. A delayed invasion of *sopE*-strain was observed after 25 min. During this time *Salmonella* migrated until the microvilli basement, there then induced moderate membrane ruffling without microvilli

effacement. Altogether, these results showed that microvilli effacement, reticular actin and disruption of tight junctions only depended on the presence of SopE. Deletion of other effector proteins also caused changes in the ruffle morphology and spatial distribution of bacteria at the apical side of the host cells. Further work is in progress to understand the molecular basis of the microvilli destruction caused by *Salmonella*.

MPV20

The *Streptococcus canis* M-*like* Protein SCM is a receptor for Mini (-plasmin)-ogen promoting bacterial transmigration

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Introduction: *Streptococcus canis* is able to cause severe invasive diseases mainly in cats and dogs. Increased incidences of streptococcal infections in humans have also been attributed to *S. canis* and have thus prompted an investigation into the mechanisms underlying their pathogenesis.
Methods and Results: Binding of plasmin(-ogen) is a common virulence trait for establishing invasive bacterial infections. Plasma-absorption analysis demonstrated a strong interaction between *S. canis* and human plasminogen. Furthermore, in comparative radioactive binding assays using human or canine plasminogen, no differences in quality of interaction could be observed, a prerequisite for establishing infections across host species. Binding of plasminogen depends on the presence of the *S. canis* M-like protein (SCM) genetically organized in the *mga-scm* locus. Surface plasmon resonance technique revealed dissociation constants in the nanomolar range indicating a specific and biologically important interaction. The plasminogen-binding site is located in the N-terminal part of SCM ranging from aminoacid 90 to 112 of the mature protein. Furthermore, Western blot analysis using truncated fragments of plasminogen revealed an interaction with the C-terminal part comprising kringle domain 5 and the catalytic domain, also known as miniplasminogen (mPlg). To the best of our knowledge, SCM represents the first bacterial receptor directly interacting with mPLG. Even though *S. canis* possess no streptokinase activity, the use of eukaryotic plasminogen activator urokinase enables the bacterium to immobilize plasmin activity on its surface. This led to the degradation of purified fibrinogen and of synthetically aggregated fibrin clots. Furthermore, electron microscopic illustration and time lapse imaging demonstrated plasmin-mediated transmigration of fibrinous thrombi by *S. canis* and, therefore, may represent a mechanism for bacterial dissemination within the host.
Discussion: Although *S. canis* is a zoonotic pathogen, very little is known about its virulence factors or mechanisms. Indeed, as described for other streptococcal pathogens (1, 2), *S. canis* utilizes similar strategies for tissue destruction and bacterial dissemination in the host. However, binding of SCM to mPlg presents a mechanistically different bacterial-plasminogen interaction. The role of such interactions in the etiopathogenesis of these infections requires further investigation.

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MPV21

Functional characterization of Burkholderia type VI secretion systems

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Burkholderia pseudomallei, the etiological agent of melioidosis, and *Burkholderia thailandensis* are closely related organisms mainly found in the soil in Southeast Asia and Northern Australia. The genome of *B. pseudomallei* encodes six type VI secretion systems (T6SSs) that contain the complete repertoire of T6S core components; orthologs of five of these systems are present in *B. thailandensis*. The type VI secretion system is a widespread and multicomponent apparatus involved in bacterial and eukaryotic cell interactions. The Burkholderia T6SSs present in a genome are evolutionarily distinct, leading us to hypothesize that each secretion system performs a discrete function. To test this, we inactivated each T6SS individually and investigated their role in virulence and bacterial fitness. We found that only one T6SS mutant, ΔT6SS-5, lost virulence in an aerosol delivered murine model of melioidosis. This is likely due to a specific role T6SS-5 plays in the subversion of or resistance against MyD88 dependent immune response since ΔT6SS-5 is highly virulent in MyD88$^{-/-}$ mice. Next, we screened *B. thailandensis* T6SS mutants against a diverse array of 31 Gram positive and negative bacterial species in growth competition assays. The proliferation and survival of *B. thailandensis* in the presence of several competitor species requires T6SS-1 in a cell contact dependent manner. Intriguingly, two of these species, *Pseudomonas fluorescens* and *Pseudomonas putida*, are also soil inhabitants in the tropics. In addition, T6SS-1 is essential for the persistence of *B. thailandensis* in mixed biofilm assays with *P. putida*. Data on T6S substrates are scarce and no Burkholderia T6S effectors have been identified to date. To shed light on the mechanism of action of T6SS-5, we aimed at identifying proteins secreted by this system. For this, we utilized quantitative Orbitrap mass spectrometry to compare the secretomes of *B. thailandensis* wild type and ΔT6SS-5. Two proteins, Hcp-5 and VgrG-5, were identified as T6SS-5 substrates. Hcp-5 and VgrG-5 are extracellular structural components of the T6S apparatus with high structural similarity to phage tail tube and tail spike proteins, respectively. Interestingly, VgrG-5 contains a putative C terminal effector domain that does not display significant sequence similarity to proteins with known function. We showed that the C terminal domain of VgrG-5 is required for *B. thailandensis* to induce the formation of multinucleated giant cells in phagocytic and non-phagocytic cells. Together the data suggest an important role of Burkholderia T6S in determining the outcome of an infection by directly targeting eukaryotic host cells or by allowing the bacteria to persist in the environment.

MPV22

Amino acid metabolism in *Streptococcus pneumoniae* as analyzed by ^{13}C-isotopologue profiling

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Streptococcus pneumoniae (pneumococci) produce a large fraction of ABC transporter systems involved in uptake and metabolism of sugars, soluble compounds and amino acids. In this study the carbon metabolism was assessed by ^{13}C isotopologue perturbation after growth of pneumococci in chemically defined media (CDM) in presence of [U-$^{13}C_6$]glucose or [1,2-$^{13}C_2$]glucose as the sole carbon source. GC/MS analysis showed a pattern of labeled and non-labeled amino acids. Lack of isotopologue labeling was indicated for His, Glu, Pro, Gly and the branched amino acids (BCCA) Ile, Leu, Val, which were all also required as supplement in CDM. Asp is not synthesized via the general pathway including pyruvate and acetyl-CoA. Instead, oxaloacetate is synthesized from phosphoenolpyruvate (PEP) and CO_2 by the PEP carboxylase. The oxaloacetate is then converted into Asp, while in a lateral reaction the essential glutamate is converted into 2-oxoglutarate. These reactions are catalyzed by the aspartate transaminase. Furthermore, the shikimate pathway could be determined by GC/MS analysis. Complete labeled aromatic amino acids are *de novo* synthesized from erythrose-4-phosphate and two molecules of PEP via the intermediate chorismate. Strikingly, unlabeled Gly [M+0] in [U-$^{13}C_6$]glucose supplemented CDM indicated the inability of pneumococci to synthesize Ser from complete labeled 3-P-D-glycerate and in a subsequent step Gly. In contrast, pneumococci synthesize Ser by glycine hydroxymethylation from 5,10-methylenetetrahydrofolate as part of the One Carbon Pool. This pathway was verified by using [U-$^{13}C_2$]glycine supplemented CDM, resulting in labeled [M+2] serine. Finally, the general carbon and amino acid metabolism was also investigated for pneumococcal mutants deficient in PavA, CodY, and G6PDH. However, the isotopologue profiles of these mutants were similar to the wild-type. In conclusion, these data show for the first time the variety of amino acid synthesis pathway in pneumococci and the essential role of pneumococcal amino acid transporter systems for bacterial fitness und host milieu adaptation.

MPV23

Sensing of enterococcal pathogens by human formyl peptide receptors

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The human innate immune system counteracts bacterial invaders by multiple antimicrobial mechanisms including polymorphonuclear leukocytes (PMN), which represent the most efficient phagocytes.

The human formyl peptide receptor 2 (FPR2) belongs to the family of seven-transmembrane G-protein coupled receptors. Recently, we could show that FPR2 is crucial for recruiting and activating PMN in staphylococcal infections, because it senses concentrations of the major staphylococcal cytolysins phenol-soluble modulin (PSM) peptides. Moreover, FPR2 adjusts PMN responses with respect to PSM release and pathogenicity of staphylococcal species.
Enterococci represent another group of important nosocomial pathogens. In this study, we show that not only staphylococci but also certain enterococci are capable of producing ligands for FPR2 thereby underscoring the important role of this receptor in antibacterial host defense.
PMN chemotaxis and intracellular calcium influx were induced in a dose-dependent manner by supernatants of *Enterococcus faecali*s and *Enterococcus faecium*. This effect could be inhibited by the FPR2-antagonist WRW4. For both, *Enterococcus faecali*s and *Enterococcus faecium* vancomycin-resistant isolates elicited considerably stronger PMN responses than vancomycin-susceptible isolates.
Calcium flux in receptor-transfected HL-60 cells showed that only *E. faecium* eliciting a FPR2-specific response whereas *E. faecalis* did not. However, both species activated the FPR2 paralog FPR1, which senses bacterial formylated peptides. The enterococcal genomes do not encode peptides with apparent similarity to PSM peptides and no peptides with PSM-like properties could be identified in enterococcal culture supernatants in sufficient quantitites for detection. To further characterize the unknown FPR2 ligands produced by *E. faecium* supernatants were treated with proteases, which completely abolished the ability to stimulate FPR2 transfected HL-60 cells thereby indicating that the unknown FPR2 ligands of *E. faecium* represent peptides.
In conclusion, we were able to demonstrate that certain enterococci produce peptide-derived microbial associated molecular patterns, which are sensed by human PMN.

MPV24

Structure-function relationship of zwitterionic cell wall polymer dependent immune modulatory mechanisms

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Recent advances on the field of glycobiology led to discovery of novel mechanisms by which bacterial cell wall glycopolymers can directly modulate the host immune response (1). It had been demonstrated that the structural prerequisite of the T cell stimulatory activity of bacterial capsular polysaccharides (CP) is the presence of positive and negative charge centres within the repeating units. We could recently show that the important pathogenicity factor (2) cell wall teichoic acid (WTA) of *S. aureus* has a similar activity and is able to activate CD4-T cells in an MHCII presentation depended way (3). This mechanism modulates the development of skin abscess formation in an *in vivo* model. Interestingly mostly pathogenic Gram-positive bacterial species seem to exhibit the ribitol-Pi repeating units with D-alanine (however WTA structures differ in side chain sugar composition or contain additional repeating unit sugars), whereas non pathogenic species often have shorter glycerol-Pi repeating units or no D-alanine modifications (1). Our data demonstrates that the glycerol-pi containing *Stapyhlococcus epidermis* WTA is not able to efficiently stimulate T cell proliferation whereas ribitol-Pi WTA of *S. aureus* is an efficient MHCII dependent T cell activating polymer. We therefore postulate that the WTA structure and expression profile play a role in defining the pathogenic potential of Gram-positive bacterial species.
In addition it is also of great interest to understand the regulatory events of WTA biosynthesis in pathogenic Gram-positive bacteria such as *S. aureus.* It is not known so far whether the amount of teichoic acids differs during the development of an infection or even between bacterial strains. Interestingly, we find that highly pathogenic *S. aureus* strains exhibit an elevated WTA amount in their cell walls. Purified protein-free cell wall fractions from these strains induce T cell proliferation and cytokine production more efficiently than cell wall from less pathogenic strains In addition we could demonstrate that cell wall fractions of these strains are more active in skin abscess formation. Thus, up regulation of WTA biosynthesis contributes to pathogenic potential of *S. aureus*.

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MSP01

Characterization of highly divergent African *Staphylococcus aureus* isolates from wild and captive animals

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Introduction: *Staphylococcus aureus* is colonizing humans and animals. Recent reports from industrialized countries have described the impact of zoonotic *S. aureus* transmissions from livestock, companion animals and meat for the colonization and infection in humans. *S. aureus* colonization of undomesticated sub-Saharan animals could pose a risk for inter-species transmission and infections in humans. We therefore characterize *S. aureus* isolates from wild and captive animals from sub-Saharan Africa to obtain data for further investigations of cross-species transmission of *S. aureus* in this area.
Methods: We screened 204 living captive (n = 39) or human-habituated (n = 59) animals and bushmeat samples (n = 106) from Ivory Coast, Gabon, Zambia and South Africa. *S. aureus* isolates were confirmed by *nuc* gene detection and sequencing of the ribosomal 16S rRNA gene. Antibiotic susceptibility was tested using VITEK2 automated systems. Isolates were genotyped using *S. aureus* protein A (*spa*) typing and multilocus sequence typing (MLST) and were characterized by their possession of genes encoding for virulence factors.
Results: *S. aureus* was found in 35% of all samples including 35 isolates from human primates (gorilla and chimpanzees, 48.6%), 34 from nonhuman primates (47.2%), two from ruminants (2.8%) and one from a wild cat (1.4%). Noteworthy, 26 isolates were *nuc* PCR negative. Resistance to penicillin, tetracycline and cotrimoxazole was 12%, 6% and 1%, respectively and was significantly associated with captive animals (OR = inf., 95% CI = 32.48-inf., $p < 0.001$). MRSA was not detected. The most prevalent toxin genes were *sei* (23.6%), *seh* (16.7%) and *seg* (15.3%). The prevalence of classical superantigen-encoding genes (*sea-see, tst*) was low. Three isolates contained Panton-Valentine leukocidin encoding genes. Among 72 isolates, we found 42 different *spa* types and so far 28 confirmed different MLST sequence types (ST). The most prevalent *spa* types were t7821 (11%), t6963 (6%) and t6962 and t7723 (6% each). The predominant STs were ST1 and ST182 (11% each) and ST9 (6%). The STs 2058, 2059, 1873, 2022, 2067, 1857, 1872 and 1874 formed a highly divergent group when using their concatenated sequences and the neighbor-joining method.
Conclusion: Our results suggest that colonization of human and nonhuman primates with *S. aureus* is frequent. One third of *S. aureus* isolates belonged to STs, which were highly divergent from those described in isolates colonizing humans. This will provide an excellent tool to investigate potential cross-species transmission in future.

MSP02

Emergence of Carbapenem- resistant Enterobacteriaceae, including NDM-1 in Austria

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Objectives: Carbapenem-resistant Enterobacteriaceae have emerged as a major public health problem throughout the world. Namely the newly described resistance mechanism conferred by New Delhi metallo-ß-lactamase 1 (NDM-1) activated worldwide interest. We report the emergence of carbapenem-resistant Enterobacteriaceae and involved carbapenem hydrolyzing enzymes in Austria.
Methods: In a 10-year retrospective analysis (2001- 2010) Enterobacteriaceae resistance data were checked for resistance to carbapenems. Detected strains were further analyzed for involved carbapenem hydrolyzing enzymes using PCR methods for the detection of eg MBL/IMP, SPM, VIM, GIM or NDM-1 respectively. Rep-PCR on the DiversiLab instrument (BioMerieux) was used to determine clonal relationship.
Results: Over a ten- year-period carbapenem-resistant Enterobacteriaceae were isolated from 13 hospitalized patients with the first isolation in the year 2005 and a remarkable increase of involved patients in 2010. Carbapenem-resistant Enterobacteriaceae comprise 8 Klebsiella pneumoniae, 4 Klebsiella oxytoca and one Escherichia coli strain. Detected carbapenemases belonged to the metallo-ß-lactamases NDM-1, VIM and IMP. K. pneumoniae carbapenemase (KPC) was first detected in 2010.
NDM-1 was detected in three patients, in two cases in K. pneumoniae, and one patient with NDM-1 positive E. coli. All three cases occurred between December 2009 and August 2010.
All detected carbapenem-resistant Enterobacteriaceae strains were multi-resistant, and exhibited susceptibility to tigecycline (13/13), colistin (10/13), amikacin (10/13), fosfomycin (5/13) and gentamicin (3/13) only.

Conclusion: Carbapenem-resistant *Enterobacteriaceae* has become a substantial global health problem. We report the emergence of such strains in Austria. Carbapenem-resistant *Enterobacteriaceae* were detected in a comparable low number of 13 patients since 2001. But regarding international data there is an urgent need of active surveillance on carbapenem-resistant Enterobacteriaceae. Both local reports and linked data of national and international surveillance networks may be successful approaches to an effective containment of these multi-resistant bacteria.

MSP03

Analysis of the population structure of *Anaplasma phagocytophilum*

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Anaplasma phagocytophilum is a Gram-negative, tick-transmitted, obligate intracellular bacterium that elicits acute febrile diseases in humans and domestic animals such as sheep, cattle, horses, dogs, and cats. Roe deer and red deer are regarded as potential reservoir hosts. Considerable strain variation has been suggested to occur within this species, because isolates from humans and animals differed in their pathogenicity for heterologous hosts.
In order to explain host preference and epidemiological diversity, we used multi locus sequence typing (MLST) for the molecular characterization of *A. phagocytophilum* strains. 237 samples from humans, domestic animals, potential reservoir hosts, and *Ixodes ricinus* ticks were analyzed.
Because of double infections the typabilitiy was only 69% (164/237). eBURST analysis revealed one clonal complex with sequence type (ST) 55 as founder. Strains belonging to this clonal complex originated from dogs (54/56), horses (10/10), humans (6/6), cats (2/2), sheep (3/42), and ticks (14/20). All samples from the US were ST 64, what might reflect their geographic origin. The phylogenetic analysis of the concatenated sequences showed two main clusters. Tick samples were found in both of them. Apart from that, cluster 2 was restricted exclusively to roe deer.
As *A. phagocytophilum* strains infecting humans, dogs and horses belonged to one clonal complex, they seem to share the same epidemiology. Their biological similarity is reflected in the results of previous cross-infections experiments. Because the concatenated sequences from *I. ricinus* ticks are found in both clusters, they might be involved in the transmission cycle of *A. phagocytophilum* strains that infect humans and domestic animals (cluster 1) and of those that infect roe deer (cluster 2). In contrast to red deer, roe deer are probably not the reservoir for granulocytic anaplasmosis in humans and domestic animals, as their concatenated sequences formed a clearly separated cluster.

MSP04

Assessment of Typing Methods for Extraintestinal pathogenic *Escherichia coli*

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The identification and description of disease outbreaks as well as the improvement of our knowledge on population genetics, epidemiology and ecology of bacterial pathogens begins with the ability to differentiate bacterial pathogens via reliable typing methods. Especially for the delineation of extraintestinal pathogenic Escherichia coli (ExPEC) and commensal E.coli this is a challenging task, because certain ExPEC and commensal E.coli strains may be geno- and phenotypically similar. We set our focus on E.coli clonal complex 73, a prominent ExPEC lineage, harboring phenotypically diverse ExPEC, asymptomatic bacteruria but also commensal E.coli variants, as a model to evaluate and improve two already established systems, i.e. “multi locus sequence typing” (MLST) and generic E.coli “multi locus VNTR analysis” (MLVA). We tested these typing systems for their ability to differentiate between the three phenotypic E.coli subgroups. Furthermore, we evaluated how additional markers improve the discriminatory potential of both typing methods. Our results will be discussed regarding the advancement of typing and molecular epidemiology of extraintestinal pathogenic Escherichia coli

MSP05

No evidence for transmissions of septic salmonellosis in children from Ghana by water from wells

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Introduction: Kumasi, Ghana, is one model region for epidemiological investigations on the transmission of infectious diseases in the tropical setting, where we analyzed bacterial causes of sepsis. *Salmonella enterica* is one of the quantitatively most important blood culture isolates from children in the rural village Agogo and the surrounding area, Ashanti Region, Ghana. However, the exact mode of transmission of this typically food-borne pathogen in that particular part of the world is widely unknown. Most children in this region do not have access to bottled drinking water. We therefore investigated contamination of drinking water from local wells.
Material and methods: Within a study period of 2 years, 192 *Salmonella enterica* blood culture isolates from children ≤ 15 years of age and 40 isolates from 13 wells were collected, partly longitudinally. Salmonella strains were repetitively isolated from selected wells. Species identity was biochemically determined by API-E (bioMeriéux) as well as H_2S synthesis on triple-sugar-iron-agar (“Kligler”-agar) in Kumasi, Ghana, and confirmed by Fluorescence-in-situ-hybridization and PCR in Hamburg. Serotyping was performed from all isolates and identical serotypes that were identified within a 1-month-range in one village were further typed by Pulsed-field-gel-electrophoresis (PFGE). PFGE patterns were compared with the software Gel Compar II (Applied Maths) using the settings DICE, UPGMA, 0.5% optimization, 1.0% position tolerance. Clonal identity was accepted at 95% identity.
Results: All analyzed wells were colonized by *Salmonella enterica*. Serotyping demonstrated that the reaction patterns of the isolates from the wells were clearly distinguishable from the blood culture isolates. PFGE-analysis of 76 clinical isolates in 8 villages showed a great diversity of different clones but also some endemic clones circulating in particular regions. Eight *S. enterica* isolates that were isolated from one particular well were discriminated to 7 distinct clones by PFGE thus demonstrating that a single well can be colonized by different *S. enterica* strains and that individual clones can persist about several weeks in a well.
Discussion: PFGE data from the blood culture isolates indicate that endemic clones circulate within the local population. The source was not identified. Serotyping gave no evidence that the water from the wells plays a major role in the transmission of *S. enterica*. The interpretation is limited because different distinct serotypes may colonize a well at the same time while only one individual clone was selected per isolation event. The high number of isolates from the wells with no matches to blood culture isolates during the whole study interval is more likely to support the hypothesis of alternative modes of transmission. Further studies addressing the role of food for the transmission of *S. enterica* in Ghana should follow.

MSP06

Enterovirus surveillance in Germany - one system of two intentions: diagnostic of CNS-infections and polio surveillance

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Background: Since the Global Polio Eradication Initiative (GPEI) has been launched in 1988, the global incidence of polio has been reduced by over 99% and the transmission of indigenous wild poliovirus was interrupted in all but four countries (Afghanistan, Pakistan, India, and Nigeria). Although the WHO region Europe has been certified polio-free in 2002, the risk of re-introduction remains (e.g. polio outbreak in Tajikistan with spread to Russian Federation, Turkmenistan, and Kazakhstan in 2010) and strategies such as vaccination and surveillance of polio have to be maintained until the global polio eradication has been achieved. In 2010 the responsibilities for the German programme of polio surveillance - namely the enterovirus surveillance - was handed over from the Governmental Institute of Public Health of Lower Saxony to the Robert Koch Institute (RKI). In this context the enterovirus surveillance system was extended and can now be used for both, the enterovirus diagnostics in patients with aseptic meningitis / encephalitis and patients with acute flaccid paralysis.

Material and Methods: The examination of one faecal sample or CSF per CNS / AFP patient is offered all paediatric and neurological clinics free of charge. Specimens are analyzed for enteroviruses using PCR, culture and virus typing. A network of 14 labs was established, each of these participating regularly in the proficiency tests.
Results: In 2010, about 47% of all paediatric hospitals and 11% of all neurology departments in Germany made use of the offer of free enterovirus diagnostics. In the five years till end of 2010 a total of ~13,500 samples were tested; on average 2,500 samples annually. In about 65% of cases stool samples were tested, the remaining 35% were CSF samples. The age distribution of patients reflects the great interest of paediatric clinics on the enterovirus diagnostics: overall, about 83% of samples originate from children <15 years of age (median = 7 years). The seasonal pattern of samples sent for testing shows the classical dominance of enteroviruses during the summer months. The overall PCR-positivity rate amounts to ~30% (33.4% for stool samples and 19.6% for CSF samples). So far, more than 2,800 samples were serotyped and >50 different serotypes were detected (ECHO 30 most common). Polioviruses were not detected between 2006 and 2010, as expected.
Conclusions: The results demonstrate that the high-quality enterovirus surveillance is a) well accepted by the clinicians and b) allows Germany to meet its obligations to WHO in establishing a reliable surveillance system to prove the polio-free status. Furthermore, the high rate of enterovirus positive results and the distribution of cases all over Germany give a good picture of the circulating enteroviruses in Germany, thus, contributing to outbreak detection and infection control.

MSP07

Characterization of extended spectrum β-lactamase producing *Escherichia coli*: comparison of phylogenetic grouping by the DiversiLab system, mononucleotide motifs and genetic markers

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Introduction: Rapid and reliable methods to identify the molecular relatedness in clinical isolates are essentially needed to investigate potential routes of transmission. Randomly amplified polymorphic DNA amplification (RAPD) techniques have been shown as a useful and comparatively simple tool for the discrimination of strain relatedness. Recently, the DiversiLab (DL, bioMérieux) system was released as an automated system for the analysis of microbial fingerprints. The main advantage, compared to RAPD, is the amplification of defined different sized repetitive fragments within the non-coding region of the genome. In the present study, the performance of the DL system as a routine typing tool for extended-spectrum ß-lactamase (ESBL) producing *E. coli* was evaluated in comparison to other methods.
Material and Methods: The phylogenetic relatedness of 107 clinical isolates of ESBL-producing *E. coli* were compared using the DiversiLab system (DL, bioMérieux), amplification of mononucleotide repeats (MNRs; yaiN, ycgW, b2345, ykgE) and three candidate gene markers (chuA, yjaA, TSPE4.C2), which were able to distinct between the main four phylogenetic groups A, B1, B2 and D. Isolates were estimated to be related by the DL system at >95 % homology using Pearson correlation.
Results: The DL system revealed two larger clusters of related isolates (cluster I, 18 strains; cluster II: six strains) as well as twelve clusters of two, three or four related strains. Isolates of cluster I belong to the phylogenetic group B2 and had none of the four MNRs. Isolates of cluster II were typed as B1 and showed amplification of all four MNRs. Other DL-clusters also showed a concordance regarding their phylogenetic group and their MNR amplification pattern.
Conclusions:
ESBL-typing by these three different methods showed excellent correlation. Amplicons of MNRs were currently sequenced to evaluate the phylogenetic inference and confirm the discrimination of the DL system. The discriminatory power of the DL system will be further evaluated in comparison to pulse-field-gelelectrophoresis and multi-locus sequence typing. Further investigations include the evaluation of the DL with an in house automated RAPD-PCR using different established primers followed by chip-gelelectrophoresis.

MSP08

Sequence typing and expression analysis of 4CMenB targets in capsule null locus meningococci

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Background and aims: The multi-component vaccine against serogroup B meningococci (4CMenB) comprises three major recombinant antigens including Neisserial adhesion A (NadA), factor H binding protein (fHbp), and Neisserial heparin binding antigen (NHBA) as well as outer-membrane vesicles, which especially induce antibodies against P1.4 of porin A. The purpose of this study was to estimate the effect of 4CMenB on capsule null locus (cnl) meningococci.
Material and methods: Forty-one strains of cnl meningococci from three countries (Burkina Faso, Czech Republic, and Germany) belonging to nine sequence types (ST) and four clonal complexes were antigen sequence typed and analysed for antigen expression by genetic typing and the recently published Meningococcal Antigen Typing System (MATS, Donnelly *et al*. 2010).
Results and conclusions: Strains of the ST-198 complex harboured an fHbp variant, which is included in the vaccine, and expressed the antigen above the positive bactericidal threshold (PBT) as defined for serogroup B strains. Expression of NHBA above the PBT was seen in strains of the ST-198 complex and ST-845. Only one strain harboured the *nadA* gene and P1.4 of porin A was not present among all analysed cnl strains. The data suggest that bactericidal antibodies will be effective only on a subset of cnl meningococci. A biological effect on the ST-198 complex and on ST-845 will depend on antibody levels at the mucosal surface and their capacity to block transmission, for which data are currently not available.

MSP09

Diversity of phage-host interactions in clinical and environmental strains of Pseudomonas aeruginosa

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Introduction: The lytic potential of novel *Pseudomonas aeruginosa* (PA) phages against a large number of novel environmental and clinical PA isolates was challenging to be studied 1.) to find new potential therapy phages, a medical approach in the fight against *PA* and 2.) to find a possible relationship between lysis phenomena in the phylogenetic tree of these hosts and the allocation of clusters of lysed strains: a phylogenetic approach using lysis as "phenotype". The latter addresses the potential role of phages in the microevolutionary diversification of PA strains into different genetic lineages (clades). Are phages relevant drivers of major phylogenetic groups? The large pool of new hosts and phages offered a test system to respond to these questions.
Material and Methods: Environmental PA strains were isolated from German rivers across different water qualities. Clinical isolates came from different sources, mostly chronic infections. All strains were genetically typed using the *Pseudomonas* ArrayTube chip. Phages ("JG series") were isolated from sewage (courtesy of J. Garbe and M. Schobert, TU Braunschweig). Phage assay: overnight cultures were adjusted to 10^9 cfu/mL, drops of 5µL phage suspension were added in 4x4 patterns per agar plate in the overlay technique.
Results: Serotypes of the strains were determined, motility phenotypes, virulence factors and biofilm formation. All strains were grouped acc. their core genomic similarity into four main clusters. The phages were known to infect PA via an LPS membrane receptor. We analysed the lysis phenotype of seven highly lytic phages of this set of 28 phages on 190 strains of PA, 99 environmental, 91 clinical. The phages showed substantial differences in the lysis phenotypes on the set of 190 hosts. The proportion of lysed strains ranged from 70% to 35%. Pairwise phage comparison indicated high or low phenotypic similarity of the phages. All results finally suggest two unrelated phage groups. One phage seemed phenotypically distinct. Clinical PA were more sensitive to phage attack than environmental. Lysis phenotypes appeared to depend on the serotype. Some serotypes showed large proportions of full lysis, others appeared to confer phage resistance, some were sensitive only to few phages. On the level of intraspecies host diversity, some genetic lineages with different phage sensitivity emerged. The host lineages differed also in the presence and abundance of specific serotypes. Two phages were far more effective in lysing clinical than environmental strains, independent of the genetic host lineage.
Conclusion: From the phage lysis patterns we conclude there is therapeutic potential of some of these highly lytic phages with broad PA host spectrum. From the lysis phenotypes we conclude that phages, via the diversity of

host receptor types, have potential to contribute to the intraspecies microevolutionary diversification within PA. From additional own studies (not shown) we have reasons to believe that the population dynamics of PA were probably shaped by environmental forces, not by clinical habitats. The results contribute to the knowledge on the evolutionary dynamics of PA phage resistance.

MSP10

Comparison of multiplex-PCR method with microarray-based approach for rapid staphylococcal cassette chromosome *mec* type assignment of methicillin-resistant *Staphylococcus aureus* isolates

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing health problem in both hospital and community settings worldwide. MRSA are characterized by the carriage of diverse Staphylococcal cassette chromosome *mec* (SCC*mec*) elements, conferring resistance to beta-lactam antibiotics. To date, ten major SCC*mec* types were described on the basis of the combination of *ccr* and *mec* complexes. Although, several PCR-based procedures were designed for typing the SCC*mec* cassettes, they were often found to be limited in their ability to characterize MRSA isolates. The goal of our study was to compare the SCC*mec* typing results produced by well-established multiplex-PCR protocol with those obtained by a microarray-based assay.
Methods: Two hundred-six non-repetitive MRSA isolates were obtained from the collection previously characterized by *spa* typing, Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) and Multiple-Locus Variable-Number Tandem Repeat Fingerprinting (MLVF). All isolates were subjected to SCC*mec*-typing using a multiplex-PCR protocol described by Kondo et al., (Kondo et al., Antimicrob Agents Chemother. 51:264-74, 2007). Microarrays (StaphyType by Alere Technologies GmbH, Jena, Germany) were implemented in 117 selected MRSA isolates.
Results: Using the multiplex-PCR protocol, 179 MRSA isolates were typeable whereas from 27 MRSA isolates, the SCC*mec* type could not be determined. The most prevalent SCC*mec* type was SCC*mec* type IV (n=108), followed by SCC*mec* type V (n=36), SCC*mec* type I (n=20), SCC*mec* type II (n=8), SCC*mec* type III (n=4), SCC*mec* type VI (n=2) and SCC*mec* type VIII (n=1). We further analyzed by the microarray-based approach all MRSA isolates of SSC*mec* types I-III,VI and VIII as well as all non-typeable MRSA isolates. Based on MLVA, MLVF and *spa* typing characteristics, representatives of the most prevalent SCC*mec* types, IV and V (n=30 and n=25, respectively), were selected for microarrays characterization. For all isolates typeable by the multiplex-PCR method, there was perfect agreement of the results between the two methods tested. Among 27 isolates non-typeable by multiplex-PCR approaches almost all (n=26) were typeable by microarrays. Among them the SCC*mec* type V was the most prevalent (n=21), followed by SCC*mec* type IV (n=4), and SCC*mec* type I (n=1). A single MRSA was non-typeable based on the microarrays results.
Conclusions: The microarray approach appeared to be more efficient than the PCR-based protocol in identification of SCC*mec* types among genetically diverse MRSA isolates. Moreover, the microarray-based method is an inexpensive and fast procedure, and can be easily implemented for investigations in routine clinical laboratories.

MSP11

Molecular Epidermiology of Rotavirus patients with acute Gastroenteritis in Northern Gyeonggi-Do

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Introduction: Rotavirus is the major cause of severe diarrhea among infants and young children. Rotaviruses, belonging to a genus of double-stranded RNA viruses in the family Reoviridae, infect the mature villus epithelial cells of the small intestine, often leading to fever, vomiting, and diarrhea in children.
Methods: A total of 5,872 stool specimens collected from patient with acute gastroenteritis in northern Gyeonggi province of Korea during 3 years surveilence (August 2007 through July 2010) were analyzed by using antigen-capture enzyme-linkes immunosorbent assay(ELISA) and RT-PCR followed by sequencing.
Results: Of the 5,872 specimens, rotavirus were selected 347 specimens as rotavirus-positive specimens by means of antigen-capture enzyme-linkes immunosorbent assay(ELISA). The peak seasons of rotavirus infection were winter and spring , from December to May. To survey genotyping of rotavirus was performed the RT-PCR using 137 samples out of 347 rotavirus-positive samples. The predominant genotypes were confirmed as G3P[8](47 samples, 34.3%) followed by G1P[8](31 samples, 22.6%), G2P[4](20 samples, 14.6%), G4P[6](12 samples, 8.76%), G9P[8](6 samples, 4.38%).
Conclusion: This study shows the occurrence and diversity of rotaviruses patient presenting acute diarrhea. Rotavirus-associated gastroenteritis occurs frequently during winter and spring. G3P[8] was the predominant genotype of the detected rotavirus. Rotavirus is responsible for the majority of epidemics in northern Gyeonggi province of Korea. These findings were concordant with previous epidermiological studies conducted worldwide; predominant genotypes were confirmed as G3P[8] followed by G1P[8]. Thus, continuous monitoring of the genetic diversity of rotavirus is important to determine the trend of genotype.

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Fig. 1. Temporal distribution of rotavirus infection in northern Gyeonggi-Do during 2007-2010
Table. 1. Disrtibution of rotavirus genotypes in northern Gyeonggi-Do during 2007-2010

Figure 1

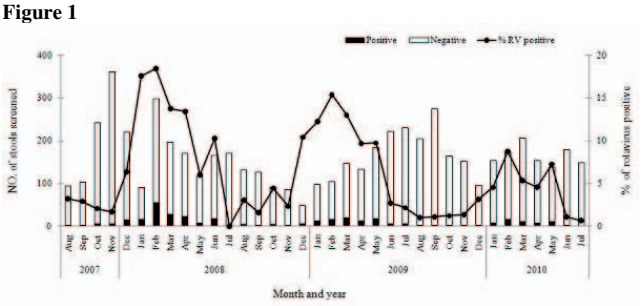

Figure 2

Table 1. Distribution of rotavirus genotypes in northern Gyeonggi-Do during 2007-2010

Genotype	Number(%) of strains by season of rotavirus infection			
	2007-2008	2008-2009	2009-2010	Total
Common	24 (88.87)	24 (68.57)	56 (84.85)	104 (75.91)
G1P[8]	15 (55.11)	6 (17.14)	12 (18.18)	31 (22.63)
G2P[4]	3 (8.33)	6 (17.14)	11 (16.67)	20 (14.60)
G3P[8]	7 (19.44)	9 (25.71)	31 (46.97)	47 (34.31)
G9P[8]	1 (2.78)	3 (8.57)	2 (3.03)	6 (4.38)
Other	12 (33.33)	11 (31.43)	10 (15.15)	33 (24.09)
G1P[4]	2 (5.56)	0 (0)	0 (0)	2 (1.46)
G2P[8]	0 (0)	2 (5.71)	0 (0)	2 (1.46)
G4P[6]	6 (16.67)	1 (2.86)	5 (7.58)	12 (8.76)
G12P[9]	0 (0)	0 (0)	1 (1.52)	1 (0.73)
G11P[28]	0 (0)	0 (0)	1 (1.52)	1 (0.73)
Patially typed strains	1 (2.78)	8 (22.86)	3 (4.55)	12 (8.76)
Mixed types	3 (8.33)	0 (0)	0 (0)	3 (2.19)

MSP12

Molecular characterization of *Streptococcus agalactiae* strains causing streptococcal toxic shock syndrome (STSS)

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Streptococcus agalactiae (group B streptococci, GBS) is a common commensal of the female urogenital tract and is well known for causing life-threatening infections in neonates. For several years now increasing infection rates of immunocompromized adult patients have been reported In rare cases an infection in neonates, pregnant women and immunocompromized elderly patients can lead to a toxic shock syndrome with necrotizing fasciitis. Streptococcal toxic shock syndrome (STSS) is a highly invasive bacterial infection with a mortality rate ranging between 30-70%. It is typically caused by *Streptococcus pyogenes* and mutations of a general virulence regulator (*cov*) have been reported in association with STSS cases. To investigate if similar mutations are found in *S. agalactiae* strains or if a certain clone is responsible for this uncommon course of *S. agalactiae* infections, we performed a detailed molecular analysis and DNA sequencing of the *cov* virulence regulator genes in a collection of 30 *S. agalactiae* STSS strains. The strains originated from 28 adults and 2 children and were collected in the US, Germany and Switzerland. Molecular typing was performed by multi locus sequence typing (MLST), capsular typing, and surface protein determination by PCR. For analysis of the *cov* gene locus the genes *covS* and *covR* were amplified by PCR and subjected to DNA sequencing.
Molecular subtyping by MLST showed that 7 of the 30 *S. agalactiae* STSS strains were ST1 and capsular serotype type V and 5 belonged to ST23 and capsular serotype Ia. The rest of the strains harbored different common MLST types. Genetic analysis showed a 1 bp deletion leading to a frame shift in *covS* in one of the strains. The two component *cov* regulator represses hemolysis and the mutation was associated with an increased hemolysis and pigmentation in the mutated strain. Another strain harbored a 3 bp deletion that had previously been reported. The rest of the strains had *cov* alleles matching the genes sequence of completely sequenced *S. agalactiae* strains in the Genbank database or were harboring silent mutations.
In conclusion virulence regulator gene mutations in strains causing streptococcal toxic shock syndrome as they have been described for *S. pyogenes* can also be found in *S. agalactiae*. A detailed molecular epidemiologic characterization of STSS *S. agalactiae* strains by MLST, capsular typing and surface protein determination showed them to be heterogenous, despite a certain predominance of ST1 and ST23 strains.

MSP13

Molecular epidemiology of *E. coli* pathovar in northern German population: old dogmata and new facts

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Introduction: Pathogenic *E. coli* species represent a major cause for diarrhea in various groups of patients. Whereas Enteropathogenic *E. coli* (EPEC) is a well known cause of diarrhea in babies, Enterohaemorrhagic *E. coli* (EHEC) and Shiga-toxin-producing *E. coli* (STEC) are known to cause infections especially by intake of food contaminated with bovine faeces. Recently, an outbreak of a novel clone of *E. coli* bearing the rare serotype O104:H4 has threatened Northern Germany and challenged the health care authorities.
We report data collected in our routine medical laboratory during the clinical investigation of stool specimens.
Material and Methods: During a period of three years, more than 20,000 stool specimens from patients with diarrhea or enteritis originating from Lower Saxony and surrounding counties were screened by real-time multiplex PCR for the presence of pathogenic *E. coli* species.
Briefly, characteristic genes for EHEC, STEC, EAEC, EPEC, and EIAC, ETEC were detected by an in-house real-time multiplex PCR method.
Results: Our PCR technique detects Enterohaemorrhagic *E. coli* and Shiga-toxin producing *E. coli* with a higher sensitivity than verotoxin-ELISA-techniques.
Pathogenic *E. coli* species were found as causative agents at all ages. Interestingly, EPEC were not only found in young children, but in adult patients as well. EHEC and STEC do not show any preference for a certain age either.
The *E. coli* species responsible for the recent outbreak bears a combination of pathogenic factors that has not been found in our 20,000 stool probes during the past three years.
Discussion: According the microbiologic-infectiologic quality standards (MIQ) of the German Society for Hygiene and Microbiology, screening of stool samples for pathogenic *E. coli* species should only be performed under certain conditions.
Following this standard, EHEC, STEC, EAEC and EPEC would not have been detected in most of our patients. For example, the MIQ standard only considers EPEC a causative agent in adult patients if they are immunocompromised or have persisting diarrhea for more than three weeks. Our data contradict this recommendation.

MSP14

Autochthonous leishmaniasis in Germany: survey of the prevalence of sand flies and infected rodents in Bavaria

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Background: Zoonotic cutaneous and visceral leishmaniasis are caused by protozoan parasites of the genus *Leishmania*, which are transmitted by sand fly vectors from reservoir animals to humans. Within Europe the disease is highly prevalent in countries and islands of the Mediterranean basin. In recent years evidence was presented that argues for a northward spread of leishmaniasis into previously non-endemic areas such as Switzerland, Slowenia and Austria. Within Germany sand flies of the species *Phlebotomus mascitii* and *P. pernicious* were detected in the Rhine valley (Naucke et al., *Parasitol. Res.* 2008). Also, a case of autochthonous human leishmaniasis (Bogdan et al., *Clin. Infect. Dis.* 2001) and three cases of autochthonous canine and equine leishmaniasis have recently been reported from Bavaria (Gothe et al., *Kleintierpraxis* 1999; Koehler et al., *Vet. Parasitol.* 2002). In the present study, we investigated whether sand flies are prevalent in Bavaria and whether wild mice carry *Leishmania* parasites and therefore might function as reservoir animals.
Methods: CDC light traps were used for trapping of sand flies. DNA was prepared from the spleens, livers and skin of different species wild mice caught in Bavaria in the years 2001 to 2004 and 2007 and analysed for the presence of *Leishmania* DNA using 18S rDNA real-time PCR and sequencing.
Results: 210 CDC light traps were placed overnight in 142 different locations throughout Bavaria in a series of field trips during the summer months (June to August) of 2009 and 2010. The selection of the locations was partially based on the predicted potential for the establishment of the vectors based on climate parameters (Fischer et al., *Geospatial Health* 2010). All light traps were negative for sand flies. The analysis of more than 400 organs from wild mice did not reveal any evidence for an infection with *Leishmania* parasites.
Conclusion: These results suggest that Bavaria is currently not an established area for the occurrence of sand flies and rodent leishmaniasis. However, the data do not exclude the possibility of sporadic cases of autochthonous *Leishmania* infections. Further surveys and risk analyses need to be performed.

MSV01

Single Nucleotide Polymorphism-Typing of Enterohemorrhagic *Escherichia coli* O157:H7/H$^-$: Refinement and Complementation of the Evolutionary Model

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7/H$^-$ belong to the highly pathogenic subgroup of Shiga toxin-producing *E. coli* (STEC) and cause severe illness, including watery or bloody diarrhea and the hemolytic uremic syndrome, the most common cause for acute renal failure in children worldwide. It is already widely accepted that EHEC O157 branched from O55:H7 to both Sorbitol fermenting (SF) O157:H$^-$ and three clusters of non-SF EHEC O157:H7 (Leopold et al., Proc Natl Acad Sci USA (2009) 106(21):8713-8). However, until now the involved hypothetical intermediate SF O157:H7 clone had not yet been identified.
Using published genome sequence data of different O55:H7 and O157 strains, a DNA sequence-based approach including 94 coding regions of the core genome was established and applied to a strain collection of 50 strains (48 SF and non-SF O157 and 2 O55) to investigate their phylogeny. Electopherogram data were analyzed and subsequent phylogenetic analyses were performed using the Ridom SeqSphere software version 0.9 beta (Ridom GmbH, Münster). Moreover, the data was compared with

previously performed multiple-locus variable-number tandem-repeats analysis (MLVA) data.
Sequence analysis of in total 51,229 nucleotides per strain resulted in 111 single nucleotide polymorphisms (SNPs) that were discovered in 76 of the 94 investigated loci. With an overall variant ratio of 0.22%, 58 SNPs were non-synonymous (52.3%) indicating a certain evolutionary pressure on each subtype. The combination of SNPs resulted in 27 different genotypes. Of these, certain SNPs or SNP genotypes could be determined to differentiate each serotype or cluster. Interestingly, SNP data enabled us to further differentiate amongst EHEC O157 subgroups, especially within EHEC O157:H7. In contrast, SNP data confirmed the already determined high genotypic conservation within SF EHEC O157:H$^-$. The SNP data was further investigated to elaborate new typing techniques using fewer loci in order to get a comparable rate of resolution. Therefore, characterization of only 25 of the 111 observed SNP localizations enabled a differentiation of all 27 genotypes. In addition, a genotype suitable to fit the hypothetical intermediate clone was identified from an animal isolate with the SF O157:H7 phenotype, thereby complementing the EHEC stepwise evolutionary model and complementing the phylogenetic step from SF O55:H7 to either SF O157:H$^-$ or non-SF O157:H7.
In summary, SNP-based typing of EHEC O55 and O157enabled us to close the gap within the evolutionary model of EHEC O157 by identification of an isolate of the hypothetical clone. Moreover, the identified SNPs and associated genotypes open up new prospects and challenges to develop alternative typing methods with less costs and comparable rates of resolution for epidemiological investigations and phylogeny studies.

MSV02

The frequency of Livestock-associated-MRSA of CC398 in clinical samples from small animals and horses is on the rise

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Introduction: Different studies revealed the emerging zoonotic behaviour of extended host spectrum genotypes (EHSGs) like livestock-associated (LA-) MRSA which belong to clonal complex (CC)398. At present, MRSA is one of the major causes of wound infections in small animals and horses, and infected animals may provide a significant threat to public health. Based on these findings, the aim of this study was to determine the proportion of LA- MRSA in wound swabs obtained from companion animals in Germany.
Material and Methods: Between Nov 2010 and May 2011, 1.814 wound swabs originating from different geographic regions in Germany were investigated for the presence of MRSA. All MRSA were further characterized by *spa*-typing, susceptibility testing and presence of genes encoding for Panton-Valentine leukocidin (PVL).
Results: Overall, the general MRSA distribution was 5.6% among clinical samples of companion animal and horse origin. Regarding distribution of the MRSA among the different animal species, canine samples (1196) showed a distribution of 4.9% (n= 59), feline samples (399) 5.8% (n= 23) and those of equine origin (219) 9.1% (n= 20) MRSA, respectively.
Interestingly, the proportion of *spa*- types frequently associated with livestock-associated MRSA (e.g. CC398, t011, t034) among the strain collection was 18,6% for canine MRSA, 4,3% for feline MRSA, and 90% for those of equine origin.
In contrast to the previously reported frequent occurrence of CC8-associated MRSA among clinical isolates of equine origin, our current results revealed a predominance of LA- MRSA in MRSA positive wound infections. A similar trend was seen in wound infections due to MRSA in dogs, which in the past had frequently been associated with CC22- and CC5- strains, while currently we identified several cases associated with CC398.
Conclusion: The detected proportions of CC398- associated MRSA in horses and dogs suggests a rapid spread of LA- associated strains into animals other than livestock and raise the question whether these EHSG-MRSA may harbour certain factors or abilities (genetic and/ or functional) mediating the obvious successful spread among different mammalian species apart from their methicillin-resistance.

MSV03

Genotyping of Pilus Islet-1 and Pilus Islet-2 in *Streptococcus pneumoniae* Clinical Isolates from Germany

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The recent discovery of long filamentous pilus-like structures extending from the surface of the pneumococcus has opened a new window in the research field of pneumococci. Two pilus regions have been identified in the pneumococcal genome: PI-1 (Pilus Islet-1) and PI-2 (Pilus Islet-2) and, at least for PI-1, their role in pneumococcal pathogenesis has been suggested. We analyzed the distribution of both pili among 419 pneumococcal clinical isolates from Germany, divided in three different groups: invasive (n=233), non-invasive (n=73) and carriers (n=113). Forty-three of the 91 pneumococcal serotypes were represented in this set of samples, including those used in the 7-valent, 10-valent and 13-valent pneumococcal conjugate vaccines (PCVs) and 23-valent PPSV23 (Pneumovax). Overall, 135 (32,2%) pneumococcal clinical isolates were positive for pili: 17,2% for PI-2, 13,4% for PI-1 and 1,7% for both pilus islets. The frequency within the invasive group (45,5%) was higher than the frequency within non-invasive (13,7%) and carrier (16,8%) groups. The 89% of the pilus-positive invasive isolates were positive for PI-2, whereas 2/3rd of the pilus-positive carrier isolates were positive for PI-1. Surprisingly, the majority (71,4%) of the pilus-positive non-invasive isolates were positive for both pneumococcal pili. The double-positive isolates belong to the serotypes 19F, 19A and 7F. Serotypes 6A and 9V are strongly related to PI-1, whereas PI-2 was present mainly in serotypes 1, 7F and 11A. Only two (9V and 19F) of the pneumococcal serotypes identified as pilus-positive in this study are present in all currently available vaccines against *S. pneumoniae*. In contrast, serotype 6A and 11A are included only in PCV13 and PPSV23 respectively. The clonality of the pilus-containing isolates identified here is being determined by MLST analysis and their correlation with antibiotic resistance profiles will also be established. Finally, these data show the scenario of pneumococcal pili distribution among pneumococcal clinical isolates in Germany and may support the potential of PI-1 and PI-2 constituents as vaccine candidates.

MSV04

Comparison of Pulsed-Field gel electrophoresis, optical mapping and spa typing for the cluster detection of Methicillin-resistant *Staphylococcus aureus*

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Background: Hospital-acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) is a growing public health concern across the world. Early detection of clusters is vital for patient protection. Pulsed-field gel electrophoresis (PFGE) is considered a 'gold standard' in detecting important outbreak events and widely used despite several limitations such as limited resolution and lack of digitized data, making intra- and inter-laboratory comparisons difficult. Spa typing, which relies on sequence variation within the *S. aureus* protein A gene (*spa*), has been proven to be a successful tool for investigating the relatedness of strains and the sequence data is easily stored in databases and communicated between researchers globally. Optical Mapping (OM) creates linear restriction enzyme maps from single DNA molecules. These maps can distinguish chromosomal events that are ~>1.5 kb in size, providing a higher resolution of data, as well as fragment organization within the genome, as compared to PFGE (~30 kb detection limit) and probably also to spa typing. Here we compare these techniques in a retrospective analysis of a HA-MRSA cluster.
Methods: Four epidemiologically related isolates, 5 unrelated clinical isolates and one isolate of indeterminable epidemiology were analyzed. Strains were previously characterized using spa typing, and 3 of 5 unrelated isolates were chosen based on an identical spa type with the epidemiologically related strains. The remaining two isolates were different spa types, yet one had the same sequence type (multilocus sequence typing), as the epidemiologically related group. The relatedness of all studied strains was determined using PFGE and OM (OpGen, Gaithersburg, MD, USA).
Results: PFGE results confirmed the close relation of all 4 epidemiologically related isolates. Three of these 4 strains, as well as the isolate of indeterminable epidemiology, had identical PFGE banding

patterns while the fourth isolate differed by a single band. The three unrelated isolates with identical spa types were more closely related to the epidemiologically-linked group (though not identical) than the 2 with different spa types. The OM data validated the PFGE data, but with a higher resolution showing additional restriction sites in strains that were identical by PFGE.
Conclusions: Our data illuminate the varying levels of data resolution that are offered by spa typing, PFGE and OM. PFGE alone was able to differentiate epidemiologically unrelated strains from the cluster strains that shared the same spa type. OM data corresponded with both the PFGE and spa typing results, yet with a higher resolution, providing more detailed phylogenetic information. This increased resolution may be useful in tracking the progress of a cluster, though further investigations are needed to verify that OM alone is sufficient to identify the spread of pathogens.

MSV05

Microevolution of *Mycobacterium tuberculosis* strains in tuberculosis patients

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Background: Transmission and phylogenetic background of clinical *Mycobacterium tuberculosis* complex isolates can be analyzed by genotyping techniques (IS*6110* DNA fingerprinting, spoligotyping, MIRU-VNTR typing). Although these methods have a discriminatory power high enough to discriminate clinical isolates on the strain level, they are not able to visualize microevolution events e.g. within one patient in the case of treatment failure and resistance development.
Methodology: To adress this question, we applied Next Generation Sequencing (NGS) to analyze the genomes of serial isolates obtained from two different patients with long duration of treatment. The time period between the first and last isolate obtained from one patient was up to 36 months. Both strains belong to the Beijing lineage; all isolates from one patient exhibited the same IS*6110* RFLP and 24 loci MIRU-VNTR typing patterns, thus excluding exogenous reinfection.
Principal findings: From all isolates high quality reads with a mean genome coverage of at least 30 were obtained. The reads were mapped to the H37rv reference sequence to identify single nucleotide polymophisms (SNPs). Demonstrating the high quality of the data obtained, we were able to identify all polymorphisms in classical resistance genes conferring resistance to first line (isoniazid, rifampicin, ethambutol, pyrazinamide and streptomycin) and second line antibiotics (ofloxacin, capreomycin). Interestingly, the emergence of SNPs in resistance genes coincides with the development of phenotypic resistance within the course of treatment. In addition, we identified more than 20 SNPs that were acquired by particular isolates in comparison to the parental (first) strain, respectively. These are actually being validated by classical sequencing.
Conclusions: Our results reveal a striking diversity in serial patient isolates that cannot be captured by standard genotyping. Functional consequences e.g. as additional resistance mechanisms or compensatory evolution are currently under investigation.

MSV06

Subpopulations of *Staphylococcus aureus* ST121 are associated with distinct clinical pictures.

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Staphylococcus aureus with the sequence type 121 (ST121) is a frequent, globally distributed, cause of skin and soft tissue infection in humans. Staphylococcal infections of skin are represented by two major clinical pictures with a different pathogenesis. It is known that superficial infections such as impetigo and staphylococcal scalded skin syndrome (*SSSS*) are associated with exfoliative toxins. Deep-seated infections, like furunculosis, abscesses and carbuncles, are preferentially linked to *Panton-Valentine leukocidin* (PVL). Therefore the existence of two different pathovars was hypothesized.
We investigated the microevolution and population structure of *S.aureus* ST121.To elucidate the mechanisms of emergence and epidemic spread we investigated the microevolution by means of single nucleotide polymorphism (SNP). SNP discovery was performed by using dHPLC for 117 genetic loci (46839 bp) within an international strain collection of 102 *S.aureus* isolates.
We identified 231 bi-allelic polymorphisms (BiP), corresponding to 1 BiP per 0.2 kilobases. The 231 bi-allelic polymorphisms were used to construct a minimum spanning tree (MST) which was associated in 86 haplotypes among the 102 strains.
Results indicate that two subpopulations have evolved into different pathovars that are associated with distinct clinical pictures. In addition we could show that much of the population structure of ST121 is local, largely endemic and spread to other countries or continents occur only on rare occasions.Furthermore, and in agreement to prior results in ST5, ST22 and ST239, mapping of *spa* types onto the MST revealed several *spa* types whose patterns seemed to reflect homoplasies.

MSV07

MRSA genome variation among and within individual patients

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It was recently demonstrated that MRSA accumulate genomic diversity sufficiently fast to document the pathogen's spatial spread over long distances, e. g., between countries or continents. It is currently unclear, however, whether genomic variation may also enable the reconstruction of transmission chains between individual patients, e. g. during an outbreak of hospital infections.
We have applied Illumina sequencing to compare the full genomes from multiple MRSA isolates from a hospital outbreak. Phylogenetic analyses indicated a marked temporal signal in the DNA sequence data. An isolate from the index patient (i. e., the presumed starting point of the outbreak) had a basal position in the phylogenetic tree, and isolates from later time points were more distal from the tree's root.
In cases where multiple isolates from individual patients were available, we observed significant genomic diversity among these. However, not each and every patient got infected by a unique MRSA, and MRSA genomes from several patients were indistinguishable or very closely related, suggesting potential transmission events.
It appears that genome sequencing holds promising potential for tracing MRSA spatial spread at a local scale.

MSV08

State-wide admission prevalence screening in Saarland, Germany (MR*SAar*Netz)

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Objective: The identification of risk factors prior to hospitalization is a central element in the prevention and control of MRSA. In Germany, screening is recommended based on the guidelines of the Robert-Koch-institute; yet, the screening intensity is largely varying between institutions. In order to introduce a regional program on MRSA control, a detailed knowledge of the MRSA burden is required. To this effect, for the first time for an entire German State, we have a conducted a State-wide admission prevalence screening (APS) in 24 hospitals of Saarland during October-November, 2010.
Methods: For screening, combined throat and nasal swabs were taken from patients immediately upon admission using a novel swab (ESwab, Copan, Italy) system for enhanced pathogen recovery. Samples were processed by a novel, fully automated, roboter driven specimen processor (WASP, Copan, Italy) and plated on selective biplate media for MRSA and methicillin sensitive *S. aureus* (MSSA). All analyses were performed in the State Hygiene laboratory at the University of Saarland Hospital.
Results: Of 20,007 specimen tested, 436 (423 throat/nose swabs, 13 wound swabs) were positive (prevalence, 2.2/100 admissions; 13,9% of total *S.aureus*); prevalence varied between institutions (0/100 - 9.0/100). These figures are higher than those previously determined in other German APS programs, and they also differ from those previously reported from other central European regions or institutions. *spa* type 003 (ST225) was most frequent among MRSA followed by *spa* type 504 (previously rarely reported). A detailed risk assessment (multivariate analysis) revealed that previous MRSA history, chronic care, and presence of a skin wound/ulcer were significantly associated with MRSA carrier status.

Conclusion: The conduct of a large regional APS study allowed for comparative determination of MRSA colonization/infection upon admission in an entire Federal State. These results are important to estimate the burden of MRSA cost and disease for the hospitals, for designing a cost-effective continuous screening program for the region, and for developing a rational approach to combat the circle of MRSA introduction from the community and care facilities into hospitals and back into the community. As a side effect, a general enhanced attention of general public, institution administrators, and policy makers towards the quality goals of MR***SAar***Netz was observed.

MSV09

Diversity of multiple-Locus-VNTR-Analysis-(MLVA)-Types in Meningococcal strains from different epidemiological settings

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Multiple Locus VNTR [Variable Number of Tandem Repeats] Analysis (MLVA) is a cheap, portable, and highly discriminatory typing method for meningococci (Schouls et al., 2006). It assesses the variation at 8 different VNTR loci within the genome. To date, however, little is known about the rate of MLVA variation and hence its usefulness for the tracing of transmissions. We attempted to describe the extent of diversity across epidemiological samples differing in the number of transmissions contained within them; to control for similar genetic background, we only compared strains sharing the same "finetype" (i.e. strains with the same serogroup, PorA, and FetA sequence type). Following samples, ordered by the presumed number of transmissions within them, were included: strains from long time carriers (20 strains from 4 carriers); strains from cases with invasive disease and close contacts (11 strains in the proximity of 5 index cases); strains from a cross-sectional study of carriers (36 strains belonging to 9 finetypes); strains from clusters of invasive disease by ET-15 meningococci (26 strains from 6 clusters); strains from finetype-specific spatio-temporal clusters of invasive disease (36 strains from 10 finetype-specific clusters computed with SaTScan version 5.1.3); and strains from randomly distributed cases of invasive disease belonging to the same finetypes as spatio-temporal clusters (72 control strains belonging to 10 finetypes). Categorical distances were computed between MLVA-profiles of strains sharing the same finetype and setting. Variation of MLVA types within a finetype differed significantly across settings ($p<0.001$, Kruskal-Wallis test; Figure). No variation of types was observed within long-time carriers and in strains obtained from index cases and close contacts containing zero and one to few transmissions, respectively. Interestingly. strains from finetype-specific clusters of disease by ET-15 showed less variation than strains from the cross-sectional carrier sample. In contrast, mean distance within strains from spatio-temporal clusters and randomly distributed cases (controls) of invasive disease was considerably higher (Figure). Our preliminary analyses describe the diversity of MLVA types in strains belonging to the same finetype obtained from different epidemiological settings; specifically, variation seems to increase according to presumed number of transmissions. To corroborate these findings and to estimate the rate of variation after one transmission, further analyses of long-time carrier- and Index-carrier-samples are under way.

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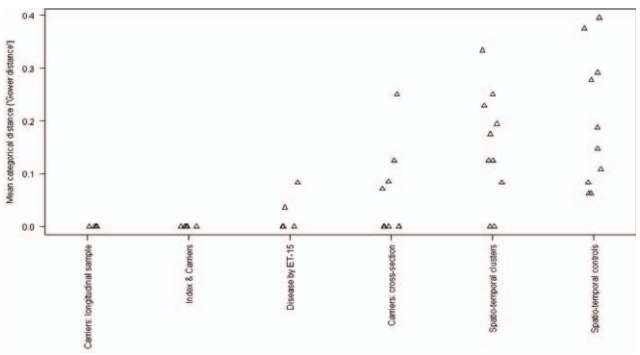
Figure 1



PRP01

Epidemiology of Vancomycin-resistant *Enterococci* in the University Clinics Jena -a longitudinal Study (March - August 2007) and an outbreak in November 2007

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Introduction: Vancomycin resistant *enterococci* (VRE) are cause of nosocomial infections. In Germany the incidence is increasing and outbreaks have been reported [1]. The aim of this study was to evaluate the situation in a large University hospital using the example of Jena.
Materials and Methods: VRE from patient samples were collected and identified phenotypically and with multiplex PCR. Antibiotic resistance was determined by broth dilution test and PCR. Epidemiological typing used pulsed field gel electrophoresis (PFGE). Clinical data were obtained from the medical records.
Results: From March to August 2007 we collected 223 VRE isolates from 116 patients (intensive care units (ICU): 93 samples (41.3 %), department of haematology/oncology (DHO): 62 samples (27.8 %), others: 68 samples (30.9 %). In November 37 VRE isolates from 32 patients of the DHO were collected. The specimen comprised stool (40.5%), wound swabs (24.3%), broncho- alveolar lavage (10.9%) nasal and skin swabs (10.2%), blood culture and intravascular devices (3.1%), miscellaneous (11%).
Species identification showed 171 *E. faecium*, 3 *E. faecalis*, 4 *E. gallinarum*. All strains were identified by api20strep, but 57 *E. gallinarum*, 16 *E. durans* and 1 *E. faecalis* were reclassified by multiplex PCR as *E. faecium*. The VRE collected in November 2007 were identified phenotypically: 6 *E. faecium*, 19 *E. gallinarum*, 2 *E. casseliflavus*.
The VanA type dominated (60%). All strains were sensitive to linezolid.
27/116 patients died, in 2 cases of sepsis with VRE. The most common nosocomial infections were sepsis, wound infection, pneumonia and urinary tract infection. 18/26 patients with wound infection underwent laparotomy prior to VRE detection. The mortality due to enterococcal infection was correlated only with sepsis ($p < 0.072$).
39/116 patients got vancomycin. Other antibiotics were: cephalosporins (54.3%), carbapeneme (40%), ciprofloxacin (39%), piperacillin (30.2%), metronidazol (21.6%), aminoglycosids (16.4%). In 15 cases VRE were treated with linezolid, but only in eight patients successfully (eradication).
The typing of 147 strains showed 9 clusters and 13 independent strains, 2 different clusters (1 ICU and 1 DHO) dominated. During the outbreak 14 strains showed known cluster 5 and new cluster 6 as well as 6 independent strains.
Discussion: Phenotypic identification is not always reliable and has to be reassessed on the DNA level. The main VRE type in our study, as in other German hospitals, was *E. faecium* of the VanA type[1]. All strains were sensitive to linezolid. Nosocomial acquirement has been proofed by the genetic clustering. This is due to antibiotic selection pressure or cross contamination. VRE are yet sensitive to linezolid, but eradication is not always possible. Therefore it is necessary to identify the patients at risk for increased mortality and to implement a dense surveillance for these patients.

PRP02

Density of antibiotic use in hospitals within the EurSafety Health-net project

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Introduction: The prudent use of antibiotics is a major preventive measure to forestall the selection and spread of multidrug-resistant bacteria. Here we assessed the density of antibiotic use in hospitals cooperating within a quality network for the prevention and control of healthcare-associated infection.
Method: Within the Dutch-German EurSafety Health-net project (www.eursafety.eu), German hospitals in the EUREGIO Gronau/Enschede provided data on the use of antibiotics in intensive care units (ICUs) and other (non-intensive care) wards. Data were assessed separately for the second half-year 2009 and the first half-year 2010. The use of antibiotics was reported by hospital pharmacies based on the Anatomical Therapeutic Chemical (ATC) code and in (milli-) gram and transformed into daily defined doses (DDD) as defined by the World Health Organization (WHO). Comparability was achieved by calculating DDD/100 patient-days (pd).
Results: Thirty-four regional hospitals within the network provided data on the use of antibiotics. The median (mean) density of antibiotic use on non-ICU wards was 48 (56) DDD/100 pd in 2009 and 47 (53) DDD/100 pd in

2010. Of note, in the non-ICU setting, extended-spectrum penicillins (J01CA), cephalosporins (J01DB-DE), carbapenems (J01DH) and quinolones (J01M) accounted (2009/2010) for 2.8%/2.4%, 39%/41.7%, 1.9%/2.2% and 11.7%/8% of the total antibiotic use, respectively. On 25 ICUs, the median (mean) density of antibiotic use was 108 (110) DDD/100 pd in 2009 and 109 (114) DDD/100 pd in 2010. On different ICUs the use of the broad-spectrum antibiotics vancomycin, teicoplanin, linezolid, carbapenems and tigecyclin together represented between 5% and 48% of the total use of antimicrobial agents.

Discussion: The total regional use of antibiotics was comparable to data from other regions published in the German GERMAP 2008 and the Dutch NethMap 2010 reports. However, between the hospitals, we observed a broad range of antibiotic use both with respect to the density and the types of antimicrobial agents used. Together with regional data on bacterial antibiotic-resistance, this data can be used by the network hospitals for discussions aiming to improve local antibiotic policies.

PRP03

Algorithm for empirical treatment for the prudent use of glycopeptides in hematologic patients: a tool in the control of vancomycin resistant enterococci

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Introduction: *Enterococcus faecium* has become a major cause of nosocomial infections. Especially patients with hematologic malignancies are at high risk because of their immunocompromised status and therefore adequate antibiotic therapy is required. However for the control of vancomycin resistant enterococci (VRE) antibiotic stewardship with a focus on prudent use becomes necessary. The aim of this study was to determine risk factors in order to develop an algorithm for optimal empirical treatment in those patients who are at risk of *E. faecium* blood stream infection (BSI).

Methods: Retrospectively demographic, clinical and microbiological data in 33 patients with an *E. faecium* BSI were compared to 66 control patients during a 5-year study period at the hematology unit. Multivariate logistic regression was used to explore the independent risk factors in order to develop a prognostic model to determine the risk of *E. faecium* BSI.

Results: Significant associations of *E. faecium* BSI were found with age, hospital stay prior to blood culture, duration of hospitalization 1 year before admission, fever prior to blood culture, severity and duration of neutropenia, CRP (C-reactive protein) at time of blood culture withdrawal, colonization with *E. faecium* prior to blood culture and diarrhea. *E. faecium* BSIs were found associated with more severe disease and higher mortality rates. Independent risk factors for *E. faecium* BSI were age > 59 years (5.47; 1.6-18.2), hospital stay prior to blood culture > 14 days (4.78; 1.3 - 18.0), fever > 1 day (4.02; 1.3-12.8), colonization with *E. faecium* 30 days prior to blood culture (OR 3.83; CI 1.1-12.8) and abdominal pain, diarrhea or neutropenia (5.95; 1.1-31.4).

Conclusion: Empirical treatment should be considered in those hematologic patients who are at high risk for development of *E. faecium* BSI. Using a prognostic model for risk stratification as designed in this study prudent use of glycopeptides becomes possible and might be helpful controlling further spread of VRE.

PRP04

In vivo transfer of Oxa-48 during an outbreak of *Klebsiella pneumoniae* with decreased susceptibility to carbapenems

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Objectives: We report an outbreak of *Klebsiella pneumoniae* with carbapenem resistance or decreased susceptibility in five patients in a tertiary care hospital in 2010. Molecular and phenotypical investigations of the clinical isolates were performed.

Methods: Antibiotic susceptibilities of *K. pneumoniae* were determined by Vitek2 and E-test. DNA from *K. pneumoniae* isolates were screened by PCR for the presence of KPC, VIM, IMP, NDM-1 and Oxa-48 beta-lactamases. Genetic relatedness was investigated using Enterobacterial Repetitive Intergenic Consensus (ERIC)- and repetitive sequence based (REP)-PCR.

Results: Ertapenem-resistant *K. pneumoniae* were isolated from rectal swabs and urine samples from five patients during an outbreak. Of these five patients, one was only colonized, two exhibited urinary tract infections and two patients were severely ill suffering from sepsis and/or pneumonia due to ertapenem-resistant *K. pneumoniae*. Infected patients were successfully treated with high dose meropenem and gentamicin, and, in case of pneumonia, in addition with inhalative colistin.

All *K. pneumoniae* were resistant to penicillins, cephalosporins, ertapenem, fluoroquinolones, and cotrimoxazol. Some of the strains exhibited elevated MICs towards meropenem, doripenem or imipenem, but still remained susceptible. Oxa-48 was identified by PCR and DNA sequencing in all *K. pneumoniae* isolates from the five patients. ERIC- and REP-PCR analysis of the *K. pneumoniae* isolates of all patients showed an identical profile suggesting a clonal spread.

In one of the patients an Oxa-48 producing *Escherichia coli* was isolated from rectal swabs in addition to the Oxa-48 producing *K. pneumoniae*. Prior to the emergence of the Oxa-48 expressing *E. coli* a less resistant *E. coli* with an ESBL phenotype could be isolated several times from this patient. This *E. coli* was negative for the presence of the Oxa-48 gene but showed an identical DNA fingerprint pattern compared with the Oxa-48 expressing *E. coli*. This strongly suggests the *in vivo* transfer of Oxa-48 from *K. pneumoniae* to *E. coli* in the patient.

Discussion: We report the outbreak of Oxa-48 producing *K. pneumoniae* in a tertiary care hospital. Epidemiological, molecular and phenotypical data suggest a horizontal interspecies transfer of Oxa-48 from *K. pneumoniae* to *E. coli in vivo*.

PRP05

Results of a 3 month universal Vancomycin-resistant enterococci screening of patients of an intensive care unit (ICU)

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Background: Increasing prevalence of clinical isolates of Vancomycin-resistant enterococci (VRE) in patients of an ICU was noticed during 6 to 9/2010. Therefore a VRE screening was implemented to determine both the rate of colonisation at ICU admission and the rate of acquisition during ICU stay.

Methods: From 10 to 12/2010 an universal VRE screening was introduced on an interdisciplinary ICU. Perianal swabs were taken from all patients at admission and once weekly until ICU discharge and analysed by culture for the presence of VRE.

Pre-existing VRE carriage was assumed if samples taken within 72 hours after admission were positive; nosocomial acquisition was defined as a positive follow-up screening after a negative result at ICU admission.

VRE-positive patients were characterised in detail and were monitored for developing VRE infections during hospitalisation.

Results: 737 patients were screened for VRE at least once and 111/737 patients (15.1%) were positive. 126/737 (22%) patients could be included in the follow-up screening.

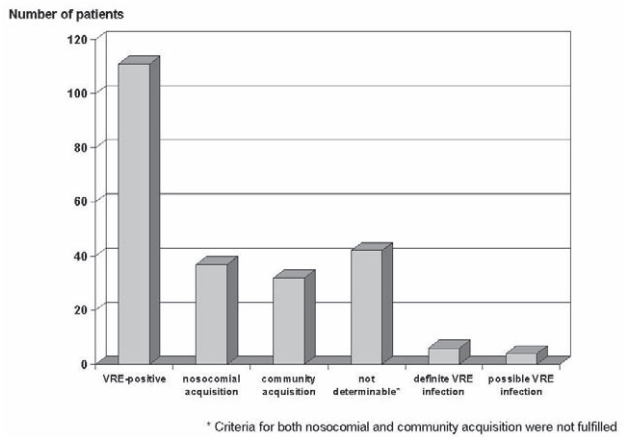
Because 42/111 (37.8%) of the VRE-positive patients had been hospitalised for more than 72 hours before the first screening swabs, the distinction community vs. nosocomial acquisition could be made only for the remaining 69 patients. 32/69 (43.5%) of these patients were VRE carriers at ICU admission, 37/69 patients (56.5%) did acquire VRE during their ICU stay. The mean time from admission to first VRE detection was 20 days (range: 4-54) in the nosocomial cases. Interestingly, none of the VRE-positive patients was previously known to carry VRE. A total of 37 (33.3%) patients had been treated with Vancomycin prior to VRE detection; 8 patients (7.2%) were known to be colonised/infected with Methicillin resistant *S. aureus*.

Only 6/111 of VRE positive patients (5.4%) developed a definite VRE infection (3 bloodstream and 3 abdominal infections); 4 patients had an asymptomatic bacteriuria. Mortality in VRE patients was 14.4% (14/111) regardless to their VRE infection or colonisation status.

Conclusions: Universal screening revealed a substantial VRE positivity rate of 15.1% including a high proportion of nosocomial acquired cases (56.5%). Reasons for the high VRE prevalence may be the previous Vancomycin therapy (33%). The clinical significance of VRE detection was low (5.4%). Neither did colonisation predict consecutive infection, nor was the VRE status associated with mortality. Therefore, we did not implement a universal VRE screening subsequent to this study period. Moreover, concerning infection control standard contact isolation precautions without single room isolation seemed to be justified. Further studies are warranted

to determine the role of the environmental contamination especially in a setting of moderate hand hygiene compliance.

Figure 1
Characterisation of VRE-positive patients



PRP06

Epidemiology and molecular characteristics of Methicillin-resistant *Staphylococcus aureus* (MRSA) infections in a university hospital: 2004-2007.

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Background: A routine surveillance for MRSA was established since 2001 at Hannover Medical School. In order to identify nosocomial transmission episodes molecular protein A (spa)-typing was introduced.
Method: MRSA infections of all inpatients were analysed according to CDC definitions and were classified into nosocomial or imported cases from 2004 to 2007. Sequencing of the repeat region of the Staphylococcus protein A gene (spa typing) were performed from microbiological isolates of all MRSA infections.
Results: In total 2,460 MRSA cases including 358 (14.6%) MRSA infections were detected. Surgical site infections were the most frequently type of MRSA infection (64.8%) followed by primary bloodstream infection (12.0%), pneumonia (11.7%) and urinary tract infection (11.5%). In addition, secondary bloodstream infections (10.6%) were detected. A significant reduction of MRSA infections was observed from 2004 to 2006 (OR 0.363; p<0.001). The spa typing revealed 49 distinct spa types. The spa type t032 (so called "Barnimer" strain) was the predominant strain type (68.4%) followed by t003 (4.5%), t020 (2.8%), t456 (2.5%), t004 (2.2%) and t022 (2.2%). The proportion of infections with secondary bloodstream infections were significantly associated with the spa type t003 (OR 6.75; p=0.004). The spa type t032 was significantly less associated to secondary bloodstream infections (p=0.041).
Conclusion: The molecular spa typing provided additional information to the epidemiological associations during routine MRSA surveillance. The spa type t003 seemed to be a more invasive MRSA strain contrary to spa type t032. Further studies were needed to verify these observations.

PRV01

Epidemiology of methicillin-resistant *Staphylococcus aureus* at hospital admission in South Brandenburg, Germany

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During the last years the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in german hospitals has raised. In order to know the impact of MRSA at the hospital admission and to develop strategies to reduce the risk of spread of MRSA inside and outside the hospitals an epidemiological study in seven german hospitals of South Brandenburg was conducted. We evaluated the incidence of MRSA carriage at hospital admission and we investigated some risk factors associated to MRSA colonisation. Moreover the epidemiological distribution and characterisation of MRSA genotypes was analyzed.
Methods: Over a two month period onset patients who accepted the study from all clinical wards were asked for risk factors and screened for MRSA. Nasal and wounds swabs were plated on MRSA chromogenic selective agar and incubated for 48 hours. Suspected MRSA colonies were confirmed by rapid tests (catalase, coagulase) and an antimicrobial susceptibility test was performed. The assignation of the MRSA isolates to the different clonal complexes was performed by analyzing their gene pattern including virulence, adhesion, resistance and other factors with DNA chip technology (Alere/Clondiag Jena).
Results: 13855 patients supported the study. The participation average was high (88.1%). The incidence of MRSA carriage at hospital admission inside the study is low (0.77%). In the multiple logistic regression analysis, risk factors like age 65 or older (relative risk [RR], 3.60; 95% confidence interval [CI], 2.29-5.67), nursing home care (RR, 6.06; 95% CI, 3.29-11.18), known MRSA colonization in the past (RR, 18.71; 95% CI, 10.85-32.26), implantable medical devices (RR, 2.52; 95% CI, 1.68-3.79), chronic wounds (RR, 4.61; 95% CI, 2.64-8.06) and metabolic diseases (RR, 2.42; 95% CI, 1.58-3.71) were significantly associated with MRSA carriage. Only 107 MRSA strain were isolated. The dominant clonal complex is ST22-MRSA-IV (Barnim strain, 71%), followed by the ST398-MRSA-V (dutch pig strain, 10%) and ST5-MRSA-II (Rhine-Hesse strain, 8%). Only one PVL-positive MRSA (ST8-MRSA-IV, USA300, 0.7%) was isolated.

Unterstützt durch das Bundesgesundheitsministerium

PRV02

Prevalence and risk factors for carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) in 17 German hospitals: results of a point-prevalence study in the rural district Hannover

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) represents the predominant multidrug-resistant organism and is a well-known causative agent of healthcare-associated infections in hospitals. However, only few data about its overall prevalence in certain geographical areas are yet available.
Objectives: To determine the prevalence of MRSA including the proportion of Panton-Valentine Leukocidin (PVL) positive strains among inpatients in the rural district Hannover (Germany). In addition we checked for patient subgroups that may be at an increased risk for MRSA carriage.
Method: A point-prevalence study was conducted in 17 hospitals from October 1, 2009 through March 31, 2010. Inpatients were screened by cultures from nose, throat and broken skin and patient data was recorded. MRSA isolates were tested for PVL and were analysed by staphylococcal protein A (spa) typing.
Results: MRSA was isolated from 118 (3.9%) of 3,013 consenting inpatients. The MRSA prevalence n different hospitals ranged from 0.8% to 12.5%. 42% of all identified MRSA carriers were detected on internal medicine wards and 30% on surgery wards. 92 (78%) were newly identified MRSA carriers. In 3 of 17 hospitals an alert system was in place. The spa typing revealed 26 distinct spa types. The spa type t032 (so called "Barnimer" strain) was the predominant strain type (67%) followed by t003 (3%), t011 (3%), t020 (3%) and t002 (2%). The PVL gene was only present in two (1.7%) MRSA strains. Factors independently associated with MRSA as determined by multivariate analysis were: (a) age older than 68 years (OR, 1.86; CI95, 1.24-2.85), (b) history of surgery (OR, 1.98; CI95, 1.29-3.02), (c) prior antibiotic therapy (OR, 1.73; CI95, 1.15-2.62), (d) history of MRSA carrier status (OR, 3.27; CI95, 1.71-5.95), (e) presence of open skin lesions (OR, 2.77; CI95, 1.64-4.52), and (f) care on an internal medicine ward (OR, 1.99; CI95, 1.30-3.02).
Conclusion: The results of our study revealed that 78% of all MRSA-positive inpatients had been missed. Hence, screening for MRSA on admission is useful to identify the imported cases and should be performed on wards harboured patients at risk, e.g. internal medicine and surgery. On the basis of our results, the respective hospital of the rural district Hannover can optimize its comprehensive infection control strategies against MRSA.

PRV03

MRSA-prevalence and ecolonisation efficiency in health-care workers

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As commonly known, MRSA is one of the most important hospital accquired pathogens. Within healthcare institutions, Methicillin-resistant Staphylococcus aureus (MRSA) can be transmitted from patient to patient or via healthcare workers' (HCW) hands, clothes, equipment and the environment. Few studies have evaluated the prevalence of MRSA colonization among HCW outside outbreak situations and the importance of

staff as a real source of transmission. Therefore, this investigation focuses on MRSA among hospital employees and evaluates the efficency of decolonisation therapy among health-care workers.
726 staff members (83 physicans, 514 nurses and 109 persons without direct contact to patients) out of nine hospitals within the German part of the Dutch-German EUREGIO participated in the investigation. Nasal-pharyngeal swabs were taken from each staff member on Mondays and subsequent Wednesdays. Risk factors for MRSA carriership were determined by standardized questionnaire. Both swabs were microbiologically processed and spa-typed in order to exclude or determine carriership. Only stable MRSA carriers with two positive swabs underwent eradication treatment. Additionally, the skin of all stable MRSA positive personnel was swabbed. In case of skin-colonization participants were released from work until successful MRSA-eradication (verified two days after the end of antimicrobial therapy); otherwise only the first two days of eradication therapy had to take place at home. Long-term eradication success was controlled one or six month after eradication.
33 out of 726 (4,5 %) staff members were MRSA-positive in al least one swab. 23 of them (3,2 %) were positive in both swabs.
Among physicians, MRSA prevalence was lower (1,2%) than in nurses (3,7 %) and other staff members without direct contact to patients (2,8 %). A positive MRSA anamnesis and acne were identified to be statistical significant risk factors for MRSA colonisation.
90 % of the stable MRSA carriers underwent decolonization therapy parallel to normal work. The therapy was successful after the first eradication cycle in 100 % of the cases and could be verified in follow-up swaps. Nine different spa-types were detected. The most frequent ones were typical hospital-acquired ha-MRSA t003 and t032 whereas lifestock-associated la-MRSA were not found at all under staff carriers.
The study showed that hospital staff is frequently colonized with typical hospital-accquired MRSA outside outbreak situations and MRSA prevalence differs among professional groups. Notable, livestock-associated MRSA were not found in the EUREGIO where a high pig density exists. Importantly, all HCW were successfully decolonized no matter if they were eradicated at home or parallel to work. Decolonisation parallel to work reduced the days of inability to work among HCW in comparison to complete decolonisation at home.

PRV04

Characterization of hospital-associated Vancomycin-resistant *Enterococcus spp.* in a tertiary care center in Hessen

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Introduction: Vancomycin-resistant *E. faecalis* and *E. faecium* (VRE) appeared first in 2005 in a tertiary care center in Hesse with increasing prevalence until yet. VRE scenarios included small outbreaks and, unfortunately, also some fatal cases. VRE screening and an isolation policy was applied in 2006. We investigated VRE isolates from these time period to identify relevant strain characteristics known to be involved in enterococcal virulence and/or epidemic spread to follow routes of transmission.
Methods: VRE isolates dating from 2005 until 2010 from 179 patients were selected. Species and antibiotic susceptibility was identified by routine microbiological procedures. Isolates were further characterized by PCRs for *vanA*, *vanB* and virulence genes/epidemic markers (esp, hyl). Moreover, PFGE and MLST were applied for typing of representative isolates. In addition, clinical and epidemiological data of each patient were collected.
Results: In total, 180 VRE isolates (1 *E. faecalis*, 179 *E. faecium*) were selected. All *E. faecium* isolates were resistant to ampicillin and carried the esp-gene (typical characteristics of hospital-associated *E. faecium*). The *hyl*-gene was detected in 145 strains. Vancomycin resistance was encoded by *vanA* (n=38) and *vanB* (n=142). Characterization by PFGE demonstrated a high genotypical heterogeneity among the isolates, but also identified clusters of related strains. MLST types were determined for isolates of PFGE subclusters revealing various sequence types but all representing hospital-associated strains (MLST CC17).
Conclusion: Our study identified a polyclonal prevalence of vancomycin-resistant *E. faecium* mainly of the *vanB*-type (79%) thus demonstrating a frequent introduction (interhospital spread) of *E. faecium*. The comparably high rate of *vanB*-VRE is contrary to other German hospital surveillance results from recent years where *vanA*-type *E. faecium* dominate. Horizontal transfer of *vanB* (also to vancomycin-susceptible *E. faecium*) in addition to a clonal spread of *vanB*-type VRE might contribute to the present VRE prevalence.

PRV05

Outbreak of *vanB*-type vancomycin-resistant *Enterococcus faecium* (VREF) expressing low levels of vancomycin resistance in a neonatal intensive care unit; Sept 2008 to May 2009

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Objective: Rates of VREF remain constant or are declining in Germany in recent years, but molecular analyses suggest a genotype switch from *vanA*-type to *vanB*-type among hospital VREF. We investigated an outbreak with VanB type VREF involving 74 neonate patients from an intensive care unit of a German hospital to resolve emergence and spread of VanB VREF.
Materials: All samples were taken at a single neonatal ICU during a 9-months period in 2008/2009. Enterococci were isolated non-selectively on Columbia Agar. Species was identified biochemically. Vancomycin MICs were determined by E-test and the genotype by a PCR-hybridization method. Altogether 75 *E. faecium* were sent to the Focal Lab for Enterococci at the RKI for further analyses including confirmation of the resistance phenotypes and genotypes, molecular typing and characterization (PFGE, MLST, plasmid typing by rep genes).
Results. We confirmed 75 VREF of which 73 possessed *vanB*. Of the 73 *vanB* VREF 36 (49%) were vancomycin-resistant according to EUCAST criteria (MIC >4 mg/L) and only 5 (7%) when using the CLSI breakpoint (=>32 mg/L). Additional resistances included ampicillin (n=73; 100%), high-level (HL) ciprofloxacin (67; 92%); HL gentamicin (61; 84%), HL streptomycin (63; 86%), erythromycin (68; 93%), and tetracycline (10; 14%). All were susceptible to linezolid and daptomycin; one was resistant to tigecycline (MIC= 1mg/L). PFGE analysis revealed one major clone prevalent over the entire study period represented by 56 isolates. Another 17 *vanB* VREF and 2 *vanA* VREF were clonally different. The major outbreak strain revealed MLST type ST192, a hospital-associated strain type widely prevalent in Germany in recent years. Plasmid typing identified prevalence of pLG1-, pRE25- and pRUM-like plasmids in the outbreak strain. Preliminary molecular characterization revealed a chromosomally located *vanB* cluster.
Discussion: An increasing number of *vanB* VREF involved in outbreaks is also reported in other European countries (Sweden, France, Poland). A molecular analysis of a recent outbreak strain from Sweden revealed *vanB*-type ST192 VREF associated with a distribution of a specific *vanB*2-Tn*5382* cluster on pRUM-like plasmids; in our case the corresponding *vanB* cluster was not plasmid-located but chromosomally-borne although ST192 isolates contained several plasmids including also a pRUM-like variant.
Conclusion: Reliable detection of low level vancomycin expression in *vanB*-type VREF is a challenge in the clinical laboratory. Its delayed detection or unnoticed appearance may support further spread of *vanB* VREF and underestimate its true prevalence among the clinical setting. Molecular (screening) methods to detect these strains are here superior to phenotypic assays including also screening agar plates with a comparably high vancomycin inoculum.

PRV06

Emerging multiresistant *Pseudomonas aeruginosa* and its hidden reservoirs in hospitals - molecular studies of a nosocomial outbreak in a high risk area.

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Multiresistant *Pseudomonas (P.) aeruginosa* is an increasing problem in health care facilities [1]. As one of the most important inhabitants of biofilms this Nonfermenter can consist in different reservoirs in clinics. The siphon traps of washing basins are colonized by different germs and may be an underestimated source of pathogen distribution. Long-term surveillance from 2007 to 2009 within a hemato-oncological setting with 24 isolation units for patients with allogenic stem cell transplantation revealed the association of emerging multiresistant *P. spp*. infections with detection of suspicious strains in siphons of patients´ rooms. All affected patients received same antibiotic prophylaxis and underwent same treatment with multiple daily throat rinsing against oropharyngeal mucositis in front of the washing basins. During this procedure it was very possible that the patients inhaled aerosols from siphon water.
Responsible for this outbreak two strains could be detected by RAPD-PCR and specific *P. aeruginosa* microarray genotyping. Comparison of outbreak strains with strains isolated from siphons of patients´ rooms showed same antibiotic resistance patterns. Resistotype 1 had broad resistance to common antibiotics with susceptibility only to Polymyxin B, whereas resistotype 2 was susceptible to Polymyxin B and Amikacin. All Carbapenem-resistant

strains were screened positive for VIM-Metallobetalactamases [2]. The outbreak could be stopped by implementation of so called Hygiene siphons at the washing basin in all of the 24 isolation units (Fa. BIOREC - Dr. SCHLUTTIG Medizinischer Apparatebau, Dresden) [3].

Conclusion: Here we report the molecular studies of an outbreak of multiresistant *P. spp.* in a hemato-oncological ward with optimal conditions concerning hygiene management. Even with maximum barrier infection control measures undertaken to avoid nosocomial infections hidden reservoirs like siphon traps of washing basins can be the source of pathogen outbreaks. The installation of Hygiene siphons in isolation units for high risk patients can help to reduce the distribution of multiresistant germs. Early molecular-epidemiological analysis is urgently needed to verify outbreak situations and is essential to implement specific and effective infection control measures.

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PWP01

Impact of *E. coli* Nissle 1917 on the gastrointestinal barrier - miRNAs as perspectives for barrier dysfunction treatment

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The gastrointestinal tract harbours a complex microbial ecosystem, engaged in continuous crosstalk with the host. The balanced relationship between intestinal epithelial cells (IECs) and gut microbes can be disturbed, resulting in the activation of the mucosal immune system which contributes to the development of inflammatory bowel diseases (IBD).

We used a T84 cell culture model to analyze the cellular responses of IECs co-incubated with the Gram-negativ probiotic strain *E.coli* Nissle 1917 (EcN). As IBD may develop after defects of barrier function and based on our microarray data obtained with co-incubated IECs we focussed on proteins (ZO-2, PAR-3, PAR-6) of the apical junctional complex (AJC). We used transepithelial resistance (TER) of T84 cells as a read-out to monitor epithelial barrier function. By microRNA-profiling of IECs after co-incubation with probiotics we identified microRNAs (miRNAs) that correspond to proteins of the AJC (qRT-PCR, Western-Blotting).

Barrier function was enforced by probiotic *E.coli* and disrupted after EPEC infection (E2348/69). Even after barrier disruption by EPEC, barrier function could be restored by incubation with probiotic EcN as monitored by an increase in TER. We confirmed the participation of microRNAs (miRNAs) in the regulation of the AJC proteins by transfecting T84 cells with miRNAs, miRNA-inhibitors and miRNA-mimics. These studies show that miRNAs are involved in strengthening probiotic effects and the barrier disrupting effect of EPEC.

This study revealed molecular responses of IECs specifically induced by the Gram-negative probiotic *E. coli* strain Nissle 1917. Further insight into the molecular targets of specific miRNAs might foster the development of new strategies for treatment of gastrointestinal diseases.

PWP02

Effects of pathogenic, and probiotic *E. coli* on Immune Cells at the gastrointestinal barrier

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The gut immune system keeps the microbiota of the gastrointestinal tract under surveillance. Besides commensal and probiotic microorganisms there are also bacterial pathogens such as enteropathogenic *E.coli* that can cause diarrhea by exploitation of host cells and subversion of defense measures. How these pathogens interact with and subvert (immune) cells of the intestinal barrier is not fully understood. In this study we address the effects of the enteropathogenic *E. coli* (EPEC) strain E2348/69 and of the probiotic *E. coli* Nissle 1917 (EcN) strain on human PMBC and human monocytic THP-1 cells.

Dendritic cells derived from human PBMC and monocytic THP-1 cells were infected with EPEC wt, EPEC mutant strains or the probiotic strain EcN. The cytotoxicity of these bacteria was evaluated by analyzing cell viability. In addition, morphological changes within the actin network were analyzed via confocal immunofluorescence microscopy and influences on the expression of proteins involved in inflammation via qRT-PCR and Western Blotting.

In contrast to infections of THP-1 cells with *E. coli* C600 or EcN, EPEC infection exhibit a dramatic increase in cytotoxicity (threefold). Analysis of the actin cytoskeleton of THP-1 cells revealed that EPEC wt as well as effector protein deletion mutants are able to induce pedestals with classic actin accumulation at bacterial attachment sites. This is not observed with an EPEC mutant lacking the effector protein Tir. Interestingly, pedestal formation can be faintly detected also with mutants without functional type III secretion system (T3SS).

Concerning the expression of cytokines we observed an upregulation of the anti-inflammatory IL-10 upon infection of DC or THP-1 cells with EcN (two and fivefold) in contrast to infection with EPEC wt. By analyzing the influence of EPEC and EcN on the NFkB signal cascade in THP-1 cells we observed an activation of the pathway upon EcN and *E. coli* C600 coincubation but an inhibition upon EPEC coincubation.

The results show that EPEC exhibit destructive activity on immune cells where virulence factors such as the T3SS-secreted effector proteins enable the bacteria to specifically target host cellular processes. The comparison of effects of pathogenic and probiotic bacteria on immune cells might reveal further insights into the molecular mechanisms that are impaired in gastrointestinal disorders.

PWP03

Role of dendritic cell activation as well as Toll-like receptor 2 and 4 expression while DSS colitis.

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Hosts live normally in symbiosis with their intestinal microbiota due to a long coevolution. Somehow this tolerance is abolished in IBD patients, which leads to a strong and long lasting inflammation irregularly interrupted by short remission phases. In order to investigate the role of dendritic cells and their TLR2 and TLR4 expression while acute phase inflammation the Dextran sodium sulfate (DSS) model was employed. Therefore DSS was administered to C57BL/6 mice for 7 days. Mice displayed significant weight loss and an increasing disease activity index. Lamina propria (LP) and mesenteric lymph node (MLN) DCs were isolated of untreated mock and DSS treated mice. LP DCs of DSS treated mice owned significantly increased levels of MHCII, CD40, CD80 and CD86 representing a more activated DC state as compared to DCs of mock mice, whereas MLN DCs did not differ concerning their activation state. Furthermore DSS mice featured higher levels of myeloid DCs in the LP as well as in the MLN compared to mock mice. As well had DSS mice a significantly increased percentage of $CD103^+$ DCs in the LP in comparison to mock mice, whereas amounts of $CD103^+$ DCs in MLN were vice versa. Surprisingly LP and MLN DCs of DSS mice expressed significantly higher amounts of TLR2 and TLR4 in comparison to mock mice. Increased appearance of $CD103^+$ DCs and higher amounts of TLR2 and TLR4 are likely to be a counter regulation of the host in order to suppress developing inflammation.

PWP04

T-RFLP-based differences in oral microbial communities as risk factor for developing painful oral inflammation under stress

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Introduction: The bacterial inventory of the human oral cavity is already fairly comprehensive and composed of about 800 different species many of which are uncultivated. However, the dynamics and variability of the oral microflora in relation to health or disease are still poorly understood. Our aim was to test the hypothesis, that the development of oral symptoms after multiple stressing of the immunosystem is related to the composition and diversity of the initial microflora.

Material and Methods

As a model system, we analyzed the oral microbiota from 58 healthy individuals during a challenging expedition in remote regions of the Himalayas (implying hygiene, diet, temperature, physical and mental stress). Plaque samples were taken at begin (Bhulebhule) and destination (3,000 meter difference in altitude) seven days later (Manang). Twenty-eight individuals remained symptom-free (Group I) while 30 participants developed dental problems, mostly gingivitis (Group II). The oral microbiota was monitored via terminal restriction fragment length polymorphism (T-RFLP) analysis of amplified 16S rRNA-genes directly from the plaque samples. Variation in T-RFLP datasets were analyzed based on the Additive Main Effects and Multiplicative Interactions (AMMI) model using the T-Rex software package (http://trex.biohpc.org/).

Results: Variation from T-RF main effects was at least 95% indicating that the majority of variation in T-RFLP profiles was due to inherent differences

in microbial communities among individuals. However, when comparing Group I and II an interaction signal of about 3% was consistently observed (under varying analytical parameters) while this was not the case when the two time points of sampling (i.e. sampling at start and end of the trekking-tour) were compared. Likewise the Shannon-Weaver-index of diversity and the Jaccard-index of similarity indicated, that Group I and II differed significantly in the heterogeneity of their oral microbial communities at begin of the trekking tour. Based on the Human Oral Microbiome Database (HOMD) distinct bacterial taxa could be elucidated as key-components explaining the observed differences.

Discussion: The data indicate that there is a discernable relationship between susceptibility to clinical symptoms and the indigenous, pre-formed oral microbiota which means that the initial composition of the oral ecosystem determines whether or not symptoms develop upon exposure to various stress-parameters.

PWP05

Role of IκBζ in induction of semimature dentritic cells and maintenance of intestinal homeostasis

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IκBζ is a member of the IκB protein family and a recently described regulator of the transcription factor NFκB. But unlike all other IκBs, IκBζ seems not only to be an inhibitor but more of a modulator of NFκB. While the production of the proinflammatory cytokine TNF-α is inhibited in presence of IκBζ, other genes like IL-6, IL-12p40 and GMCSF are highly up regulated. We hypothesize that IκBζ at least partially contributes to DC semimaturation, as this process was shown to be dependent on presence of IL-6 and absence of TNF-α. Furthermore this DC differentiation type promotes rather tolerogenic than proinflammatory T-cell responses.
Due to this fact IκBζ might play a crucial role in maintenance of intestinal homeostasis. To clarify the role of which might lead to the development of new strategies in treatment of inflammatory bowl diseases we analysed and compared in a first step the IκBζ mRNA and protein expression in naïve, semimature and mature DC using quantitative PCR and western blot analysis. Additionally the expression of IκBζ in restimulation of semimature DC was investigated.
Future experiment will involve IκBζ deficient mice, for generation of BMDC and the analysis of the impact of IκBζ on acute DSS colitis.

PWP06

Genome analysis of probiotic Symbioflor 2 *Escherichia coli* DSM17252

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The probiotic drug Symbioflor 2 (*E. coli* DSM 17252) contains six *Escherichia coli* genotypes named *E. coli* G1/2, G3/10, G4/9, G5, G6/7 and G8, all functionally representing one population. Symbioflor 2 is a liquid preparation containing live *E. coli* cells, which were recently classified as biosafety level 1 organisms. The product is well established as a probiotic drug for years and it is widely used especially for the treatment of Colon irritable in adults and children.
The genomes of all six genotypes have been sequenced by pyrosequencing and sequencing by synthesis technology and overall twelve different plasmids were identified. Whole-genome comparison of the six genotypes allow a subdivision into four different genomotypes. We could also show that all Symbioflor 2 *E. coli* clearly belong to the cluster of apathogenic *E. coli*.
However, it is largely unknown which *E. coli* genotype is responsible for the products probiotic character and which genes and proteins are involved in the development of a probiotic trait. *E. coli* G3/10 has already attracted interest due to its ability of inducing defensin production in cell culture and in human volunteers. Using an *in vitro* adherence assay with human intestinal epithelial cells we could further show that *E. coli* G3/10 significantly decreases the adhesion of enteropathogenic *E. coli* strain E2348/69 (EPEC) to host cells, comparable to the probiotic strain *E. coli* Nissle 1917. The EPEC adherence assay is a suitable surrogate test system to evaluate probiotic action of a given microorganism. The sequenced genomes together with *in vitro* data will help us to gain further insight into the probiotic behavior of Symbioflor 2.

PWP07

Molecular analysis of population dynamics in microbial communities during oral biofilm formation

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Oral biofilms are supposed to be involved in a number of oral diseases such as plaque formation, dental caries and periodontitis. Thus, a more detailed knowledge of oral microbiota in biofilm formation is important for a better understanding of pathogenesis.
In the present pilot study the composition of human microbiota during intraoral biofilm formation was analysed in 7 healthy volunteers (females, aged 24 to 26) using bovine dental enamel slabs harvested after 3 and 30 minutes, 2, 6 and 24 hours. In parallel, interproximal dental plaque and saliva were analysed. The microbial communities were characterized by T-RFLP (Terminal Restriction Fragment Length Polymorphism) analysis of 16S rRNA genes applying three different restriction enzymes, *Hae*III, *Msp*I and *Hha*I. T-RFLP signals of the phylogenetic groups were assigned to sequence data obtained by DGGE (Denaturing Gradient Gel Electrophoresis) analysis or in silico analysis of published sequence data, respectively.
Initially, 70 samples were investigated by DGGE revealing that the variety of bands was restricted to few signals in all dental biofilm samples whereas a higher diversity of bands was found in saliva and proximal interdental plaque. Semiquantitative analysis by T-RFLP confirmed lower microbial diversity in dental biofilms compared to other oral compartments. 55 specific *HaeIII* T-RF signals could be assigned to various phylogenetic groups and we could show that microbial community structure was different between dental biofilms, saliva and interproximal dental plaque. The abundance of streptococci was highest in dental biofilms whereas *Bacteroidetes* were predominantly found in saliva. The relative abundance of *Veillonella spp.* varied during biofilm formation with lowest frequencies in 6 hours biofilms. Other phylogenetic groups e.g. the group of *Haemophilus/Neisseria* were detected with equal frequencies in all samples. The similarities in the community structure in different oral compartments were estimated by calculation of similarity index Chao-Jaccard revealing the lowest similarity between microbial communities in saliva and interproximal dental plaque compared to 2 hours dental biofilm. The highest similarity was calculated for microbial communities in 6 hours dental biofilms versus 24 hours dental biofilms.
We conclude that characteristic signatures of microbiota can be found in saliva, interproximal dental plaque and also during oral biofilm formation. Culture-independent analysis of microbiota may provide new insights into the microbial diversity in various communities which was shown here for different oral compartments and growing oral biofilms.

PWP08

Influence of lactobacilli on Candida viability and yeast to hyphae transition

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Background: Candida albicans, a diploid asexual fungus is a part of the normal flora including the vaginal surfaces. In healthy individuals, C. albicans normally does not cause disease. However, when the balance of the normal flora is altered e. g. during antibiotic or hormonal therapy, C. albicans can cause painful cutaneous or subcutaneous infections including vulvovaginitis. In germany around five million women suffer from candidal vulvovaginitis. Lactobacilli belong to the physiological flora of the vagina. While some studies show the beneficial effect of lactobacilli on bacterial vaginosis. However, the role of lactobacilli in candidal vulvovaginitis is not known. One well known pathogenicity factor of candida spp. is the transition from yeast to hyphal growth. Therefore we analyzed how far lactobacilli may influence the yeast-hyphal transition has been described as a factor that facilitates the invasion process.
Methods: In total we used five different C. albicans strains which were isolated from women presenting different microbial environment in their vagina. The different Candida spp. were incubated with distinct Lactobacillus strains (L. gasseri, L.crispatus und L. jenseni) isolated from the vagina as well. We analyzed how far the different Lactobacillus strains

influence the viability (XTT_reduction assay) of Candida as well as the expression of hyphae-specific genes (ALS3, HWP1, HYR1)
Results: Only L. crispatus inhibited the growth of all Candida albicans isolates under study and diminished their viability significantly. In contrast L. gasseri led to a significant increase in viability of all Candida albicans strains. L. jensenii exhibited no influenec on fungal growth. The effect of the different Lactobacillus spp. on yeast to hyphae transition was strongly dependent on the type of the respective Candida albicans strain.
Conclusion: From our results one may conclude that lactobacillus spp. may either favour or inhibit pathogenicity of Candida albicans.

PWP09
Impact of the intestinal microbiota on mucosal homeostasis

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In addition to genetic predisposition, environmental factors such as commensal bacteria contribute to the development of inflammatory bowl disease (IBD).
The gut of mammalians is colonised by a complex flora of microorganisms containing 500 - 1000 different bacterial species. These bacterial populations contribute to the health of the host, among other things, by promoting proper immune systeme development and limiting pathogen colonization. *Bacteroides vulgatus* mpk was shown to have the ability to prevent Colitis, whereas *E. coli* mpk induces intestinal inflammation in interleukin-2-defiient (IL-2$^{-/-}$) mice. The mechanism however remains unclear.
In the current study we analyse the composition of the intestinal microbiota of T-cell transferred Rag1$^{-/-}$ by 454-Seqeuncing of 16S RNA genes. With this method we want to reveal differences between (1) the gut microbiota of mice that develop colitis compared to mice that stay healthy and (2) the composition of the intestinal microbiota before and during development of colitis.

PWV01
New culture-independent analysis of microbial communities in cystic fibrosis lung modifies the current view of polymicrobial infection

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Cystic fibrosis (CF) is a recessive inherited disease caused by mutations of the CFTR gene. In CF patients morbidity is severely influenced by chronic polymicrobial lung infection. Routine microbiological testing is focused on the detection of few typical pathogens using culture-based techniques. Thus, complexity of microbial community in cystic fibrosis lung cannot be displayed. However, by the use of new culture-independent techniques unbiased semi-quantitative analysis of complex bacterial communities are now available which could help to better understand the role of microbiota in colonisation and pathogenicity of CF.
In the present pilot study 51 respiratory samples of 37 CF patients in three different age groups (0 to 10, 11 to 20 and >20 years) were analysed both by culture-based and new culture-independent methods (T-RFLP).
Corresponding to previous studies, an age-dependent infection rate was found by culture-based analysis for the classical CF related pathogens with highest rates of *S. aureus* infections in the 0 to 10 year old group and rising *P. aeruginosa* infection rate in older patients (11-20 and >20 years). This finding was supported by the culture-independent analysis of bacterial nucleic acid in respiratory samples using T-RFLP, however, we also found new aspects of respiratory infections by the culture-independent method with increasing rates of anaerobic bacteria in CF patients at older age which potentially contribute to clinical progression of CF lung disease; however, prospective clinical studies are required to confirm this new assumption. Culture-independent analysis also provides a quantitative view to the relative abundance of specific pathogens in bacterial communities of CF patients. High relative abundance of Staphylococci (24%), *Neisseria / Haemophilus* (16%), Streptococci (9%), *Bacteroidetes* (6%) and *Veillonella* (5%) were found whereas the *Pseudomonas* species were detected with surprisingly low frequencies in samples (1%) of all age groups.
We conclude that culture-independent analysis (e.g. T-RFLP) will fundamentally change the current view of polymicrobial lung infection in CF patients due to the unbiased detection of potentially new respiratory pathogens and due to the new quantitative aspects. We suppose that very soon culture-independent analysis of the respiratory microbiota could become a new diagnostic standard for optimized diagnostics and therapy of CF patients.

PWV02
Microbiome of the murine lung

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Introduction: Commensals comprise an important component of the micro-ecology of epithelia. Yet, opposite to the upper respiratory tract and the gut, the lower respiratory tract including lung has been considered free of foreign microorganisms, so it is vastly unexplored. To understand how lung micro-ecology affects host defense and infection, it is essential to identify lung commensals.
Material and methods: We identified bacterial species in sterilely excised murine lungs by combining various culture systems and 16S rDNA analysis using cloning or 454 pyrosequencing method. We compared inbred mice maintained under specific-pathogen-free (SPF) or inbred and wild-derived mice of distinct geographical origin from a non-SPF-facility as well as mice caught in the wild. To localize bacteria in lungs, Fluorescence in situ Hybridization (FISH) was used. Finally, bacterial communities in each group of mice were compared by divergence-based diversity analyses on the species level.
Results: We isolated aerobes and facultative anaerobes including Pasteurellaceae, Enterobacteria and Firmicutes from lungs of Plön mice. Additional species including non-cultivatable Betaproteobacteria and Enteric bacteria were identified by 16S rDNA cloning analysis. In addition, we confirmed that major species of lung commensal is Betaproteobacteria, or Ralstonia sp. by high-throughput pyrosequencing method. Notably, we detected bacterial communities on alveolar epithelia by eubacteria specific FISH in the absence of inflammatory lung pathology. Comparison of bacterial community between mouse groups shows that overall composition of Phyla or Family level was similar regardless of mouse origin, but rarefaction curve by diversity or richness measure shows significant differences between each group.
Conclusion/Discussion: We screened mouse lung microbiome by traditional culture method and robust genetic analysis. Ultimately, we expect that our lung isolates or major species by non-culture method can be introduced into mouse model experiment for lung commensalism as well as coinfection with lung pathogens. Identification of commensal bacteria in murine lungs will pave the path to study influence of respiratory tract microbiome on infection and immunity of the lung.

PWV03
The salivary microbiome as an indicator of human-microbe co-evolution

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Introduction: Human-associated bacteria hold great potential for shedding light on the history of our ancestors (i.e. migration pattern). The concept of human-bacterial co-evolution has been demonstrated for *Helicobacter pylori* however its analysis requires stomach-biopsies. We currently test the hypothesis that oral microbes may function as chronometer of human evolution thereby allowing a fine-scale differentiation of human populations.
Material and Methods: Saliva samples from ten volunteers each from 12 areas world-wide, representing diverse ethnic groups are the initial focus of this study. Variations in the 16S-23S rDNA internal transcribed spacer region of *Fusobacterium nucleatum* and the *gdh*, (encoding for the glucose-dehydrogenase) and the *gtf* (encoding for the glycosyl-transferase) of the mitis-streptococci are analyzed by culture-independent methods. In addition, T-RFLP for whole community genetic profiling will be applied.
Results: The ITS region of *F. nucleatum* and the gdh of mitis-streptococci could be directly PCR-amplified from saliva DNA-extract using newly designed as well as published primers. All ten individuals tested from the countries Germany, Turkey, Poland, Bolivia, and Congo were positive for F. nucleatum. Nine of ten individuals from Germany and Congo were tested positive also for the *gdh*. Sequence analysis of ten clones per individual showed a high intra- and inter-individual diversity for both genes. Based on phylogenetic tree reconstruction most clusters consisted of sequence types

which were representative for multiple countries, however, one country was typically dominating in a cluster. In addition, we also found unique clusters explicitly consisting of sequence types specific to one country.
Discussion: Oral microbes found in saliva may exhibit a clonal structure and thus be representative for a distinct geographic region. Phylogenetic tools such as UniFrac are necessary to compute the distances within and between hosts in order to discern geographical and ethnic associations. Given that saliva is increasingly preferred in sampling humans as a source of DNA for epidemiologic and population genetic studies, the salivary microbiome may hold additional valuable information for providing new perspectives on unsolved human migration patterns - an issue of great social, anthropological, and medical importance

PWV04

Structure function analysis of the bacterial protease Lactocepin in the context of probiotic mechanisms

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Introduction: Clinical studies revealed that the probiotic mixture VSL#3 is protective in the context of inflammatory bowel disease (IBD). We previously showed that reduced cecal inflammation in VSL#3-fed IL-10$^{-/-}$ mice correlates with reduced levels of interferon-inducible protein (IP)-10 in cecal epithelial cells. Mechanistically, IP-10 degradation in intestinal epithelial cells (IEC) was mediated by cell surface proteins of VSL#3-derived *Lactobacillus paracasei* (*L.p*). The aim of the present study was to identify the active probiotic structure triggering the loss of the pro-inflammatory chemokine IP-10.
Methods: IEC stimulation experiments, cell-free incubation assays, ileal explant culture and *in vivo* experiments using a murine ileitis model were used to investigate the probiotic mechanism of L.p and its physiological relevance. Chromatographic fractionation and LC-MS/MS analysis were used to identify the active probiotic structure. Isogenic mutants were generated to proof the protective activity of the identified structure.
Results: In addition to fixed bacteria also conditioned media of L.p (CM L.p) mediated the loss of IP-10 in IEC. Further *in vitro* experiments including PMSF inhibitor studies revealed that L.p expresses a secreted and cell wall associated serine protease of L.p that selectively and directly degrades IP-10 in an IEC-independent manner. Beside IP-10, CM L.p selectively targets an array of pro-inflammatory chemokines (eg. I-TAC, Fractalkine), whereas all tested cytokines were not cleaved. Furthermore, explant culture experiments using inflamed intestinal tissue from experimental IBD models revealed that established IP-10 gradients in the tissue are also destroyed by this host cell-independent mechanism. The physiological relevance of this anti-inflammatory mechanism was proven by intraperitoneal injection of the active bacterial supernatant into an experimental ileitis model, resulting in selective degradation of ileal IP-10, lymphocyte recruitment and ileal inflammation. Chromatographic fractionation of the probiotic supernatant and subsequent LC-MS/MS analysis indicated lactocepin of L.p to be the active probiotic protease. The generation of a lactocepin-negative mutant from a similarly active, but transformable, human *Lactobacillus casei* isolate resulted in loss of its IP-10 degrading capacity, finally proving that probiotic lactocepin is the active anti-inflammatory bacterial structure.
Conclusion: The present study reveals the selective degradation of pro-inflammatory chemokines including IP-10 by probiotic lactocepin as a specific structure-related probiotic mechanism of a clinically relevant probiotic. The identification of lactocepin as a probiotic structure could enable a more structure-based evaluation of probiotic bacteria for therapeutical interventions in the context of chronic inflammation

PWV05

Flagellin and *tcpC* are essential factors of the protective effect of *E. coli* Nissle strain 1917 in DSS- induced colitis

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Background: The probiotic *E. coli* Nissle strain 1917 (EcN) is as effective as mesalazine in maintenance of remission in ulcerative colitis and shortens the duration of diarrhea in young children. We studied in a preclinical model of acute colitis whether EcN protects from disease and analysed the bacterial mechanism underlying the anti-inflammatory capacity.
Methods: C57BL/6 and *TLR5*$^{-/-}$ mice were fed with either EcN or EcNΔfliC or EcNΔtcpC and treated with 3, 5% DSS. Body weight and disease activity index were assessed daily. At the end of the experiment the colon length and weight was measured and inflammation was determined by histological analyses of the colon. Furthermore activation and maturation of lamina propria and mesenteric lymph node dendritic cells and T cells was analysed.
Results: In wild type mice *E. coli* Nissle protects from DSS induced colitis whereas the protection is reduced in *TLR5*$^{-/-}$ mice. In line with this the ΔfliC mutant strain was less effective in protecting the host from disease as compared to the EcN wild type strain. However a second bacterial factor *tcpC* also contributes to the protective effect of EcN as the ΔtcpC mutant strain was not able to protect from disease. Administration of the double mutant ΔfliCΔtcpC of EcN evidences this conclusion.
Conclusions: EcN ameliorates a DSS induced acute colitis via flagellin and the secreted protein *tcpC*. However contribution of further bacterial factors to the anti-inflammatory effect of EcN can not be excluded.

PWV06

More than a marine propeller - the flagella of the probiotic *Escherichia coli* strain Nissle 1917 is also the major adhesin mediating binding to mucin2.

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The flagella of the probiotic *E. coli* strain Nissle 1917 (EcN) is not just responsible for motility but also for EcN's ability to induce the production of human beta-defensin 2. Here, we report a third function of this organell in EcN.
Ex vivo studies with EcN, its isogenic non-flagellated mutant EcNΔ*fliC*and the complemented mutant proved the flagella to be essential for efficient adherence to cryosections of human gut biopsies. A hyperflagellated EcN variant adhered even more efficient to this cryosections than EcN WT. In contrast, in vitro studies showed that neither overexpression nor the lack of flagella has a significant effect on the adhesion to the human epithelial cell lines Caco-2 and T24. The observed discrepancy is most likely due to the lack of mucin on these cell lines, whereas mucus might still be present in the cryosections. This interpretation is supported by the finding, showing flagella-dependent adhesion of EcN to the human epithelial cell line LS174T, which produces mucins in vitro. Binding of the EcN flagella to mucin was further demonstrated by the inhibition of EcN adhesion to cryosections after preincubation of flagellated EcN with mucin2 in a mucin2-dose dependent manner. In addition, we were able to show the hyperflagellated EcN variant to adhere much more efficient to mucin2 compared to EcN WT, whereas the non-flagellated mutant EcNΔ*fliC*adhered barely. In contrast, the *E. coli* strain CFT073 harboring and expressing an identical *fliC* gene adhered as inefficient to mucin2 as EcNΔ*fliC* for unknown reason(s). Finally, electron microscopic examination of EcN revealed this *E. coli* strain to express only one flagella at one cell pole. This is in contrast to the general assumption, that *E. coli* are peritrichously flagellated bacteria.
We conclude, the flagella of EcN is its major adhesin for binding to mucin2, the major constituent of mucus in the small and large human intestine. Therefore, this organelle might be of importance for efficient, transient colonization of the human gut.

PWV07

Natural ligands of the aryl hydrocarbon receptor control intestinal lymphoid organogenesis

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Intestinal homeostasis is a prerequisite for the active extraction of metabolites by the absortive intestinal epithelium and for protection against intestinal pathogens. However, the underlying molecular networks are ill defined. RORγt-expressing innate lymphocytes (RORγt$^+$ ILC) play an important role in promoting intestinal homeostasis. RORγt$^+$ ILC constitutively produce interleukin (IL-)22, a cytokine instructing epithelial expression of antimicrobial genes required for the protection against intestinal infections with attaching and effacing (A/E) bacteria. Furthermore, subsets of RORγt$^+$ ILC have lymphoid tissue-inducing (LTi) function required for the postnatal development of intestinal lymphoid tissue harboring microflora-reactive IgA-producing B cells. While bacteria control the generation of B cell-containing isolated lymphoid follicles (ILF) from B cell-free cryptopatches, it is unknown whether the development of cryptopatches is programmed or also instructed by environmental cues. The family of Per-Arnt-Sim (PAS) domain-containing proteins is conserved throughout all eukaryotic species and it is believed that this group of proteins has evolved to control adaptation of multicellular organisms to environmental challenges. One of its members, the aryl hydrocarbon receptor (AhR) functions as a ligand-inducible transcription factor that, upon binding of its ligands, instructs transcriptional programs that include the induction of xenobiotic metabolizing enzymes. Although the role of the AhR in the response to environmental toxins is appreciated, its role in the mucosal immune system is not well defined. We addressed the role of the AhR for development and function of RORγt$^+$ ILC by generating mice lacking the AhR in RORγt$^+$ ILC ($Ahr^{\Delta LTi}$). Interestingly, $Ahr^{\Delta LTi}$ lacked genesis of cryptopatches and ILF. While RORγt$^+$ ILC developed independent of the AhR before birth, they required AhR signals for postnatal expansion which was a prerequisite for the formation of CP and ILF. Postnatal maintenance of RORγt$^+$ ILC was controlled by dietary phytochemicals (e.g., plant-derived glucosinolates and polyphenols) that served as natural AhR ligands. AhR-mediated signals stabilized Kit expression by RORγt$^+$ ILC and mice with defective Kit signaling had crippled development of ILF. AhR-deficient mice were highly susceptible to infections with *Citrobacter rodentium*, a mouse model of A/E infections such as those with enterohemorrhagic strains of *E. coli*. Our results demonstrate that plant-derived, dietary AhR ligands dynamically regulate postnatal development of lymphoid follicles by controling the pool size of RORγt$^+$ ILC with LTi function. Thus, sensing of dietary compounds allows dynamic adaptation of immune cells to environmental cues required to establish intestinal homeostasis and resistance to intestinal infections.

PWV08

Enterococcus faecalis Metalloprotease compromises epithelial barrier and contributes to intestinal inflammation

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Background: Proteolytic degradation of extracellular matrix by matrix metalloproteases is a serious consequence of intestinal inflammation. The aim of our study was to investigate whether proteases produced by commensal gut bacteria contribute to the pathogenesis of IBD. For this purpose we focused on the zinc dependent metalloprotease Gelatinase (GelE) from *Enterococcus faecalis* (*E. faecalis*).
Results: In order to investigate the impact of bacterial GelE on the development of chronic intestinal inflammation we monoassociated gnotobiotc wild type (Wt) and Interleukin 10 deficient (IL-10$^{-/-}$) mice with GelE expressing *E. faecalis* strain OG1RF and isogenic mutant strains that lack GelE expression including TX5264 (gelE deletion) and TX5266 (fsrB deletion). Histological analysis revealed a significant reduction of distal and proximal colonic tissue pathology of IL-10$^{-/-}$ mice in the absence of GelE. Wt mice did not develop colitis demonstrating the commensal character of the *E. faecalis* strains. Epithelial barrier function seemed to be affected by GelE as the presence of extracellular E-Cadherin protein domain was significantly reduced in IL-10$^{-/-}$ mice. Furthermore, using LC-MS/MS analysis, we could indentify cleavage sites for GelE in the sequence of recombinant murine E-Cadherin suggesting the possibility for a direct GelE-mediated degradation. Stimulation of distal colon segments with proteolytically active GelE revealed the loss of barrier function in mice susceptible to intestinal inflammation (IL-10$^{-/-}$, TNF$^{\Delta ARE/Wt}$, NOD2$^{-/-}$) before tissue pathology has developed. The loss of barrier integrity was associated with reduced levels of extracellular E-Cadherin. Finally, Ptk6 cells exhibited decreased transepithelial electrical resistance and increased translocation of permeability markers after stimulation with GelE from OG1RF background. We could show specificity for the GelE-mediated loss of barrier function by using an *E. faecalis* lipoprotein as unrelated control and the broad spectrum MMP inhibitor Marimastat to block GelE activity.
Conclusion: *E. faecalis* GelE impairs intestinal mucosal barrier function associated with the degradation of extracellular E-Cadherin protein domain. Our data clearly demonstrates the role of GelE, as a commensal-derived protease, in the development of intestinal inflammation in the disease-susceptible host.

PWV09

Molecular mechanisms leading to semi-mature murine dendritic cells and their role in intestinal homeostasis

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Dendritic cells (DCs) can provide different phenotypes. They can display an immature DC (iDC) phenotype or an activated mature DC (mDC) phenotype. Recently a third phenotype has been discovered, termed semi-mature (smDCs). These smDCs are able to take up antigen, but not to process it and they show reduced expression of T cell activating co-stimulatory molecules and a reduced expression of MHC class II. SmDC fail to polarize T cells. Sm BMDCs show reduced cleaving of the invariant chain (Ii) compared to mDCs, a major regulator of the MHC class II transport to the cell surface, so leading to reduced MHC class II surface expression. The cleaving of Ii is catalyzed by the endosomal protease CatS, which is regulated by the endogenous inhibitor Cystatin C. Indeed, mice lacking Cystatin C provide a significant higher susceptibility towards DSS induced colitis.
Therefore we suggest that semi-maturation plays an important role in maintaining the intestinal homeostasis and that regulation of Cathepsin S could be a potential target for the treatment of colitis.

PWV10 – PRESENTED AS POSTER

Susceptibility of IL-10 deficient mice to *Helicobacter hepaticus*-induced colitis depends on intestinal microbiota composition

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Background: *Helicobacter hepaticus*, a member of the enterohepatic *Helicobacter* species (EHS), has been reported to induce inflammatory bowel disease (IBD) in some strains of immunocompromised mice, including interleukin 10 (IL-10) deficient mice, but variable results have been reported from different centers, suggesting that the intestinal microbiota composition might influence susceptibility to *H. hepaticus* induced colitis.
Methods: IL-10 deficient C57/BL6J mice were infected with *H. hepaticus* ATCC51449 by oral gavage. Necropsies were performed 10-18 weeks after infection, followed by assessment of histopathology and quantitation of *H. hepaticus* colonization density. Microbiome composition analysis of uninfected mice housed under SPF conditions at the animal facility of Massachusetts Institute of Technology (MIT) or Hannover Medical School (MHH) was performed by partial amplification of 16S rDNA genes with universal primers and subsequent deep sequencing with Roche/454 pyrosequencing technology.
Results: IL-10 deficient C57/BL6J mice bred at MIT developed robust typhlocolitis after infection with *H. hepaticus* ATCC 51449, while mice housed at Hannover Medical School (MHH), while susceptible to colonization with *H. hepaticus*, did not develop any clinical or histological signs of colitis. Microbiota composition analysis was performed for caecum and colon samples from 8 uninfected mice from MIT and MHH, respectively. An average number of 4202 sequences were obtained per

tissue sample (mean length, 234 bp), and 98.5% of sequences could be classified into phyla. Flora composition differed markedly between MIT and MHH mice. MHH mice shared 600 (20%) of detected species with the MIT mice, and showed an overall lower richness of the bacterial flora compared to the MIT mice. Several bacterial phyla were exclusively detected in either MHH or MIT mice. Most notably, all caecum samples from MIT mice yielded large numbers of sequences assigned to the Deferribacteres phylum, while this phylum was completely absent from MHH mice.

Conclusion: C57/BL6J IL-10 ko mice housed under SPF conditions in two different institutions differed markedly in their susceptibility to *H. hepaticus*-induced intestinal pathology. These differences were associated with highly significant differences of microbiota composition, which are likely responsible for attenuating responses to *H. hepaticus* in mice housed at one of the institutions. The data highlight the importance of characterizing the intestinal microbiome when interpreting the results from murine models of IBD.

QSP01

Usefulness of automatisation in a university diagnostic laboratory: more than meets the eye

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Introduction: Laboratrory automatisation has made rapid and important advances in the past few years with increasing technology, standardisation of specimen collection and integration of multiple processes from simple inoculation of media to imaging and identification of microorganisms using sophisticated technology such as MALDI-TOF in a semi-automated fashion. University laboratories are traditionally not very high throughput diagnostic services and often have personell structuring which is markedly different to that of their counterparts in the private sector. Thus they have not been the target for many commercial companies manufacturing these technologies. There is however a need for efficiency and improved quality provided by standardised procedures that are optimally furnished by automated systems.

Methods: A university diagnostic laboratory providing a service to approximately 1800 in-patient beds, most of which (1200) are in a tertiary care / university setting and the remainder in a satellite teaching hospital also providing tertiary care, underwent a trial of an automated inoculation apparatus, with a view to further automatisation and integration of advanced technology.

Results: The implementation of the system proceeded in two main phases; firstly the introduction of a standardised specimen collection system essential for the full implementation of any automatisation. Thus in the primary phase the eSwab (Copan-Italia) was introduced universally approximately 18 months before the second phase in which the automatic inoculator or specimen processor (WASP - walk away specimen processor - MAST/Copan-Italia) was set up in the diagnostic laboratory. After initiation, which included a unidirectional connection to the LIS, testing of the media plates for compatiility and training of the technical staff, the system was run in parallel with the manual inoculation for a standard period in order to run comparisons for quality control. Thereafter the system was put “on-line” and run in the diagnostic services as the sole specimen processor. Staff re-allocation and processng tme, specimen quality and errors were all documented and analysed.

Discussion: The introduction and full implementation of an automated specimen processor in the microbiology diagnostic service for a typical large university hospital has far reaching consequences to the work-flow and staff time-allocation. Especially in this setting, the use of highly qualified staff for repetitive tasks demanding low skill is inefficient. University laboratories are generally unable to employ staff commensurate for low-skill tasks and furthermore are not in the position to cut costs through staff reductions. In this setting automatisation can be of great benefit by freeing technical staff for more tasks more appropriate to their training and also to the demands of a tertiary care / reference laboratory.

QSP02

INSTAND proficiency testing program for syphilis diagnostics: Summary report 2007-2010

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Introduction: *T. pallidum* spp. *pallidum*, is the causative agent of syphilis, a sexual transmitted disease of variable clinical presentation. As the spirochete resists conventional cultivation and to overcome test specific limitations, laboratory diagnosis is based on a stepwise approach using immunological assays. However, independent clinical evaluation of such tests is not mandatory within the EU and currently, a large number of assays of variable analytical quality are on the market. Here we present a summary report of the results obtained by the German proficiency testing program for syphilis serology between 2007 and 2010

Methods: From 2007 to 2010 eight proficiency testing surveys were conducted in Germany by the central reference laboratory for bacteriological serodiagnostics now situated at the Institute for Laboratory Medicine at the Northwest Medical Centre in Frankfurt, Germany, in cooperation with the Institute of Standardisation in the Medical Laboratory e.V. (INSTAND e.V.), Düsseldorf and with reference laboratories of the Bacteriologic Infection Serology Study Group of Germany (BISSGG). In each testing, two samples obtained from infected patients or healthy donors were sent out to the participants for analysis under routine laboratory conditions. Assessment of reference test results was performed according to standard operating procedures of INSTAND and current guidelines of the German chamber of physicians as published recently (Müller *et al*., 2010).

Results: Screening tests such as TPPA and TPHA *(*mean accuracy, 96.9% [qualitative], 88.9% [quantitative]), and ELISAs (mean qualitative accuracy, 94.7%) were more reliable than VDRL testing (mean accuracy, 92.1% [qualitative], 87.2% [quantitative]), the FTA-ABS (mean accuracy, 94.1% [qualitative], 85.2% [quantitative]), and immunoblots (mean qualitative accuracy, 96.1%). On average, 8.2% of participants misclassified active syphilis as past infection without indicating the need for further treatment. Moreover, 13.5% of laboratories wrongly reported evidence for active infection in cases with past syphilis or in sera from seronegative blood donors.

Discussion: With the liberalisation of the in vitro diagnostics market in the EU a wide range of serological assays for syphilis diagnostics of different diagnostic quality was promoted for use in diagnostic laboratories. In this study we confirm previous investigations that, although positive trends are obvious, the sensitivity and specificity of currently applied assays for syphilis diagnostics can still vary substantially, depending on the stage of disease, the type of test and the kind of manufacturer. Our results demonstrate that ongoing external quality control of routine diagnostic laboratories and in depth clinical evaluation of diagnostic assays is clearly needed to monitor test quality and to ensure a high level of quality in syphilis diagnostics.

QSV01

External quality control of immunodiagnostics in rare and fastidious pathogens (bordetella, mycoplasma, coxiella, campylobacter, and yersinia): a metaanalysis of proficiency testing trials

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Introduction: There is a long tradition in the participation of medical laboratories in external quality control of well established parameters and diagnostic procedures in medical microbiology. This tradition is now additionally enforced by the new guidelines of the German chamber of physicians. Evaluations of proficiency testing trials of indirect detection methods for rare and fastidious pathogens however are sparse. Most importantly the participation in such surveys is not mandatory in Germany although there is a lack of test standardization and very few data are available on the quality of such assays. Here, we present a summary report on the results of the INSTAND proficiency testing trials in the serodiagnostics of bordetella, mycoplasma, coxiella, campylobacter and yersinia

Materials and methods: From 2000 to 2010 twenty-five proficiency testing surveys addressing the serodiagnostics of the above mentioned pathogens were conducted in Germany by the central reference laboratory for bacteriological serodiagnostics now situated at the Institute for Laboratory Medicine at the Northwest Medical Centre in Frankfurt/Main, Germany, in cooperation with the DGHM, the Institute of Standardization in the Medical Laboratory (INSTAND e.V.), Düsseldorf and the reference laboratories of the Bacteriologic Infection Serology Study Group of Germany (BISSGG). In each survey, two samples obtained from infected patients or healthy donors were sent out to the participants for analysis under routine laboratory conditions. Assessment of reference test results was performed according to standard operating procedures of INSTAND and current guidelines of the German chamber of physicians as published recently.

Results: During the study period on average the following numbers of laboratories participated in the different serodiagnostic trials: yersinia: N=197; campylobacter: 73; bordetella: N=117; mycoplasma: 118; coxiella: 47. For the detection of specific antibodies in yersinia-, campylobacter-, and bordetella-serology mainly ELISA and immunoblots were used by the participants whereas in coxiella-serology more traditional tests such as CFT and IFA were predominantly applied. Ranges of average pass rates for diagnostic testing and diagnostic comments were as follows: yersinia: 58-85%; bordetella: 70-95%; mycoplasma: 56-90%; coxiella: 60-100%; campylobacter: 43-93%.

Discussion: Although, proficiency testing trials cannot fully substitute clinical evaluations of immunodiagnostic tests, the results of our metaanalysis demonstrate a very variable degree of standardization and

assay quality in the serodiagnostics of rare and fastidious bacterial pathogens. Most importantly, our observations provide interesting insights into the comparability of different assay systems the interpretation of quantitative and semi quantitative test results and the correct clinical interpretation of the serological findings pointing to limitations of these diagnostic procedures in the routine diagnostic microbiological laboratory.

QSV02

Prevalence of virulence genes among methicillin-resistant *Staphylococcus aureus* isolates from nasal carriage of healthy humans in Euregio

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Staphylococcus aureus is a leading cause of human disease in the hospital setting as well as in the community. It has been shown an association between nasal colonization of *S. aureus* and infection with the same strain. Of particular concern is a worldwide increase in the number of infections caused by methicllin-resistant *S. aureus* (MRSA). In addition, to the long-known healthcare-associated clones, there is observed the emergence and spread of community- and livestock-associated MRSA. The ability of the clinically important strains to persist within hosts is largely due to the expression of a battery of virulence factors which promote adhesion, acquisition of nutrients, and evasion of host immunological responses. The objective of this study was to determine prevalence of the virulence genes among the most successful and sporadic MRSA clones in the Dutch-German Euregio A total of 52 non-repetitive MRSA isolates recovered from nasal carriage from healthy persons in Euregio between 2003 and 2009 were tested. Isolates selection was based on the *spa* typing results and was intended to include representatives of the most predominant spa types recovered in Euregio (t003, t032, t011, t034, t004, t008) as well as representatives of sporadic *spa* types. The virulence genes (tst1, sea, seb, sec, sed, sef, seg, seh, sei, sej, sek, sel, selm, seln, selo, seq, ser, selu, PVL, lukM, lukD, lukE, sak, chp, scn, etA, etB, etD, edinA, edinB, edinC, arcA-ACME, arcB-ACME, arcC-ACME, arcD-ACME) were detected by the microarrays (StaphyType by Alere Technologies GmbH, Jena, Germany). Different combinations of virulence genes were identified among MRSA isolates. All isolates carried at least 5 virulence genes with the exception of MRSA isolates of *spa*-Clonal Complex (CC) 011 (with *spa* types t011 and t034). Isolates of *spa*-CC 011 did not carry any virulence gene with the exception of a single isolate (with *spa* type t011) which harboured the sed and arcB-ACME genes. We observed association the more predominant MRSA clone the more virulence genes were detected: t003 - 15 genes (sea, sed, seg, sei, sej, selm, seln, selo, ser, selu, lukD, lukE, sak, chp, scn); t032 - 12 genes (sec, sed, seg, sei, sel, selm, seln, selo, selu, sak, chp, scn); t004 - 10 genes (sed, seg, sei, selm, seln, selo, selu, sak, chp, scn). Although, most of the la-MRSA carry no tested virulence factors, some la-MRSA harbour important virulence factors which shows the genomic plasticity of this MRSA subtype and possible adaptive changes in the future.

RKP01

Identical toxigenic *Corynebacterium ulcerans* strain in a patient with diphtheria-like illness and her asymptomatic cat

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Diphtheria and diphtheria-like illness is caused by *Corynebacterium* species harbouring the diphtheria toxin (DT) encoding *tox* gene. In recent years, diphtheria-like infections with toxigenic *Corynebacterium ulcerans* have outnumbered those caused by toxigenic *C. diphtheriae* in many industrialized countries. Originally associated with consumption of raw milk and dairy products or contact to cattle, *C. ulcerans* has increasingly been isolated from domestic animals such as pet dogs and cats. So far, isolation of an identical toxigenic *C. ulcerans* strain from an animal and its owner is documented only for dogs and a pig.
Here we report the first case of proven transmission of a toxigenic *C. ulcerans* strain between a pet cat and a human causing pharyngeal diphtheria-like illness.

RKP02

Antimicrobial resistance patterns of *Corynebacterium diphtherieae*, *C. ulcerans* and *C. pseudotuberculosis* strains of human and animal origin.

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Objectives: Human infections due to *Corynebacterium diphtheriae* and the zoonotic germs *C. ulcerans* and *C. pseudotuberculosis* are rare but potentially life-threatening events requiring specific antibiotic treatment, and in cases suspicious for diphtheria the prompt antitoxin therapy. In the last years, we have observed an increased number of *C. ulcerans* strains isolated from patients with wound infections and diphtheria-like illness, mainly harbouring the diphtheria toxin (DT) encoding *tox* gene. Benzyl penicillin and erythromycin are still recommended for specific antibiotic treatment, to eradicate the pathogen in patients and to prevent infection among contacts, although only very few antimicrobial susceptibility data are available.
Methods: In this study 106 isolates of toxigenic and non-toxigenic clinical isolates of *C. diphtheriae* (n=35), *C. pseudotuberculosis* (n=47) and *C. ulcerans* (n=24) from humans and animals collected in the National Consiliary Laboratory for Diphtheria from 2001 to 2010 were examined. Minimal inhibitory concentration (MIC) of benzyl penicillin, ceftriaxone, erythromycin, tetracycline, clindamycin, and ciprofloxacin were determined using the Etest system. In the absence of standardized breakpoints, antibiotic susceptibility was determined by using the CLSI criteria for broth microbouillon dilution susceptibility testing for *Corynebacterium* species.
Results: Benzyl penicillin, erythromycin and ciprofloxacin seem to be highly active substances in all three *Corynebacterium* species (susceptibility rates of 98.1, 98.1 and 96.2% respectively). For *C. diphtheriae* tetracycline and ceftriaxone showed lowest activity in vitro (susceptibility rates of 71.4% and 42.9%), while clindamycin was least active against *C. ulcerans* (susceptibility rate of 16.7%). In contrast, *C. pseudotuberculosis* strains were completely susceptible to all examined antibiotics.
Conclusion: Our data emphasize the need for an accurate species identification of potentially toxigenic corynebacteria and underscore the necessity of antibiotic testing. Although clindamycin is not a first-line drug in diphtheria therapy, it is favoured for the treatment of wound infections, but due to our results *C. ulcerans* is not surely covered by this substance. Benzyl penicillin and erythromycin can be still recommended for specific antibiotic treatment in Germany, but antibiotic resistance may occur.

RKP03

Culture of a gastric non-*H. pylori* Helicobacter from a gastric biopsy specimen of a 14 year old patient

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Case report: A 14 year old girl presented with persistent epigastric pain since several months in our hospital. A treatment with omeprazole for 14 days showed no clinical improvement. An esophagogastroscopy was performed, revealing a mild erosive gastritis. Biopsy specimens from the stomach were sent for histopathology and helicobacter culture and susceptibility testing. In a direct gram stain of the homogenized gastric biopsy specimen long gram negative corkscrew-like rods atypical for *H. pylori* were found. After 7 days of incubation on agar media containing human erythrocytes and inactivated horse serum first colonies appeared. Cytochrome oxidase, catalase and urease tests performed from the grown bacteria were positive. The strain was susceptible to clarithromycin, metronidazole, amoxicillin, levofloxacin, tetracycline and rifampicin (Etest method). For species identification partial *ureA* and *ureB* gene PCR followed by sequencing was performed as described by O`Rourke et al. (2004). Following our phylogenetic analyses, this isolate can be identified as *Helicobacter felis*. *Helicobacter felis* belongs to the "gastric non-*H. pylori* helicobacters", formerly also classified as *H. heilmannii* type II (Haesebrouck et al. 2009). *H. felis* is frequently found in the stomach of cats and dogs. Infections in humans have been reported, but culture is difficult due to the fastidious nature of this organism. To our knowledge only two non-*H. pylori* strains were cultured from human gastric biopsy

specimen so far. Both strains were classified as *H. bizzozeronii* (Jalava et al. 2001; Kivistö et al. 2008). This case report demonstrates that direct gram stain of gastric biopsy specimen is important to identify gastric non-*H. pylori* helicobacters. Furthermore, culture of fastidious non-*H. pylori* helicobacters using our standard protocol is possible.

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RKP04

Which factors interfere with metronidazole susceptibility testing of *H. pylori* by the Etest method?

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Introduction: Metronidazole is part of the Italian triple therapy, which is a recommended first line scheme for *H. pylori* eradication. In Germany, the primary resistance rate for metronidazole is 29% (data from the 3rd European multi-centre study on antibiotic susceptibility of *H.* pylori). However, phenotypic susceptibility testing is currently not approved by the FDA due to "variance in test results, lack of reproducibility and clinical relevance". But phenotypic susceptibility testing is the only option for detection of resistance, because the molecular mechanisms of resistance are not conclusively explained. Therefore, the aim of our study is to evaluate factors that influence the precision and reproducibility of metronidazole susceptibility testing using the Etest method.
Material and Methods: 20 randomly selected *H. pylori* isolates cultured from routine gastric biopsy specimens (no selection concerning resistance patterns, pre-treatments, patient data etc.) are actually under study. Etests of each isolate are performed repeatedly at the same time point (precision) and at different time points (reproducibility). Further variables included are the culture media (Mueller-Hinton + 10% horse blood vs. Isosensitest + 10% horse blood) and the incubation period (48 hours vs. 72 hours).
Results: The minimal inhibitory concentrations (MIC) detected on Mueller-Hinton and Isosensitest agar after 48h and 72h of incubation showed a high correlation. A small but systematic difference between the media and incubation periods was found. MICs on Isosensitest agar were higher compared to Mueller-Hinton agar (factor 1.1), and 48h of incubation led to lower MICs than 72h of incubation (factor 0.86). The range of MICs was significantly broader between series at different time points than within one series.
Discussion: There is a small but systematic influence of culture media and incubation period on metronidazole testing using the Etest method, but correlation of MICs is high. To evaluate the reproducibility further data are needed.

RKP05

Use of Multilocus Sequence Typing (MLST) and Mycobacterial Interspersed Repetitive Units-Variable Number of Tandem Repeats (MIRU-VNTR) for typing of *M. avium* strains

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Introduction: One of the most frequent isolated species within nontuberculous mycobacteria is *M. avium*, which is ubiquitously found in the environment and has been shown to be responsible for a variety of human diseases, mainly in immunocomprised patients, but also in patients with no obvious immune deficiencies. *M. avium* comprises the subspecies *M. avium* subsp. *avium*, *M. avium* subsp. *silvaticum*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *hominissuis.* Routes of infection are rarely known. For understanding of transmission mechanisms and for deeper insights into the pathogenicity genetic characterization of the infectious strains is a prerequisite. Therefore, the aim of our study was to assess the applicability of MLST and MIRU-VNTR for distinguishing *M. avium* strains.
Material and Methods: Genotyping was performed by MLST. For this, fragments of several housekeeping genes were analyzed by sequence analysis. Comparing the DNA sequences of each locus we estimated the degree of variability of these genes in isolates of different *M. avium* subspecies. By MIRU variable numbers of tandem repeats of 6 loci were analyzed. Into the study we included *M. avium* isolates of children suffering from *M. avium* lymphadenitis sent to the NRZ in the years 2003, 2004, and 2009. Furthermore, strains isolated from slaughter pigs and from birds were analyzed. Subspecies analysis was done by PCR targeting IS900/901 elements.
Results: Firstly bird isolates were proven to be *M. avium* subsp. *avium* by IS900/901 PCR. Various single nucleotide polymorphisms could be detected by MLST analysis in the genes investigated. Differences in the genes concerning their variability were used to decide the applicability for future investigations. The MIRU-VNTR analysis showed no great impact due to a moderate variability.
Discussion: By molecular methods subspecies of *M. avium* can be identified. MLST enables typing of strains that in future may be used for example for analysis of transmission or reactivation vs. new infection.

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RKP06

Reduction of antibiotic resistance among S. pneumoniae isolated from IPD in children and adults as a result of the German national immunization program for pneumococcal conjugate vaccine.

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Introduction: We evaluated the impact of the immunization of children <2 with pneumococcal conjugate vaccine (3+1 doses) started in July 2006 on antibiotic resistance levels among *S. pneumoniae* isolated from children and adults with invasive pneumococcal disease (IPD) in Germany.
Materials and methods: Isolates from IPD cases in children <16 years and adults >= 16 years were sent to the German National Reference Center for Streptococci by microbiological laboratories all over Germany. Serotyping was performed using the Neufeld Quellung reaction and MICs were determined using the microdilution method.
Results: Numbers of IPD cases with serotypes included in the 7-valent pneumococcal conjugate vaccine (PCV7) were strongly reduced in children under two years after the start of childhood vaccination. In the years before vaccination (1997-2006) an average 66.4% were PCV7 serotypes, in 2010-2011 only 7.5%. In children 2-4 years these percentages were 72.1% before and 4.6% after, in children 5-15 years, 36.0% before and 9.8% after. In adults (16 years and older) an average 46.0% of cases had PCV7 serotypes before vaccination. After vaccination this was reduced to 12.6%. Among isolates from children macrolide resistance was reduced from an average 26,1% in 1997-2006 to 10,5% in 2010-11. Among adults macrolide resistance was reduced from 17.4% before to 9.0% after vaccination. Penicillin resistance levels have not changed much after vaccination in either children or adults. However, the serotype distribution among these isolates has changed considerably as well.
Discussion: Five years after the introduction of PCV7, the reduction in numbers of reported cases of IPD with vaccine serotypes in children under 2 years was almost 90%. The observed reduction of IPD cases with vaccine serotypes in older children and adults indicates a herd protection effect. Due to the disappearance of vaccine serotypes macrolide resistance levels have been reduced considerably. Penicillin resistance levels have not changed, since the disappearance of penicillin resistant vaccine serotypes was compensated by the appearance of penicillin resistant serotype 19A.

RKP07

Establishment of a red deer DNA banc as source for future studies on emerging zoonotic and vector borne pathogens

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Background: Ongoing global warming is thought to cause environmental changes which could foster the emergence and re-emergence of zoonotic as well as vectorborne infectious diseases affecting human and animal health. Besides domestic livestock, wild animals such as deer or wild boar serve as reservoir for such infectious diseases. To date there is little data available on the prevalence of zoonotic and vector borne pathogens such as *Anaplasma phagocytophilum*, *Francisella tularensis*, Babesia spp, Rickettsia spp, or TBE-Virus. Data obtained in seroprevalence studies indicate that wild animals are susceptible to several zoonotic and vector borne pathogens, however do not provide informations to which extend wild animals serve as reservoir.
In this study we aim at 1) establishing a DNA banc from well defined material from red deer and 2) to perform first investigations on the prevalence of zoonotic and vector borne pathogens in these materials. Standardized collected and stored DNA-samples may serve as a material collection for longitudinal studies on endemic and emerging zoonotic and vector borne pathogens.
Methods: Lymph nodes from red deer (Cervus elapus) were collected directly after shooting and stored at -20°C until further use. DNA (600µl) was extracted (Biosprint, Quiagen) from each single lymph node and stored

in 20 vials as a DNA-banc, which is connected to a data-banc containing data like sex, age, health status, or place of shot regarding the deer. These samples are used to screen for zoonotic and vector borne pathogens like Anaplasma, Francisella, or DNA-viruses by PCR.

Results: So far, around 1500 lymph nodes from 323 red deer were collected and the deer data banc was established. The DNA banc actually consists of 323 samples, one lymph node from each deer. First screening revealed that none of the 323 tested samples contained measurable F. tularensis DNA, but 39 (12.1%) samples were positive for A. phagocytophilum DNA as confirmed by sequencing. Further pathogen-screening actually is on the way and data will be provided. Moreover, 200 tissue samples were subjected to RNA-extraction and are currently screened for tick borne encephalitis virus.

Conclusion: The detection of *A. phagocytophilum* DNA in the screened samples indicates that lymph nodes from red deer is suitable for screening purposes with respect to A. phagocytophilum. The absence of *F. tularensis* DNA in lymph nodes however does not conclusively rule out red deer as reservoir.

Due to the high mobility and broad distribution of red deer, it might serve as a sentinel for emerging zoonotic and vectorborne pathogens. The presented DNA banc is an important prerequisite for future investigations on emerging pathogens.

RKP08

Lyme Borreliosis in Europe: Is it on the rise?

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Background: Lyme Borreliosis (LB), caused by spirochetes of the Borrelia burgdorferi s.l. complex is the most common tick-borne infectious disease of the northern hemisphere. To date there are at least five humanpathogenic species: *B. burgdorferi s.s., B. afzelii, B. garinii, B. spielmanii* and *B. bavariensis*. In Germany Borrelia are transmitted by the hard tick *Ixodes ricinus* . Infection with Borrelia can result in clinical manifestations of the skin (Erythema migrans, Borrelia lymphocytoma, Acrodermatitis chronica atrophicans), nervous system (neuroborreliosis), joints (Lyme Arthritis) or heart (Lyme Carditis).

Currently concise data on the incidences of LB in European countries is rare.

In this review we aimed at collecting data on the incidence of LB in all European countries emphasising data on neuroborreliosis.

Methods: Data were collected by structured literature search in PubMed, national health registries, public health institutions and personal expert-contacts.

Results: Data on the incidence of LB was obtained for 29 European countries. Reporting regimens are very heterogenous among European countries and there is little information on the reliability of the employed reporting systems (e.g. case definitions). Geographic distribution shows lowest incidences of LB in southwest Europe (Portugal < 0.7 x 10^5, 2007) and along the Mediteranian coast. Incidences increase northward (Sweden 69 x 10^5, 1995) as well as from Western Europe (England & Wales,1.72 x10^5, 2009) towards Central Europe (Switzerland, 115x 10^5, 2008). The highest incidence was reported from Slovenia (312 x 10^5).

With respect to Neuroborreliosis (NB) there is data from 9 European countries. Data collected in studies and national registries show that reported incidence of NB varies between 0.6 x 10^5 in France and 11 x 10^5 in Sweden. In 2009 incidence of NB was 1.1x10^5 in Norway and 3.8 x 10^5 in Denmark. Incidence of NB in Germany was 3.3 x 10^5 in 1998; more recent data from Eastern Germany show an incidence of early NB of 0.3 x10^5 (2009).

Longitudinal data on the incidence of LB in Europe indicate that some countries observe a clear increase, e.g. Slovenia, whereas incidence data from other countries remained fairly constant over the past ten years. Some countries reported a rise in LB incidence until 2006, since then reported data either remained within the same range (Norway) or even declined (Germany).

Conclusions: Although data on the incidence of LB are available from many European countries, currently it is hard to provide a reliable picture mainly due to the various methodologies of data acquisition preventing conclusive comparison. For the purpose of comparable data, well designed sentinels or prospective studies are urgently needed.

RKP09

Climate change - What about borreliae and ticks?

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Background: Lyme Borreliosis (LB), caused by spirochetes of the Borrelia burgdorferi (Bb) s.l. complex is the most common tick-borne infectious disease of the northern hemisphere. To date there are at least five assured humanpathogenic species: *Bb* s.s*., B. afzelii, B. garinii, B. spielmanii and B. bavariensis*. In Germany Borrelia are transmitted by the hard tick *Ixodes ricinus* (IR). This study aimed at collecting data on the density of ticks in various Bavarian regions, to assess the infection rate of IR ticks with Bb s.l. and to assess regional distribution of the Bb s.l. genospecies in Bavaria. These standadised collected data should also serve as a basis to follow the influence of climate change on ticks and tick-borne diseases.

Material: Questing IR ticks were collected by standardized flag dragging at 22 sites in southern Bavaria. In Bavarian Forest National Park (BFNP) IR ticks were collected along an altitudinal gradient (300m - 1340m). At each site at least four plots (100m^2 each) were flagged and data on environmental variables like humidity or temperature was collected. DNA extracted from IR was screened for the presence of *Bb* s.l. by nested OspA-PCR followed by RFLP for genospecies identification.

Result: 3056 ticks (48 larvae, 1970 nymphs and 1038 adults) were collected in 2010. Tick activity followed a bimodal distribution (april/june and sept/oct). In the BFNP ticks could be collected up to 940m. IR tick activity in BFNP was considerably lower than in Upper Bavaria or at Lake Constance. Preliminary analysis of 1550 ticks (717 nymphs, 687 adults)) from four sites revealed regional infection rates between 2.0% - 5.3% in nymphs and 3.1% - 17.6% in adults. Results indicate that infection rates are higher in regions of urban land use than in rural areas e.g. BFNP. Preliminary results on the analysis of 82 positive PCR samples by RFLP revealed the presence of *B.b.s.s., B. afzelii, B. garinii, B. bavariensis, B. spielmanii* and *B. valaisiana* in IR ticks. In comparison to data collected in 2003/4 in the English Garden (Munich) infection rates with Bb.s.l. genospecies tended to be lower, while prevalence of *Bb*.s.l. genospecies did not change.

Conclusion: IR ticks are abundant all over Bavaria following bimodal seasonal activity. Higher abundance of IR ticks in areas of anthropogenic influence indicate an effect of landuse on IR tick density. In comparison to earlier investigations from Bavaria neither Bb infection rate of IR nor *B.b.* Spp. prevalence changed significantly. This observation indicates that climate change so far had no measurable effect on these parameters.

This study is part of the Bavarian collaborative research project Vectorborne infectious diseases in climate change investigations(Project 08-18) funded by the Bavarian State Ministry of Environment and Health.

RKP10

Incidence of Lyme borreliosis after the bite of a *Borrelia burgdorferi* infected *Ixodes ricinus* tick in Romania

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Introduction: Data from Romania regarding Lyme borreliosis - like prevalence of *B. burgdorferi* (*Bb*) s.l. genospecies in ticks or incidence of the different clinical manifestations - are scarce so far. The aim of our study was to evaluate the clinical and serological outcome of patients bitten by Bb infected ticks, with regard to different *Borrelia* genospecies identified in these ticks.

Material and methods: All ticks collected from patients presented to the Clinic of Infectious Diseases Cluj Napoca were morphologically identified by an entomologist and investigated for Bb infection using Real Time-PCR targeting a 153 bp fragment of the *hbb* gene. The analysis was performed using LightCycler480 thermal cycler. Melting curve analysis of the amplicons allows genospecies identification. All patients bitten by *Bb* infected ticks and a control group -randomly selected patients bitten by *Borrelia* negative ticks- were asked for follow up one year later (up to may 2011). A questionnaire was completed for each person by a medical investigator regarding clinical manifestations of Lyme borreliosis, treatment follow up in case of confirmed diagnosis, or chemoprophylaxis followed after the tick bite. For investigating IgM and IgG Borrelia antibodies two-tiered testing was performed (Virotech Borrelia afzelii IgM ELISA, Borrelia afzelii + VlsE IgG ELISA tests, and Euroimmun IgM and IgG Anti-Borrelia EUROLINE-RN-AT Western Blot tests).

Results: 532 ticks were morphologically examined: 518 *Ixodes ricinus*, 10 *Dermacentor marginatus* and 3 *Haemaphysalis* spp. ticks, one unidentified tick due to destruction. Out of 389 DNA extracts (378 *Ixodes ricinus*, 7 *Dermacentor marginatus*, 3 *Haemaphysalis* and one unidentified tick) 43 were positive by hbb real time PCR for Bb sensu lato. The positive samples were 42 *Ixodes* and the one unidentified tick. The *Dermacentor* and *Haemaphysalis* ticks were all negative for *Borrelia spp.* in hbb RT PCR. In *Ixodes* ticks the Bb s.l. prevalence was 11.1%. The positive *Ixodes* ticks were 15 in adult stage, 26 nymphs and 1 with unidentified stage due to partial destruction. Species identification, based on melting curve analysis revealed mainly *B. afzelii* (62.7%), but also *B. garinii* (16.2%), *Bb* sensu

stricto(6.9%; for the first time identified in Romania), *B. spielmanii/B. valaisiana* (11,6%) and *B. lusitaniae* (2,3%). In case of infection with B. *spielmanii* or *B. valaisiana* the melting curve analysis could not differentiate between the two species because of overlapping melting curves. No mixed infection was detected.
Results on the clinical outcome and *Borrelia* serology of the individuals bitten by *Borrelia*-positive ticks will be presented and compared to the control group. Preliminary results suggest that there is a low risk to develop Lyme borreliosis after a *Borrelia*-infected tick bite in Romania.

RKP11
External quality assessment of pertussis serology in Germany

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Objective: Testing pertussis serology performance in routine laboratories after the availability of an International Reference Preparation.
Methods: In cooperation with INSTAND, reference preparations were used in an external quality assessment programme (EQA). Two reference preparations, the WHO Reference Reagent (06/142) and a preparation from NIBSC with very low level of antibodies were reconstituted centrally and sent as liquid samples to a total of 200 participants. Results were reported qualitatively and quantitatively. Quantitative results were converted into IU/ml wherever possible using the suggestions from the producers of diagnostic kits.
Results: Results were reported from a total of 183 participants. ELISAs with mixed antigens were used for measuring IgG-antibodies by 111 participants. IgA-antibodies were measured by 110 participants using mixed antigen ELISAs, and 113 participants used these ELISAs for measuring IgM-antibodies. A total of 47 or 44 participants used ELISAs with purified PT for measuring IgG- or IgA-antibodies, respectively. Immunoblot assays were used to estimate IgG, IgA and IgM antibodies by 62, 63, and 11 participants, respectively. Five participants used indirect immunofluorescence measuring IgG, IgA and IgM antibodies, and one laboratory used complement fixation. Quantitative results were reported in different units, and only the ELISAs using purified PT gave results that were comparable to the expected values in IU/ml.
Conclusions: Many laboratories used methods that are not recommended by the EU reference laboratories and report results that cannot be related to the WHO reference preparation.

RKV01
Epidemiology and surveillance of meningococcal disease in Germany

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Invasive meningococcal disease (IMD) is notifiable in Germany. Most cases undergo detailed molecular typing permitting surveillance of circulating strains and identification of clusters by means of scan statistics. Routine vaccination against Serogroup (Sg) C IMD of 1-year old (y.o.) children was recommended in 2006, with individual catch-up recommended for older children. Vaccination coverage in 2009 in ≤18 y.o. was 50.5%, highest among 2-5 y.o. (78%)[1]. IMD incidence (>99% laboratory confirmed) in Germany decreased from 0.93 cases/100.000 inhabitants in 2001-2003 to 0.72 in 2004-2006, 0.56 in 2007-2009 and 0.47 in 2010. The peak in incidence usually seen in the first quarter was absent in 2010. IMD incidence was highest in <2 y.o. with a smaller peak in 15-19 y.o. Case fatality ranged from 6.9%-9.7% in 2001-2010, higher for SgC than SgB IMD (11.6% versus 7.9%, p_X2=0.0007). Disproportionately greater decreases in SgC than SgB incidence were observed in 1-5, 6-14 and 15-19 y.o. since 2006, but not in <1 or >19 y.o. Dominant *N. meningitidis* lineages were similar to those in other European countries. The most common SgB lineage 3 clone (B:P1.7-2,4:F1-5) continued to cause a disproportionately high number of cases in western North Rhine-Westphalia in 2009-2010. The most common SgC fine type C:P1.5-2:F.3-3, clustered in parts of Bavaria in 2005, 2006 and 2009, decreased markedly in most regions in 2010. IMD-incidence in Germany reached a record low in 2010, with MenC vaccination explaining only a small part of this decrease. There is no evidence for herd immunity in unvaccinated groups. The low incidence in early 2010 may have been related to the earlier than usual occurrence of the pandemic influenza wave in the fall of 2009.

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RKV02
Retrospective performance of HRM-PCR to discover two outbreaks of listeriosis in Austria, 2008 - 2010

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Introduction: For public health laboratories in charge of listeriosis surveillance, high-resolution melting PCR (HRM-PCR) analysis is a promising new technique to get by with low specificity of serotyping and various disadvantages of pulsed field gelelectrophoresis (PFGE).
Material and Methods: From 2008 to 2010, two different outbreaks of listeriosis have been reported from Austria: 1. An outbreak of febrile gastroenteritis in guests of a local restaurant after consumption of contaminated home-made jellied pork, including two cases of bacteriologically proven invasive listeriosis. 2. A multi-national outbreak of invasive listeriosis due to contaminated acid curd cheese sold by supermarkets. The product carried two genetically different outbreak strains that were responsible for 12 cases (clone 1) and 13 cases (clone 2) of bacteriologically proven invasive listeriosis from Austria (total: 25 cases). For all samples belonging to the outbreaks the *inlB* HRM-PCR products were subsequently sequenced for verification. Retrospectively, we studied performance of serotyping and HRM-PCR *inlB* sequence-type (inlB) analysis to detect these outbreaks that have been confirmed by PFGE.
Results: 112 isolates of Listeria monocytogenes from patients with invasive disease from Austria have been tested during the study period. Serotype distribution in the total study population: 1/2a - 55.3 %, 4b - 28.6 %, 1/2b - 15.2 %, 1/2c - 0.9 %. InlB distribution: 15 - 18.8 %, 9 - 17.9 %, 1 - 13.9 %, 5 - 9.8 %, non-typable - 8.0 %, 2, 3 and 7 - 7.1 % each, and else types 10.3 % altogether. Both invasive isolates of outbreak no. 1 were serotype 4b and inlB 2. All 12 isolates of clone 1 of outbreak no. 2 were serotype 1/2a and inlB 15, and all 13 isolates of clone 2 were serotype 1/2a and inlB 9. In addition, we found 30 isolates of serotype 4b and 37 isolates of serotype 1/2a that were not related to the mentioned outbreak clones. With regard to HRM-PCR analysis, 6 of 8 inlB 2 isolates, 9 of 21 inlB 15 isolates, and 7 of 20 inlB 9 isolates did not belong to the outbreaks.
Conclusions: HRM-PCR analysis appears to be a sensitive, fast and inexpensive screening method to discover possible outbreaks. In our study, it better distinguished outbreak clones from unrelated strains than serotyping, but it still requires confirmation by more discriminatory methods like PFGE.

RKV03
Population analysis of community-acquired MRSA in Germany: Data from the National Reference Centre for Staphylococci

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Introduction: Community associated MRSA (CA-MRSA) have emerged worldwide from the end of the 1990ties. Although particular clonal lineages were associated with particular geographic areas (e.g. ST30: "Oceanic", ST8: "USA300", ST80: "European", ST59 "Asiatic") further spread leading to dynamics of clonal lineages over time is observed. Here we report on CA-MRSA clonal lineages recorded in Germany over the past three years.
Methods: The respective isolates were sent to the Reference Centre for further characterisation between January 2009 and May 2011 (n = 320). The antimicrobial susceptibility, the presence of several resistance and pathogenicity associated genes, the SCC*mec*-type, spa- and multilocus sequence-type were analyzed. For selected isolates *lukSF-PV* was sequenced to determine the PVL sequence variation. PFGE after *Sma*I-digestion of the chromosomal DNA was also performed for the comparison of selected clonal lineages.
Results: The dominant CA-MRSA were the European clone ST80, isolates attributed to ST8, and to ST30. Compared to data of the last years, we observed an increasing frequency of ST8 and surprisingly ST30. At the first look CA-MRSA ST8 were suspicious for the "USA300" clone. However, a considerable portion of them was lacking *arc*A and *mph*B. These isolates do not represent a subpopulation of "degenerated" ST8 (loss of the ACME cluster and of the resistance plasmid) as initially assumed. Although finding of the same rudimentary inverted repeat in the *fos*B vicinity and of *sek* and *set* suggest relatedness. SCC*mec*IVc in the *arc*A-negative isolates instead of SCC*mec*IVa, which is typical for USA300, indicates convergent evolution. Furthermore CA-MRSA clones which belonged to ST1, ST5, ST22, ST152

and ST398 emerged occasionally. In this respect the impact of LA-MRSA ST398 on the epidemiology of CA-MRSA needs further attention. We detected a Linezolid-resistant (*cfr*-positive) LA-MRSA ST398 from pneumonia and two PVL-positive MSSA ST398 from furunculosis. We also noticed sporadic cases of CA-MRSA ST45, ST59, ST72, ST88, ST154, ST772. Moreover we found spa-types which were not described for CA-MRSA so far, but we assigned them to well-known clonal complexes according to their sequence type. Cases of infections in older inpatients, e.g. with prosthetic joint infections, indicate a changing epidemiology of CA-MRSA infections which is already known from the USA.
Conclusions: Our results show the diversity among the CA-MRSA in Germany. Moreover CA-MRSA seem to enter the nosocomial setting. Identifying common and new emerging CA-MRSA is an ongoing challenge in the changing epidemiology of MRSA, and prevention of further spread needs tight surveillance.

RKV04

Phylogenetic analysis of the *cap* region II of *Haemophilus influenzae* serotypes c and d

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The capsule-specific synthesis (*cap*) regions II of *Haemophilus influenzae* serotypes c and d (Hic and Hid) were unknown before this study. The Hic capsule consists of dimeric repeating units of O-acetylated N-acetyl-β-D-glucosamine (β-D-GlcNAc) and α-D-Galactose (α-D-Gal), whereas the Hid capsule is essentially a polymer of β-D-GlcNAc and N-acetyl-D-mannosaminuronic acid (D-ManANAc) dimers. The polysaccharide of Hid shares common monomers with Hie capsule, and high similarities between the capsule loci of Hie and primarily of *P. multocida* B and E have been found previously. We here present an analysis of the *cap* regions II of Hic and Hid and, based on similarity searches, discuss the phylogenetic development of bacterial capsules.
Sequencing of the *cap* region II of Hic ATCC 9007 and Hid ATCC 9008 was carried out by PCR and primer walking.
The sequence of Hic revealed four open reading frames (ORFs), named *ccs1-4*. Similarity searches showed a phosphotransferase function for Ccs1. Ccs2 seems to be a member of glycosyltransferase family 2. These findings point at a role of both proteins in the synthesis of the capsule polysaccharaide, where a phosphodiester linkage connects O-acetylated β-D-GlcNAc to α-D-Gal. Ccs4 was found to be subject to phase variation and showed similarities to O-acetyl transferases of the CysE/LacA/LpxA/NodL family, which includes the capsule O-acetyltransferase of *Neisseria meningitidis* serogroup W-135.
Of the five ORFs *dcs1-5* found in Hid, similarity searches revealed an UDP-N-acetyl-D-glucosamine 2-epimerase function for Dcs1 and UDP-N-acetyl-D-mannosaminuronic acid dehydrogenase function for Dcs2. Their putative role in the conversion of β-D-GlcNAc to β-D-ManANAc corresponds to the repetitive disaccharide units β-D-GlcNAc and β-D-ManANAc of the capsule polysaccharide.
The similarity of genes and gene organization found in Hic and Hid to capsulation genes and operons in *Actinobacillus spp.* as well as to more distant genera including *N. meningitidis* suggest horizontal gene transfer during capsule evolution across the bacterial classes.

RKV05

Insight into the work of the National Reference Centre for *Salmonella* and other enteric bacterial pathogens

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Within the National Reference Centre (NRC) for *Salmonella* and other Enteric Bacterial Pathogens located at the Robert Koch-Institut, a broad spectrum of human pathogenic bacteria is investigated, such as *Salmonella*, intestinal *E. coli*, *Shigella*, *Yersinia*, and *Campylobacter*. Additionally since 2009, the NRC has been the German partner of the Binational Consilary Laboratory for *Listeria* located at The Austrian Agency for Health and Food Safety in Vienna, Austria. Methods for lab-based surveillance of the above mentioned pathogens are developed, validated, and used to monitor changes of pathogen occurrence, alterations within the pathogen (e.g. virulence gene profile), and antibiotic resistance. Further, clusters or increased abundance of disease cases are investigated to examine their epidemiologic relationship. Within the year 2010, about 6000 bacterial samples from human disease cases were analyzed and among others the following interesting observations with respect to typing of the strains were made:
1) Reduction in occurrence of the formerly leading *Salmonella enterica* serovar Enteritidis
2) Increase in occurrence of monophasic *Salmonella enterica* serovar Typhimurium strains
3) Increased observation of *Salmonella* infections in children caused by rare serotypes and the link to reptile presence in households
4) Virulence gene fingerprinting of disease-associated intestinal *E. coli* and characterization of intermediate *E. coli* strains which contain virulence genes or plasmids from different pathotypes
5) Work on outbreaks or family-associated cases due to *Salmonella* (75), *E. coli* (31, EHEC and EPEC), *Campylobacter* (10), *Shigella sonnei* (2), and *Listeria* (2).

RKV06

Tasks of the National Reference Centre for *Helicobacter pylori* - an overview

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In 2001 the German National Reference Centre (NRC) for *Helicobacter pylori* launched ResiNet, a German multicentre study aiming to keep resistance development in *H. pylori* under surveillance. In collaboration with 14 microbiological centres all across Germany, ResiNet monitors antimicrobial resistance rates in *H. pylori* and identifies risk factors for their development. This is of paramount importance, since resistances have a massive negative impact on therapy success. The NRC is dedicated to constrain the increase of resistances by recommending susceptibility testing in patients after the first treatment failure and in patients who have undergone antibiotic therapies at multiple occasions due to unrelated bacterial infections, as noted down in the S3-guideline “Helicobacter pylori and gastroduodenal ulcer disease”.
Even though phenotypic susceptibility testing, as standardised by the NRC, has been shown to be reliable and reproducible, it may fail due to extra-long transport times, incorrect handling of gastric tissue samples and contaminations. To overcome these shortcomings, the NRC has set up molecular genetic methods to examine antimicrobial resistance in *H. pylori* genotypically by identifying resistance associated mutations. The request for genotypic resistance testing in some 5000 patients’ samples underlines the increasing demand and need for these rescue diagnostic procedures, which are continuously enhanced.
Since 2006, the NRC has been offering gastroenterologists a unique service: on request, they are sent individual treatment recommendations based on the antimicrobial resistance patterns of their patients’ *H. pylori* isolates. Data on the outcome of the so far more than 1500 therapy recommendations will be analysed and will provide precious data to work out optimised treatment recommendations for difficult-to-treat patients.
10 years after being founded, the NRC for *H. pylori* established itself as an indispensable institution needed to (i) improve the diagnosis of *H. pylori* related infections; (ii) to control the development of antimicrobial resistance in *H. pylori*; (iii) to standardise antimicrobial susceptibility testing procedures; and (iv) to give treatment recommendations in difficult-to-treat *H. pylori* infections.

ZOP01

Characterization of livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) using multiple-locus variable number of tandem repeats analysis (MLVA)

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Staphylococcus aureus is an important human pathogen and the emergence of methicillin-resistant *S. aureus* (MRSA) has become a major concern especially in healthcare settings. Recently, MRSA have also emerged in livestock (e. g. swine and poultry) and are spreading to humans from this reservoir. Therefore, precise typing methods to unravel the transmission dynamics of zoonotic *S. aureus* strains are of great importance. Widely used and portable tools to track epidemic isolates are multilocus sequence typing (MLST) and sequence typing of the *S. aureus* protein A (*spa*) gene. However, studies have shown that zoonotic MRSA-isolates from livestock mainly belong to the MLST clonal complex 398 and are mostly associated with a limited subset of *spa* types (predominantly with t011, t108 and t034). To assess the range of genetic diversity among these MRSA isolates, we investigate in whether multiple-locus variable number of tandem repeats analysis (MLVA) provides a greater resolution than *spa*-typing.
In this study, we apply the *S. aureus*-MLVA scheme from Schouls et al. [1] that includes eight different gene loci and is based on automated fragment analysis on a capillary sequencer. We aim to investigate the diversity of livestock-associated MRSA isolates derived between 2005 and 2010 from more than 50 pig stables comprising locations in various federal states of Germany.
First results of MLVA show that by *spa*-typing indistinguishable strains of different geographic regions can be clustered into at least two different MLVA-types. MLVA discriminated isolates associated with *spa*-type t011 from two different pig farms assigning them to MLVA-types 398 and 2215.

Yet, the MLVA profiles identified so far differ in only one locus which supports the theory of a clonal origin of livestock-associated MRSA.
MLVA typing of zoonotic MRSA might therefore contribute to a better understanding of its genetic diversity against a geographical and temporal background. Our findings will finally add valuable information to investigate the transmission dynamics of a zoonotic pathogen that has only recently overcome a species barrier.

References
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ZOP02
Similarities of the dense granule protein GRA9 between the close relatives *T. gondii* and *N. caninum*

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Neospora caninum (*N. caninum*) is an obligate intracellular apicomplexan parasite which is very close related to *Toxoplasma gondii* (*T. gondii*). The definitive hosts of *N. caninum* are dogs and cattle display the main intermediate hosts. This work deals with dense granule proteins which are known to have important roles in host-parasite-relationship. Previously, we described the dense granule protein GRA9 in *T. gondii.* In this study we identified the putative homologue in *N. caninum*.
The genomic sequence of *Nc*GRA9 is located on chromosome XII and consists of two exons. By Southern Blot it was proven that it is present as a single copy gene. After production of a *N. caninum* specific anti-GRA9 antiserum against the recombinant protein it could be shown by Western Blot that the *Nc*GRA9 gene encodes a 40,2 kDa protein. Furthermore, we defined *Nc*GRA9 as a member of the excreted-secreted antigens (ESA) of *N.caninum.* By confocal microscopy it was observed that in extracellular parasites the protein is accumulated in a dotted pattern which points out that *Nc*GRA9 is located in the dense granule organelles. Furthermore, we could show that after invasion of the parasites into the host cell *Nc*GRA9 is secreted into the parasitophorous vacuole (PV). Thus *Nc*GRA9 fulfils all main characteristics of GRA proteins.
In sum, we could verify in this study that *Nc*GRA9 and *Tg*GRA9 are structurally similar, show immunological cross reactivity and are similar distributed within the respective parasites which indicate a close relationship between both proteins.

ZOP03
Interaction and localization of the *Toxoplasma gondii* protein GRA9 within the parasitophorous vacuole

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The apicomplexan parasite *Toxoplasma gondii* (*T. gondii*) is one of the most successful protozoan parasites infecting all warm-blooded animals, including humans. In host cells the parasite develops within a parasitophorous vacuole (PV), which causes handicaps in nutrient supply. GRA- and ROP-proteins, the main components of the PV are delivered from secretory organelles and are important in the connection between parasites and host cell organelles.
We have described a new 41 kDa *T. gondii* protein called GRA9 which stably associates with the network of membranous tubules and the PV membrane (PVM). We show here that GRA9 co-localize with the dense granules proteins GRA1, GRA2, GRA3, GRA5 and GRA7. Recent co-immunoprecipitation data indicate that GRA9 is a major interaction partner of GRA7 and GRA3 which both were found to be important in nutrient transport between the host cell and the parasite.
To explore GRA9 secretion into the PV and the interaction with network structures and the PVM in more detail, we exogenously expressed HA-tagged GRA9 as well as C-terminal truncated versions of GRA9 in RH tachyzoites. Results indicate that the C-terminus of GRA9 is essential and sufficient for the correct GRA9 secretion into the PV. GRA9-proteins lacking the last one hundred amino acids, including two hydrophobic domains show an alteration in secretion behaviour. The C-terminal truncated GRA9-protein was found exclusively within the cytoplasm of the parasites and could not enter the lumen of the PV.

ZOP04
Advances in the biomolecular interaction analysis of immunoglobulin-binding protein EibG from Shiga toxin-producing *Escherichia coli*

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Shiga toxin-producing *Escherichia coli* (STEC) can cause diarrhea and the hemolytic uremic syndrome (HUS) in humans and belong therefore to a group of pathogens of major interest. Several STEC strains express seven related immunoglobulin (Ig)-binding proteins: EibA, C, D, E, F, G and H. Sharing the facility to bind IgG by nonimmune manner, these proteins occur separately or combined within a single strain. Additionally to the potential evasion of host immune response, EibG is involved in bacterial adherence to host intestinal epithelial cells. Furthermore *eibG*-positive strains show chain-like adherence pattern (CLAP) depending on different *eibG*-allels. Sequencing of entire *eibG* resulted in 21 different *eibG*-allels allocated in three subtypes (*eibG*-α, -β and -γ). Adhesion assays with human and bovine epithelial cells showed different CLAP-phenotypes depending on *eibG*-subtypes. These results were confirmed by inserting *eibG* into *E. coli* with alternative genetic background. The possible functional role of glycosylation of IgG, harboring N-glycans in the CH2 domain of the Fc fragment, in EibG affinity was investigated with native and enzymatically deglycosylated IgG. Peptide N-glycosidase (PNGase) treatment of IgG did not influence EibG-IgG interaction, indicating an EibG binding domain different from the site of glycosylation within the CH2 domain of IgG. Next EibG affinity to IgG subtypes of different vertebrate species was determined by ELISA under variable conditions aimed at identification of Igs with high binding affinity to EibG that may facilitate efficient isolation and purification of EibG from cell lysates. Pending determination of the amino acid sequences of EibG variants will be performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Thus, peptide mapping and comparison to sequences of other Eib-proteins should help to unravel the molecular mechanisms of IgG-binding and cellular adherence. Furthermore, in search of the cellular receptors of epithelial cells, the potential affinity of EibG to glycosphingolipids of different biological origin will be explored by thin-layer chromatography (TLC) overlay assay techniques.

ZOP05
Do complement factor H 402Y and C7 M allotypes predispose to (typical) hemolytic uremic syndrome?

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Typical hemolytic uremic syndrome (HUS) is mainly caused by infections with enterohemorrhagic *Escherichia coli*, whereas in atypical, non-bacteria-associated HUS, complement plays a dominant role. Recently complement has also been shown to be involved in typical HUS. In this study, mostly weakly significant associations with homozygosities of complement allotype C7 M and inversely with factor H 402H were found, suggesting that 402Y and C7 M allotypes predispose to (typical) hemolytic uremic syndrome.

ZOP06
Establishment of a National *Giardia intestinalis* Database and Biobank for functional epidemiology

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Giardiasis is a common and worldwide distributed protozoan infection of the gastrointestinal tract that is caused by the flagellate parasite Giardia intestinalis. Clinical signs are extremely variable, ranging from asymptomatic to chronic symptoms including severe diarrhoea and abdominal pain, and the contributing factors and mechanisms of pathophysiology are largely unknown. The zoonosis potential of distinct Giardia intestinalis genotypes has been highlighted, however, definition of specific parasite virulence factors and host risk factors has been hampered by the lack of functional epidemiological studies.
The aim of this project is to built-up a unique database-linked biobank collection of Giardia intestinalis from human clinical cases and isolates from the field or from domestic animals and thus connecting epidemiological data and molecular characteristics with in-vitro functional

test using the respective parasite isolate cultures. Subsequently, we will exploit the data and clonal parasite collection for functional studies, e.g. to correlate genotype-specific variations in potential virulence factors with functional characteristics.
Currently in the first phase of the project, we are collecting, characterizing and cultivating Giardia isolates from humans and animals contributed by cooperation-partners in Germany and Switzerland. We successfully established an in-vitro culture protocol for the expansion of clonal Giardia trophozoites derived from human faecal specimens. First genotyping results of cloned parasites at the glutamate dehydrogenase gene locus indicate a successful establishment of assemblage B sub-cultures, which are more difficult to isolate than assemblage A due to slower growth behaviour in vitro.
Eventually, this biobank collection will provide a unique tool for functional epidemiological studies of virulence factors.

ZOP07

Knowledge is there but where is the knowledge? Knowledge management in microbiology

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Introduction: The rapid expansion of biological data resulting from thousands of genome sequencing projects and recently developed high-throughput techniques is prodigious. Furthermore, the numbers of article citations in public literature databases like PubMed increases exponentially. Hence, the risk for the individual specialist to lose control of the available information in his field of interest is rising constantly. To address these problems databases and software tools were constructed to structure and consolidate the data. But the numbers of these databases is growing as well and their quality is by far not self-evidently. Here we show our recent developments using text and data mining technologies to make knowledge accessible.
Methods: Detailed knowledge is stored in full-text articles only. Thus we enhanced our in-house text mining system to work on all available and licensed full-text articles in our special field of interest. Then we extracted a large set of entity relationships and created a relational network. Additionally, we extended a data mining platform to perform multiple genome analyses in parallel. Finally, we integrated the text mining into the data mining platform to enhance the analytic power of the complete system.
Results: We obtained all available full-text articles (about 1,800) of our main research field regarding to Coxiella burnetii and Q fever. Based on selected review articles, we evaluate and compare the automatically extracted relation network with an interaction network created by human curators in-house. This allows us to determine in-depth the bias of the automated approach.
Conclusions: The data mining techniques used here allows scientists to deal with many species or strains at once. With the incorporation of text mining they are further able to quickly highlight important functions, causalities and differences as well as to reveal previously hidden knowledge.
Hence the here presented tool assist users strongly during their studies on novel aspects and hypotheses in their research field when providing the complete accessible knowledge in a fast and structured manner.
Now, knowledge is there where knowledge should be.

ZOP08

Core genome determination of zoonotic pathogens using whole genome data in combination with a new web application

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Background: The increasing accessibility of next generation sequencing tools has led to an abundance of genome data, including both fully finished genomes and unfinished assembled contigs. Definition of a core genome, pan genome and/or singleton genes are often necessary to perform comparative or phylogenetic analyses of multiple strains within the same species. We developed a program that facilitates comparative genome analysis utilizing both annotated as well as un-annotated sequence data, while also providing maximal options and parameters for the user for further downstream analysis.
Methods: This program utilizes NCBI's BLAST algorithm to query the genome(s) of interest using a list of ORFs or sequences (defined by the user, or based upon derived from annotated genomes queried). The user controls the parameters for the 'core' definition by setting a threshold for percent identity, percent overlap, etc. Perl programs pair BLAST hit data with corresponding subject ORF data, then sort the results into separate categorized files. If querying annotated genomes, the user may choose various output file formats such as a list of orthologous core ORFs, the sequence of the core ORFs for a reference strain, and/or these core ORFs sequences concatenated.
Results: The Perl programs were implemented in a web interface based on Java, creating a user-friendly graphical-user interface. An evaluation with 15 *Staphylococcus aureus* and 4 *Escherichia coli* O157:H7 genomes with default parameters (95% minimal sequence similarity, not >95% similar to another ORF within the same chromosome (percent identity and overlap), 100% presence in the investigated genomes/sequences) yielded 1,131 and 3,200 core ORFs for *S. aureus* and *E. coli*, respectively. All non-core ORFs are reported to the User in categorized files (e.g., multi-copy ORFs, alternative start and/or stop codons, gaps in alignments, <95% sequence similarity).
Conclusions: The Microbial Core Genome analysis tool, MCGenome, can be used to comparatively analyze multiple annotated genomes or interrogate a single un-annotated one. The process was created to be completely transparent by giving the user control over their parameters and their data, while maintaining a user-friendly web interface. The source code is available from the authors.

ZOP09

Subcutaneous sparganosis in a Japanese immigrant caused by *Spirometra erinaceieuropaei*

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Introduction: Sparganosis is a neglected parasitic disease caused by the plerocercoid stage (sparganum) of *Spirometra* sp., a pseudophyllidean tapeworm. The disease is most often found in East Asia, were humans become infected by eating raw or undercooked meat of fish, amphibians, or reptiles which contain the larval stage. The spargana invade the brain, eye, viscera, and the subcutis, and can cause serious illness. A rare and complicated form, disseminated proliferative sparganosis, is caused by a branching, maldifferentiated plerocercoid provisionally termed *Sparganum proliferum*.
Methods: Several 4-5 cm long, flattened pseudosegmented helminths were extracted from a subcutaneous nodule of the infraumbilical region of a 60-year-old female Japanese immigrant. The parasite was formalin-fixed and embedded in paraffin for further histological examination. In addition, DNA was extracted and subjected to a cestode-specific mitochondrial 12S rRNA gene- and cytochrome c oxidase subunit 1 (*cox1*)-PCR for species identification.
Results: Morphological and histological analysis of the helminth showed typical aspects of a cestode, i.e. calcareous corpuscles in a spongy stroma surrounded by an aspinous tegument. As a characteristic feature of a pseudophyllidean cestode larva (plerocercoid or sparganum), the anterior region showed an invagination and no proper scolex had been developed. The organism was pseudosegmented without any strobilar structures. Sequence analysis of the 440 and 425 bp amplicons of the 12S rRNA gene- and *cox1*-PCR showed 100% and 99% identity with *Spirometra erinaceieuropaei* (AB374543.1 and AF096237.2, AF096238.2). Histological sections of the parasite were also used to set up an immunofluorescence test. When incubated with the patient's serum a tegumental signal was detected at concentrations of 1:100.
Conclusion: *Spirometra erinaceieuropaei* has a cosmopolitan distribution, but most human cases occur in Asia possibly due to local eating habits. PCR analysis unambiguously identified the species responsible for the patient's disease and thus excluded the more pathogenic *Sparganum proliferum*, a larval cestode for which the adult strobilar stage is unknown.

ZOP10

Case report - Human *Dirofilaria repens* infection in Romania

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Introduction: Human dirofilariasis is a zoonotic infectious disease caused by the filarial nematodes of dogs *Dirofilaria repens* and *Dirofilaria immitis*. Depending on the species involved human infections usually manifest as one cutaneous or visceral larva migrans that forms a painless nodule in the later course of disease. Dirofilariae are endemic in the Mediterranean, particularly in Italy. They are considered as emerging pathogens just increasing their geographical range.
The case: We present one of the few known cases of human dirofilariasis caused by *D. repens* in Romania. The patient developed unusual and severe clinical manifestations that mimicked pathological conditions like cellulitis or deep venous thrombosis.
Conclusion: Human doctors as well as veterinarians should be prepared to come across cases of Dirofilariasis in Romania, as well as in other European countries regarded nonendemic so far. As our case illustrates, infection should even be considered in patients with signs of parasitic

infection, which do not exactly correspond to the description of Dirofilariasis in the text books.

ZOP11

Characterization of sorbitol-fermenting Shiga toxin-negative *eae*-positive *Escherichia coli* O157 strains isolated from humans

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We characterized 31 Shiga toxin (Stx)-negative sorbitol-fermenting (SF) *E. coli* O157 strains isolated during 1987-2007 from patients with hemolytic uremic syndrome (HUS) (n = 16), diarrhea (n = 10) and asymptomatic carriers (n = 5) in Germany. H serotyping and *fliC* genotyping identified five different H types including H7, H16, H34, H39, and H45; all strains with *fliC*H7 were H$^-$ (non-motile). The strains carried four different *eae* alleles (γ, ε, κ and a) which were associated with particular serotypes/*fliC* types: strains O157:H$^-$[*fliC*H7] carried *eae*-g, O157:H16/H$^-$[*fliC*H16] and O157:H34 *eae*-ε, O157:H39 *eae*-κ, and O157:H45 *eae*-a. All O157:H$^-$ [*fliC*H7] strains possessed *sfpA*, a marker for the gene cluster encoding Sfp fimbriae, and most of them carried EHEC-*hlyA* and *cdtV* encoding EHEC hemolysin and cytolethal distending toxin, respectively. None of these virulence genes was present in O157:non-H7 isolates. Moreover, strains of the different serotypes differed by their ability to adhere to human intestinal epithelial cells in vitro. Phylogenetic analysis of the 31 Stx-negative SF O157 strains using multilocus sequence typing demonstrated five different sequence types (STs 11, 10, 752, 1041, 593) which corresponded to serotypes O157:H$^-$[*fliC*H7], O157:H16/H$^-$[*fliC*H16], O157:H34, O157:H39 and O157:H45, respectively. Comparison of the SF Stx-negative *E. coli* O157 strains with the HUSEC collection using minimum spanning tree demonstrated that all O157:H$^-$[*fliC*H7] strains clustered in ST11 with SF and nSF STEC O157:H7/H$^-$, whereas strains of the other serotypes were phylogenetically unrelated to STEC O157. Only strains O157:H$^-$[*fliC*H7] and O157:H16 were associated with HUS. Our data demonstrate that the group of *E. coli* O157 pathogenic for humans is broader than previously thought and includes, besides classical nSF STEC O157:H7 also SF *E. coli* O157 of several other serotypes. Stx-independent diagnostic strategies involving PCRs targeting *rfb*O157 and *eae* are method of choice to identify these pathogens

ZOP12

Evolutionary events in Enterohemorrhagic *Escherichia coli* (EHEC) O26:H11

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Enterohemorrhagic *Escherichia coli* (EHEC) is a highly pathogenic and Shiga toxin (Stx) positive subgroup of intestinal pathogenic *E. coli*. EHEC infections often lead to diarrhea, casually hemorrhagic colitis and the hemolytic uremic syndrome (HUS). Whereas EHEC O157:H7 is the most common serotype in HUS patients, EHEC isolated from patients with diarrhea exhibit more frequently non-O157 serotypes; among them O26:H11 is the most common serotype in Germany. In contrast to the well-studied EHEC O157:H7, only little is known about the genome content and genome plasticity (e. g. loss of Stx-encoding genes [*stx*]) of this serotype. We therefore investigated the genomes of two O26 strains isolated at an interval of 8 days from the same HUS-patient by whole genome sequencing.
Using 454 next generation and classical Sanger sequencing, we determined the complete genome sequences of the two O26:H11 isolates. Whole genome comparisons using Mauve 2.3.0 software were performed to investigate differences in the gene content and to detect single nucleotide polymorphisms (SNP). SNPs were additionally confirmed by PCR product sequencing of the particular region. For confirmed SNPs in coding regions, phenotypic tests were applied to confirm their impact on the phenotype.
Whole genome comparisons revealed the loss of a total of 71 genes in the subsequent isolate after 8 days of infection. The majority of them were located on a genomic island, which also included the *stx* operon. Moreover, we determined three non-synonymous SNPs and three SNPs in non-coding regions. The non-synonymous SNPs were located in *rpoS* (RNA polymerase sigma factor), *mreB* (rod shape-determining protein), and *malT* (transcriptional regulator). Interestingly, the SNP in *rpoS* (position 443, TAG to TGG) resolved a stopcodon to an intact gene. RpoS plays a crucial role in survival against stress and controls a large regulon, among them KatE (catalase HPII), which is dependent on RpoS. Because of the reconstitution of the *rpoS* gene function, a positive catalase test was detectable in the subsequent isolate. MalT, the transcriptional activator of maltose-regulon genes, may be also involved in protection against stresses. In contrast, the change in MreB, which is involved in cell division, chromosome segregation, and is responsible for the typical rod shape of cells, is not understood yet. Interestingly, synonymous SNPs, which usually represent the molecular clock and the normal mutation rate within a chromosome, were not detected indicating that external factors were responsible for the pathogens´ changes.
In conclusion, our analysis showed the enormous plasticity of genomes during infection because O26:H11 was able to undergo genomic changes with phenotypic effects within days. Further studies will focus on the impact of the phenotypic changes to the host-pathogen interaction.

ZOP13

Human *Dirofilaria repens* infections in Germany

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Introduction: *Dirofilaria (D.) repens* is a filaria of dogs and other carnivores, which is transmitted by various mosquitoes. *D. repens* can accidentally infect humans, which usually results in a subcutaneous nodule containing a single infertile parasite. *D. repens* is endemic in many tropical and subtropical countries as well as in the Mediterranean. Recently single cases of infections were reported from north of the Alps.
Methods and Results: We recently received multiple samples of *D. repens* worms, which had been removed from infected humans. The patients showed various clinical symptoms, such as dermal and subconjunctival larva migrans, formation of subcutaneous nodules at diverse locations as well as formation of nodules in lung and testis. In most cases our phenotypic diagnosis was confirmed by PCR and sequencing of the mitochondrial 12SrRNA-gene. In the recent years we diagnosed more cases of human *D. repens* infections than ever before, suggesting an increasing rate of infections. It has been postulated that climate warming will lead to spread of *D. repens* infections even to regions north of the Alps. We therefore analyzed more than 40 000 mosquitoes captured in Southwest Germany in the years 2009 and 2010 for the presence of filariae using PCR. We did not find evidence for the presence of significant numbers of *D. repens*-infected mosquitoes.
Conclusion: The number of *D. repens* infections seems to be increasing and doctors should be prepared to diagnose and treat corresponding infections in travelers. In Germany mosquitoes do not (yet?) pose a significant risk for transmitting *D. repens*.

ZOP14

Protein complexes in brains are composed of heterogeneous cellular prion protein phenotypes

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The main characteristic of prion diseases is the accumulation of pathological prions (PrP^{Sc}). The molecular mechanism leading to PrP^{Sc} is considered to be caused by conversion of a normal host-encoded prion protein (PrP^{C}). PrP^{C} acts as substrate in this protein transformation with the result of generating few distinct PrP^{Sc} types. However, PrP^{C} is composed of several overlapping types in brain protein complexes but little is known about the composition of the subtypes.
In this study we separated PrP^{C} proteins obtained from human, sheep and cattle on the basis of differential solubility in detergents. The subtypes were characterized by immunoblotting using various antibodies recognizing different PrP epitopes followed by densitometrical signal quantification and determination of the glycoprotein banding patterns.
Single and serial applications of the detergents N-octyl-β-D-glucopyranoside and CHAPS facilitated high solubility of glycosylated PrP^{C} isoforms whereas high contingents of nonglycosylated human and half of total bovine PrP^{C} remained non-soluble. Most proteins of all species became highly soluble with N-lauroylsarcosine and sodium dodecyl sulphate. Furthermore we identified an aggregated, highly soluble PrP^{C} isoform in the supenatants of all analysed species.
Our findings demonstrate that heterogenous PrP^{C} phenotypes overlapping in brains may be separated regarding their solubility characteristics and convey distinct solubility subtypes. Protein differentiation by solubility experiments can therefore facilitate the separation of PrP^{C} types, can provide detailed information on protein composition and can offer new prospects for transformation efficiency to PrP^{Sc} by conversion assays.

ZOP15

Bacterial effector molecule interference in EHEC: EspPα cleaves and inactivates EHEC-Hly

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This year's high number of infections with enterohemorrhagic *Escherichia coli* (EHEC) in Germany recalls into mind that EHEC are able to cause large outbreaks with severe diseases such as hemorrhagic colitis and the hemolytic uremic syndrome (HUS). Shiga toxins have been identified as major virulence factors and have been studied intensively over the last years. However, the detailed pathomechanisms are still not fully understood and the contribution of additional virulence factors is still elusive. As it is known that the function of bacterial toxins can be modulated by host or bacterial proteases, we studied a potential interplay of two EHEC virulence factors, namely the hemolysin from EHEC (EHEC-Hly) and the serine protease autotransporter EspPα. EHEC-Hly belongs to the family of RTX (repeat-in-toxin) and is a pore-forming cytolysin. It has been shown that EHEC-Hly lyses microvascular endothelial cells as well as erythrocytes, suggesting a role in pathogenicity. EspPα is believed to interfere with the blood coagulation cascade of the host by cleaving factor V and with the complement system by degrading and inactivating C3 and C5. Here we demonstrate that EspPα cleaves EHEC-Hly and that this degradation abolishes the cytolytic activity on erythrocytes and microvascular endothelial cells in a cellular infection model. Functional inactivation of EHEC-Hly occurs when EspPα was supplemented to cultures expressing EHEC-Hly and when both virulence factors were coexpressed. EspPα is also able to degrade the more stable, vesicle-associated, form of EHEC-Hly, which was described recently by our group. Degradation of EHEC-Hly by EspPα occurs in the hydrophobic domain, which is essential for the interaction of the hemolysin with the host cell. EHEC in contact with human intestinal epithelial cells simultaneously upregulated EspPα and EHEC-Hly indicating that both molecules might interact under physiological conditions. Our data suggest that interference of effector molecules might be an additional way to regulate virulence functions and bacterial pathogenicity.

ZOP16

In vitro-evaluation of recombinant *E. coli* Shiga toxoids as candidates for vaccination

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Shiga toxin- (Stx-) producing *E. coli* (STEC) form an *E. coli* pathovar which is characterised by the production of potent cytokines. Stx-producing, ernterohaemorrhagic *E. coli* (EHEC) are the cause of serious infections with life-threatening complications in humans, e.g. the haemolytic uremic syndrome (HUS). Cattle are considered the most important reservoir for STEC worldwide. Recent studies clearly suggest that Stx modulates (suppresses) the bovine immune system resulting in a persistence of infection and long-term STEC shedding. Since the first infection of calves coincides with the lack of Stx-specific antibodies, we hypothesize that vaccination of calves prior to STEC infection may prevent the establishment of a persistent infection. The aim of this study was to generate recombinant Shiga toxoids (rStx1mut & rStx2mut) by site-directed mutagenesis of the enzymatic domain of the toxin and to assess their immunomodulatory and antigenic properties in comparison to recombinant wild type Stx (rStx1WT & rStx2WT). Bovine primary immune cells were used as test systems. In ileal intraepithelial lymphocytes (n=3) rStx1WT and rStx2WT induced a significantly elevated transcription rate of *il-4*. rStx1WT as well as rStx2WT reduced the expression of Stx-receptor CD77 on different types of peripheral blood B- and T- cells (n=6). On teflon bag generated primary bovine macrophages (n=5), rStx1WT or rStx2WT reduced the CD14 expression. In comparable concentrations, neither rStx1mut nor rStx2mut demonstrated any of these effects. Antibodies from sera of naturally infected cattle recognized the Shiga toxoids equally well as the recombinant wild type toxins in a competitive ELISA. While retaining their antigenicity, recombinant Shiga toxoids lost the immunomodulatory properties in cattle. We conclude that Shiga toxoids are promising vaccination candidates for use in cattle to reduce persistence and long-term STEC shedding.

ZOP17

Efficiency of filtration technique for isolation of leptospires from surface waters: Role of different membranes with different pore size and materials

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Background and Objectives: *Leptospira* species spirochetes are helical and motile with dimensions of approximately 0.2-0.3 µm in diameter by 6-30 µm in length. Research has focused on efficient techniques for detecting and isolating *Leptospira* from surface water. Although antibodies for serovars of *Leptospira interrogans* have been developed as clinical diagnostic and research tools, they have not been applied for water sample analysis. Culturing methods for *Leptospira* are also available, but the recommended incubation periods are exceedingly long (16-26 weeks). The primary challenges for carrying out water sampling for *Leptospira* spirochetes include isolation, concentration, and quantitative detection of small numbers of target microorganisms in water. The main goal of this experimental research focused on challenge for application of different kinds and different pore size of membrane filters for isolation of *Leptospira* from surface water samples.
Materials and Methods: The filter materials evaluated included polyvinylidene fluoride (Durapore 0.22 µm and 0.40 µm pore diameters), nitrocellulose (0.22 and 0.45 µm pore diameters), nylon mesh (37 µm), and glass fiber (1.0 µm). Culture of *Leptospira* were prepared using liquid and semi-solid Ellinghausen and McCullough medium as modified by Johnson and Harris. Suspensions of pure culture then passed through different kinds and different pore size membrane filters. Finally, *Leptospira* population enumerated with Petroff-Hausser bacterial counting chamber. Some filters were examined by scanning electron microscopy to verify that leptospires were present following filtration.
Results: This research showed important aspect of using filtration method for isolation of *Leptospira* from surface water. Our results suggest for isolate nearly 100% of *Leptospira* from surface water, the optimal pore diameter should be less than o.45µm.
Conclusions: Although filtration method can be used to isolation leptospires from surface water samples, it is unclear whether this is a useful method for detection all of leptospires exist within surface water samples. Accordingly, a large proportion of leptospires can be retained by membrane filter with a pore diameter commonly used to isolate leptospires from surface water samples (o.45µm pore size).

Keywords
Filtration Technique; *Leptospira*; Surface Waters

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ZOP18

Investigation of environmental distribution of the genus *Leptospira* in surface waters of Golestan province in Iran

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Introduction and Objectives: Leptospirosis is considered a reemerging disease that infects people who have contact with contaminated surface water. Annually, tens of millions of human cases occur worldwide, with case fatality rates ranging as high as 20%-25% in some regions. Leptospirosis is characterized by some researchers as the most common water borne illness in the world. Leptospirosis is caused by serovars of at least eight species of spirochetes from the genus *Leptospira*, which are the environmentally transmitted form of the pathogen. Waters contaminated with the urine of leptospiruric animals is the source of leptospires, and the role of water as a vehicle of transmission is well known. In this research, environmental studies were conducted to distribution of the genus *Leptospira* in surface waters of Golestan Province in Iran.
Material and Methods: Environmental study was conducted in the Golestan province of Iran. Within the provinces, 10 bodies of surface waters were selected for sampling and 30 samples were collected. After preparation, their cultured in EMJH medium was prepared with

enrichments and selective supplements. The leptospires was determined with observed on a microscope equipped for darkfield microscopy. Then, isolated tested with biochemical experiments and identified isolates of leptospires. Results verified by PCR and sequencing of PCR products.
Result: The climate, temperature and pH of the surface water samples were major factor tested and were within the range reported to be capable of supporting leptospires viability. A total 30 samples were collected, and 6 samples (20%) were found to be positive by culture, microscopically and biochemically experiments. Final verification carried out by sequencing of PCR products.
Discussion: We attempted to examine the distribution of leptospires in surface waters within Golestan Province of Iran, by an enrichment culture method using a standard volume of sample material. Little direct information is available on their actual distribution in water and on the relative density of leptospires in these habitats. Such information, gained by a study of the distribution of members of the leptospires, could be of value in elucidating the survival of the leptospires in nature outside the host and provide a greater understanding of the biology of the genus as a whole.

Keywords
Leptospira, leptospirosis, surface waters, Golestan province, Iran.

ZOV01

Staphylococcus aureus and MRSA in thawing liquid of broiler chicken carcasses and their relation to clonal lineages from humans

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Objective: As known from the origin of zoonotic enteric pathogens broiler chicken carcasses can be a substantial source for infections in humans.Here we report the finding of *S. aureus*/ MRSA various clonal linages in thawing liquid of chicken broilers which are also known from infections in humans.
Methods: Thawing liquid from broiler chicken purchased in supermarkets (119 samples from 5 different producers) were investigated for contamination by direct plating on sheep blood agar and in parallel onto Chrom-agar MRSA. Isolates were subjected to typing by means of *spa*-Typing, MLST and genotyping by use of the Alere-microarray, antibiotic susceptibility testing (microbroth MIC) and in case of MRSA genotyping of SCC*mec*-elements
Results:
•· LA-MRSA ST398 was found in 41 from 119 samples. The isolates presented *spa*-types t011, t034, t324, t571, t2576, contained SCC*mec*-element V and the pattern of virulence associated gens typical for this clonal linage. Resistance phenotypes were: PEN, OXA, ERY, CLI, TET, (GEN).
•· *S. aureus*/ MSSA ST12 were present in 7 from these samples. The isolates extributed spa-type t160 and were phenotypically resistant to PEN only or susceptible. The pattern of virulence associated gens were *ent*A/ *ent*B, *clf*A/ *clf*B, *can*, fubA, *slp*A, *slp*B, *aur, sdr*C but not *sak*, *scu* and *chp*
•· *S. aureus* ST 5 were detected in 7 from 119 samples. The exhibited spa-Type t002 and different resistance phenotypes including CIP, ERY, CLI.
The pattern of virulence associated gens typical for this clonal linage.
Conclusion: The proportion of ST398 among HA-MRSA in Germany is 41%; among community MRSA even 17%. Professional exposition and/or familial association is not always documented. MLST ST12 was represented by 2 isolates among 198 *S. aureus* (MSSA) from infections in humans typed by the Reference Center in 2011.
Thus contaminated broiler chicken carcasses can be a source of acquisition of LA-MRSA ST398 and for *S. aureus* with less pronounced host specificity able to cause relevant infections in humans.
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ZOV02

BacSNiP - a new and reliable method for SNP-typing of *Coxiella burnetii*

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Introduction: Single nucleotide polymorphisms (SNPs) are widely used in order to differentiate bacterial species. Today there are various typing methods in use, including MALDI-TOF, Pyrosequencing, or TaqMan assays. We established a novel SimpleProbe melting curve based Real-Time-PCR method working with *Coxiella burnetii*, the causative organism for the Q fever disease.
Material and Methods: Seven *Coxiella* SNPs were used for assay development and evaluation of the method. Primers and each one SimpleProbe were designed and verified with DNA from a reference strain, containing the appropriate nucleotide substitution, before testing a set of 20 strains which had been already genotyped using the MLVA method. The PCR-Mix (10 µl) contained 1 µl LightCycler FastStart DNA Master HybProbe (Roche) with 3 mM $MgCl_2$ (0.8 µl), 1 µl of the BacSNiP Mix and up to 5 µl template DNA. The assay was run on a LightCycler 480 system (Roche) using the SimpleProbe detection format and reading the melting temperatures.
Results: Each nucleotide substitution could be identified by a distinct melting peak, showing up to 5°C temperature differences to the normal variant. Replicates (three) of the controls showed uniform results. The twenty tested strains could be classified into seven different genotypes which were in concordance to their origin and clonal lineage. The detected sequence variations were confirmed by Sanger DNA sequencing.
Conclusions: The presented SimpleProbe based BacSNiP method is a robust and reliable technique for analyzing single isolates as well as for high throughput screening using prefilled 96-well plates. The results were more consistent than those obtained by MLVA as confirmed by Sanger sequencing. Costs are similar or lower compared with other techniques such as MALDI-TOF and Pyrosequencing.

ZOV03

Novel murine infection models provide deep insights into the „Ménage à Trois“ of *Campylobacter jejuni*, microbiota and host innate immunity

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Background: Although *Campylobacter jejuni*-infections have a high prevalence worldwide and represent a significant socioeconomic burden, it is still not well understood how *C. jejuni* causes intestinal inflammation. Detailed investigation of *C. jejuni*-mediated intestinal immunopathology is hampered by the lack of appropriate vertebrate models. In particular, mice display colonization resistance against this pathogen.
Methodology/Principal findings: To overcome these limitations we developed a novel *C. jejuni*-infection model using gnotobiotic mice in which the intestinal flora was eradicated by antibiotic treatment. These animals could then be permanently associated with a complete human (hfa) or murine (mfa) microbiota. After peroral infection *C. jejuni* colonized the gastrointestinal tract of gnotobiotic and hfa mice for six weeks whereas mfa mice cleared the pathogen within two days. Strikingly, stable *C. jejuni* colonization was accompanied by a pro-inflammatory immune response indicated by increased numbers of T- and B-lymphocytes, regulatory T-cells, neutrophils and apoptotic cells as well as increased concentrations of TNF-a, IL-6, and MCP-1 in the colon mucosa of hfa mice. Analysis of $MyD88^{-/-}$, $TRIF^{-/-}$, $TLR4^{-/-}$, and $TLR9^{-/-}$ mice revealed that TLR4- and TLR9-signaling was essential for immunopathology following *C. jejuni*-infection. Interestingly, *C. jejuni*-mutant strains deficient in formic acid metabolism and perception induced less intestinal immunopathology compared to the parental strain infection. In summary, the murine gut flora is essential for colonization resistance against *C. jejuni* and can be overcome by reconstitution of gnotobiotic mice with human flora. Detection of *C. jejuni*-LPS and -CpG-DNA by host TLR4 and TLR9, respectively, plays a key role in immunopathology. Finally, the host immune response is tightly coupled to bacterial formic acid metabolism and invasion fitness.

Conclusion/Significance: We conclude that gnotobiotic and "humanized" mice represent excellent novel *C. jejuni*-infection and -inflammation models and provide deep insights into the immunological and molecular interplays between *C. jejuni*, microbiota and innate immunity in human campylobacteriosis.

ZOV04

Role of putative RNA-binding proteins in *Campylobacter jejuni* gene expression and virulence

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Background: RNA-binding proteins like the RNA chaperone Hfq exert global control of different forms of post-transcriptional regulation of gene expression in many bacteria, by facilitating the interaction between small RNAs and mRNAs. While the epsilon-Proteobacteria *Campylobacter* and *Helicobacter* do transcribe small RNAs, they do not have a recognisable Hfq ortholog. Using bioinformatic approaches, we have identified 3 genes (Cj0138, Cj0667 and Cj1103) in the *C. jejuni* genome which encode proteins with a predicted RNA-binding motif, and have investigated their role in gene expression and colonization/virulence using gnotobiotic IL-10 deficient mice.
Methodology/Principal findings: Mutant strains were generated with an inactivated gene, with two copies of the gene, and one where the inactivated gene was complemented *in trans*. Growth of the mutants was similar to that of the wild-type strain, but while inactivation of Cj1103 did not affect motility or growth phenotypes, overexpression of Cj1103 resulted in a significant decrease in autoagglutination (AAG). Transcriptomic and proteomic characterisation of these strains suggested changes in expression of flagellar genes, which may explain the AAG phenotype.
Infection of gnotobiotic IL-10 deficient mice with the *C. jejuni* wildtype strain caused acute bloody diarrhea, histopathology and inflammatory responses in the colon. All mutants colonized the mouse intestine to similar levels as the wildtype strain, but differed in their potential to induce diarrhea and inflammatory responses in the colon. Clinical signs of disease and levels of pro-inflammatory cytokines in the colon were significantly reduced in the Cj0138 mutant when compared to the wildtype strain.
Conclusion/Significance: Although there are subtle phenotypes associated with these putative RNA-binding proteins, there is currently no proof that these proteins function as general RNA-chaperones, as the predicted pleiotropic phenotypes were not observed. The results obtained in the infected gnotobiotic IL-10-deficient mice indicate a role of the genes Cj0138, Cj0667 and Cj1103 in *C.jejuni* virulence.

ZOV05

Animals as putative source of Adherent-Invasive Escherichia coli (AIEC) – a group of pathogens associated with Crohn's disease in humans

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Adherent-Invasive Escherichia coli (AIEC), which have been associated with Crohn's disease, show traits similar to human and animal extraintestinal pathogenic E. coli (ExPEC) regarding their phylogenetic origin and virulence gene profile. However, previous studies have shown that human ExPEC strains rarely share the AIEC phenotype, whereas only limited data are available on the AIEC-like phenotype properties of animal E. coli strains, both from extraintestinal and intestinal sources. We therefore analyzed the AIEC phenotype of 79 ExPEC strains from birds, cats, dogs, and swine, which were selected with respect to multi locus sequence types (STs) that had previously been linked with AIEC strains from patients with Crohn's disease, including ST131, ST73 and ST10. In addition, 45 E. coli enteritis isolates from cats, dogs – in case of ST complex (STC) 73 (n=26) representing a phylotype typically observed among strains causing cystitis and pyelonephritis in humans – and swine, were included. While only 1.3% of ExPEC strains showed an AIEC phenotype, as shown by the ability to adhere to and to invade intestinal epithelial cells, as well as to survive and replicate within murine macrophages, up to 57.8% of animal enteritis strains (33.3% swine; 35.3% dogs; 81.8% cats) phenotypically resembled the AIEC pathotype. Interestingly, none of the STC73 ExPEC strains showed this phenotype, whereas 72.4% of STC73 strains from enteritis were confirmed as AIEC-like strains. Due to the non-arbitrary strain selection no conclusion can be drawn considering the real prevalence of AIEC-like strains in the intestinal tract of animals, but their frequent detection provides support for a lack of host-specificity and a zoonotic risk. Further studies including well characterized diseased animals are needed in order to detect putative reservoirs of AIEC strains, even in the intestine of asymptomatic carriers, and to evaluate the dimension of the risk with respect to the implementation of prevention and control measures and thus to public health.

ZOV06

Shiga toxin 1 and 2 - Different toxins, different toxicities?

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Enterohemorrhagic *Escherichia coli* (EHEC) cause hemolytic uremic syndrome (HUS), the most common cause of acute renal failure in children[1]. Shiga toxins (Stxs) are key virulence factors of EHEC and endothelial cells are the major toxin targets[2]. We performed a direct and comprehensive comparison of cellular injury induced by two major Stx types, Stx1 and Stx2, in human brain microvascular endothelial cells (HBMECs) and EA.hy 926 macrovascular endothelial cells. Scanning electron microscopy of microcarrier-based cultures, digital holographic microscopy of single cells, and quantitative apoptosis/necrosis assays demonstrate that Stx1 causes both necrosis and apoptosis, whereas Stx2 induces mainly apoptosis in both cell lines. Moreover, microvascular and macrovascular endothelial cells have different susceptibilities to the toxins: HBMECs are more susceptible to Stx2, whereas EA.hy 926 cells are more susceptible to Stx1[3]. These findings have implications in the pathogenesis of HUS, and suggest the existence of yet to be delineated Stx type-specific mechanisms of endothelial injury beyond inhibition of protein biosynthesis.

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DVP22

Molecular diagnosis of the etiological agents of infectious endocarditis using commercial universal rRNA gene PCR plus sequencing tests *

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Introduction: The incidence and relatively high mortality of infectious endocarditis (IE) has remained relatively constant over the past 30 years. Blood culture diagnosis is negative in 2.5 to 31% of cases because of the inability of fastidious microorganisms to grow and growth inhibition of pathogens due to prior antibiotic administration. Molecular methods are considered highly sensitive diagnostic tools. Here, we evaluated SepsiTest™ and UMD Tissue™ (Molzym, Bremen) for the analysis of whole blood and heart valve samples from patients who were operated on for endocarditis.
Objectives: Evaluation of SepsiTest™ and UMD Tissue™ for the diagnosis of the etiological agents of IE.
Methods: The study included 30 patients who were classified into 20 definitive IE and 10 non-IE cases according to the modified Duke criteria. Whole-blood (WB) and heart valve (HV) samples were analysed according to the SOP of the laboratory (Vitek 2; bioMérieux). Real-Time PCR analyses were performed according to the instructions of the manufacturer. In case of positive samples, the amplicons were sequenced (GATC, Constance) and strains identified by using BLAST search tools (www.ncbi.nlm.nih.gov/BLAST; www.sepsitest-blast.de).
Results: The sensitivity of PCR (85%) was nearly twice as high as that of culture (45%), which in 10/20 IE cases presumably stayed negative because of growth inhibition of the pathogens by antibiotics. PCR provided the basis for reclassification of 5/10 non-IE cases into IE cases. Culture-negative infections were identified by PCR, including single infections due to streptococci and Gram-negative bacteria (Escherichia coli, Haemophilus parainfluenzae) and mixed infections involving two Gram-positive bacteria or Candida spp. with Gram-positive bacteria.
Conclusions: The commercial tests proved to be of value for the rapid diagnosis of IE, particularly in cases of culture-negative infections.
* The results have been published elsewhere (J. Clin. Microbiol. doi:10.1128/JCM.00830-11)

DVP23

Simultaneous detection of eight potential bioterrorism agents by real time PCR

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The Bacillus anthracis attacks in the United States in 2001 demonstrated the need of fast and reliable diagnostic methods for the detection of potential biological agents. Not only Bacillus anthracis but also Burkholderia (pseudo-) mallei, Brucella ssp., Francisella tularensis, Yersinia pestis, Coxiella burnetii and the Orthopoxvirus Variola major are listed as biological agents with high risk of misuse in bioterrorism attacks by the Centers for Disease Control and Prevention (CDC). Since then a number of PCR-based detection assays for these agents were published, some of them are designed as multiplex assay for two or three biological agents. However, an assay for the simultaneous detection of eight different agents has not been developed so far.
We designed and optimised real-time PCR TaqMan-Assays for the detection of Bacillus anthracis, Burkholderia (pseudo-) mallei, Brucella ssp., Francisella tularensis, Yersinia pestis, Coxiella burnetii and Orthopoxviruses based on different chromosomal targets for each pathogen. Primers and probes were lyophilizied on 96well plates for use with the LightCycler® 480-System. In total we tested over 400 DNA samples including at minimum 20 DNA samples of each of the eight biological agents respectively. All assays were tested for specificity and limit of detection (LOD).
Our findings show only positive PCR results for the respective target bacteria and no cross reaction with closely related species or other clinically relevant bacterial pathogens. Sensitivity experiments showed detection limits down to 10 copies per reaction for most of the assays. In total we designed a real-time PCR TaqMan assay combination for the detection of eight different potential bioterrorism specimen in one real-time PCR run. This assay is easily to handle because of lyophilizied primers and probes and for the first time, it is possible to detect all these highly virulent pathogens at the same time with very good detection limits.

MPP90

Analysis of antibiotic tolerance in Staphylococcus – towards the characterization of S. aureus persister cells

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Bacterial cultures contain a subpopulation of dormant cells, so called persisters. These non-growing cells are phenotypic variants of the wild type capable of surviving killing by bactericidal antibiotics. The persister state is reversible and dormant cells might be expected to form stochastically during growth of a culture, prior to the antibiotic exposure. We aimed to study mechanisms governing persister formation and their cellular, physiologic, and genetic properties in Staphylococcus aureus.
Tolerance of different planktonically grown S. aureus strains (SA113, two small colony variants (SCVs) hemB and menD, as well as HG001, HG002, and HG003) to various antibiotics was tested at exponential- or stationary-phase. Antibiotics applied were daptomycin, ciprofloxacin, tobramycin, rifampicin, and penicillin in a range of 1- to 100-fold MIC. The biphasic killing kinetics, highly indicative of persister cells, were observed in stationary-phase upon treatment of SA113 with 100-fold MIC daptomycin and of menD challenged with 100-fold MIC tobramycin. Time-dependent CFU analyses revealed a strong killing effect of daptomycin on exponentially growing SA113 and HG001-003 while SCVs' killing was retarded. Tobramycin treatment appeared to eradicate SA113 less efficiently than SCV cells. Challenging of HG001-003 with 10-fold tobramycin resulted in SCV-like cells upon cultivation on solid media.
These results indicate that stationary-phase cells are more antibiotic tolerant than exponential- phase cells due to the elevated levels of persisters. Thus, growth phase as well as strain background and genotype appear to be important factors in the formation of S. aureus persister cells.

PWV11

– PRESENTED AS ORAL PRESENTATION IN WORKSHOP 24 -

Crohn's disease relevant *Enterococcus faecalis* trigger differential host response in germfree and conventional NOD2 deficient mice

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Background: The pattern recognition molecule NOD2 plays an important role in maintaining of intestinal homeostasis and mutation of the *NOD2* gene has been associated in humans with increased susceptibility to Crohn's disease. Mice deficient for NOD2 molecule show no symptoms of spontaneous intestinal inflammation, underscoring the multifactorial etiology of Crohn's disease. However, several studies in mice have shown that NOD2 plays an important role in controlling the composition of the intestinal microbiota by regulating expression of antimicrobial peptides and in the host defense against several intestinal pathogens such as *Yersinia pseudotuberculosis*, *Citrobacter rodentium* or *Helicobacter hepaticus*. Involvement of enterococci as common members of the intestinal microbiota in the development of intestinal inflammation has been already proved using IL10-/- mice monocolonized with *Enterococcus faecalis.*
Methods: One new *Enterococcus faecalis* strain (CD11) was identified by screening fecal samples from patients with Crohn's disease for gelatinolytic activity. Specific pathogen free and germ free NOD2-/- mice were orally infected either with *Enterococcus faecalis* CD11 or OG1RF (a well-characterized, fully sequenced strain) at the age of 4 weeks.
Results: Specific pathogen free NOD2-/- mice are highly susceptible to oral inoculation with CD11 strain and die all within 3 weeks post infection, while mice infected with OG1RF strain as well as monocolonized mice (both with CD11 or with OG1RF) are protected from mortality. In a short-term experiment (1 week), mice infection with CD11 was followed by significant weight loss, elevated levels of serum-amyloid A and IL6 in the blood and high regulation of TNF, IL6 and IL1-beta in the gut.
Conclusions: These results clearly demonstrate a link between *E. faecalis* stain specificity and the ability of this commensal to trigger disease in a genetically susceptible host.

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