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ABSTRACTS

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WS01.01

Biofilm formation abilities and disinfectant tolerance in german Candidozyma auris isolates

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Introduction: *Candidozyma auris* (*C. auris*) is considered an emerging fungal pathogen, leading to nosocomial outbreaks. Clinically, *C. auris* is associated with resistant phenotypes limiting treatment options. While six distinct clades are described, only Clade I, III and IV seem to be relevant in outbreaks. It is suggested that the ability to aggregate and form biofilms may contribute to resistance and environmental persistence. The usage of disinfectants is one of the major methods to clear *C. auris* from the environment and thus prevent surface associated transmissions.

Aims: Gaining insights into the effectiveness of surface disinfectants against German *C. auris* isolates from the National Reference Center for Invasive Fungal Infection (NRZMyk) and their impact on biofilm formation, we evaluated representative *C. auris* subsets concerning aggregation status, differences in biofilm forming capacities and susceptibility to disinfectants in biofilm states and performed evolution assays exploring underlying evasion strategies.

Materials and Methods: A representative strain-set (n=51, clade 1-5) was categorized into 4 sub-groups with early outbreak (< 3-month transmission history), late outbreak (> 3-month transmission history), non-outbreak and multi-drug resistant (MDR) isolates. These were classified into aggregative and non-aggregative phenotypes to assess their general adherence abilities and then evaluated by crystal violet assay (CV) concerning biofilm capacities (OD595). Bead assays were used to measure the effect of three common hospital disinfectants (Incidin 0.5%, Perform 0.5%, Gigasept 2%) on *C. auris* biofilm survival in accordance with the manufacturer"s exposure times measuring log reduction. An evolution assay was deployed challenging robust phenotypes.

Results: Non-aggregative phenotypes were more prevalent (n=42) and associated with transmission prone clade 1, while clade 3 isolates harboured most aggregative strains. CV revealed no significant difference between groups including outbreaks strains (mean OD595: MDR 0,273; non-outbreak 0,183; early outbreak 0,106; late outbreak 0,074). However, biofilm forming capacity was more prominent in aggregative phenotypes, MDR (max. OD595 1,465) and non-outbreak (max. OD595 0,396) subgroups. All disinfectants showed good anti-candida activity with a mean log reduction of 6,124 for Gigasept; 5,768 for Incidin; 6,124 for Perform. 4 isolates showed robustness against Incidin with a min. log reduction of 1,17; 1,903; 1,937 and 1,717. Based on ongoing evolution experiments, clinical *C. auris* phenotypes may overcome Incidin or Gigasept as stressors, while Perform seems to stay effective after prolonged exposure.

Conclusion: Our findings suggest that aggregation and biofilm formation are not necessarily associated with transmission phenotypes. Clinical strains seem to be prone to disinfectant tolerance while exposed to sublethal concentrations, especially true for certain substances warranting further investigation.

Fig. 1

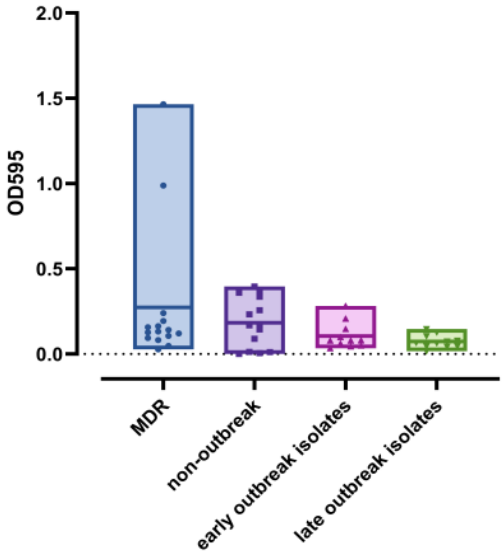

figure 1: crystal violet assay

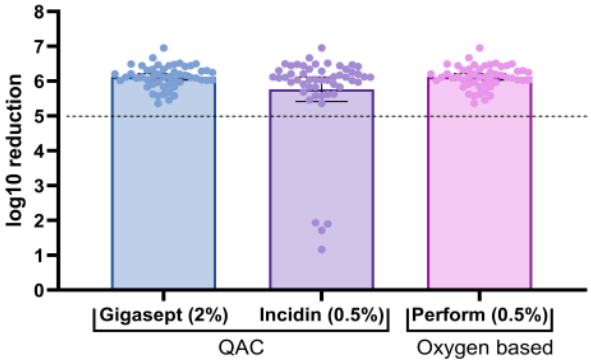

figure 2: bead assay

WS01.02

Not more resistant – But more persistent? Environmental survival of hypervirulent Klebsiella pneumoniae

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Background: Hypervirulent *Klebsiella pneumoniae* (hvKp) has emerged globally as a highly pathogenic variant capable of causing severe invasive infections in otherwise healthy individuals. While classical *K. pneumoniae* (cKp) is primarily associated with healthcare-associated infections, increasing reports from the Centers for Disease Control and Prevention [1] and the World Health Organization [2] indicate the emergence of nosocomial transmission clusters involving hvKp. However, little is known about whether hypervirulence itself influences environmental persistence or susceptibility to disinfection measures relevant for hospital infection prevention.

Objectives: This study investigated whether hvKp differs from cKp regarding (i) susceptibility to clinically relevant biocides, (ii) UV-C inactivation efficacy, and (iii) persistence on dry surfaces, to assess whether hypervirulence contributes to healthcare-associated transmission potential.

Materials and Methods: Clinical hvKp (n=15) and cKp (n=15) isolates were comparatively analyzed. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined for benzalkonium chloride, chlorhexidine, glutaraldehyde, and isopropanol. UV-C susceptibility was evaluated by comparing $\log_{10}$ reductions after standardized irradiation. Surface persistence experiments were performed on dry abiotic surfaces over a six-week observation period with serial quantification of surviving bacteria. For statistical comparison between cKp and hvKp isolates, the non-parametric Mann–Whitney U test was applied.

Results: No significant differences between hvKp and cKp were observed regarding susceptibility to the tested biocides. MIC and MBC values were comparable across both groups. Similarly, UV-C irradiation achieved similar $\log_{10}$ reductions in hvKp and cKp isolates ($p = 0.724$), although considerable intra-group variability was observed. In contrast, hvKp demonstrated significantly prolonged persistence on dry surfaces. Across all evaluated time points, ranging from 1 day to 6 weeks, hvKp showed consistently higher survival rates than cKp. AUC analysis confirmed significantly enhanced environmental persistence of hvKp isolates ($p < 0.001$). Differences became particularly evident during prolonged incubation, suggesting enhanced long-term survival under environmental stress conditions.

Conclusions: Although hvKp did not differ from cKp regarding susceptibility to biocides or UV-C irradiation, the significantly enhanced surface persistence observed for hvKp may increase the risk of healthcare-associated transmission. Strict adherence to infection prevention measures, including adequate disinfectant exposure times, and structured surveillance strategies therefore appear essential in clinical settings.

[1] CDC (2024). Emergence of hypervirulent *Klebsiella pneumoniae* ST23 carrying carbapenemase genes

[2] WHO (2024). Antimicrobial Resistance, Hypervirulent *Klebsiella pneumoniae* - Global situation

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WS01.03

Beyond reference strains: Variable disinfectant susceptibility in clinical vancomycin-resistant *Enterococcus faecium* (VREfm) isolates

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Background: Due to limited therapeutic options, vancomycin-resistant *Enterococcus faecium* (VREfm) infections present a significant challenge in hospital settings, making effective prevention strategies essential. Disinfectants are widely used to control the spread of VREfm on abiotic surfaces. However, the efficacy of disinfectants is primarily tested using planktonic reference strains of *E. hirae*, and Efm, despite known differences in disinfectant susceptibility between strains. Furthermore, there is limited knowledge of the activity of clinically applied disinfectants against clinical VREfm isolates of diverse sequence types (ST) and mature biofilms. This study therefore investigated the efficacy of commonly used disinfectants against VREfm isolates in both planktonic and biofilm-associated states.

Methods: A total of 68 clinical and multicentre VREfm isolates were analysed. The activity of 2 % Gigasept® used for instruments, 0.5 % and 2 % Incidin™ used for surfaces, and 70 % ethanol used within hand disinfectants against planktonic cells and mature biofilms was assessed. For biofilm VREfm, a glass bead-based assay was employed. The isolates were treated with the recommended in-use concentrations and exposure times of the disinfectants. Furthermore, sub-inhibitory concentrations at the recommended exposure times were applied to simulat disinfection routine failure. Disinfection efficacy was determined by calculating the log10 reduction.

Results: Of the disinfectants tested, 2 % Gigasept® and 2 % Incidin™ were effective against planktonic clinical VREfm isolates. Both disinfectants reduced growth in 100 % and 97 % on biofilm VREfm, respectively. Using 70 % ethanol and 0.5 % Incidin™ (in-use concentrations), the clinical disinfection efficacy was 16 ± 7 % and 92 ± 8 % for planktonic VREfm, and 68 ± 8 % and 92 ± 4 % for biofilm VREfm, respectively.

Only 1% Gigasept® was effective against all isolates tested at sub-inhibitory concentrations. Although 1 % Incidin™ reduced planktonic VREfm, it was only effective against 91 ± 7 % of biofilm VREfm. Ethanol failed to reduce any planktonic VREfm and reduced one-third of biofilm VREfm 33 ± 18 %. An efficacy of 71 ± 11% (planktonic VREfm) and 66 ± 21% (biofilm VREfm) was calculated for 0.25% Incidin™. Reduced susceptibility was observed across several sequence types, including the clinically high-prevalence VREfm VREfm ST80 and ST117.

Conclusion: In addition to reference strains, clinical VREfm isolates should be included in disinfectant efficacy testing, as reference strains may not adequately reflect clinically relevant susceptibility patterns. Reliable activity against clinical isolates appears to require higher disinfectant concentrations, while lower concentrations may require additional mechanical support to achieve sufficient reduction. The reduced susceptibility exhibited by certain strains may be indicative of the emergence of tolerance to disinfectants commonly utilised in clinical practice.

WS01.04

Antimicrobial coatings as complementary strategies to disinfectants for high-touch-exposed surfaces: Novel coatings and novel testing approaches

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Background: Surface disinfection of high-touch surfaces in clinical, industrial, and public spaces is crucial to prevent undesired transmission of pathogens and improve hygiene; however, disinfection cycles do not protect such surfaces from contamination between applications. Antimicrobial coatings can prospectively protect high-touch surfaces between disinfection cycles by killing pathogens upon contact. Harmonized international standard tests exist for assessing the efficacy of such products, but they do not realistically mimic real-life conditions and still lack proper assessment of resistance development, an important shortcoming in light of the global threat of antimicrobial resistance (AMR).

Methods: Within the EU-funded STOP project (Surface Transfer Of Pathogens, Grant Agreement 1010579610), a novel approach has been proposed for the development of antimicrobial coatings for long-lasting application on high-touch surfaces. The approach encompasses multiple strategies, including silver-loaded nanoparticles functionalized with antimicrobial peptides and superhydrophobic surfaces that prevent pathogen adhesion, which are currently undergoing field trials. The development of these products was accompanied by a novel test for studying surface transfer of pathogens that mimics fingertip-mediated transmission of microbes under real-life conditions, as well as tests for predicting both selection of antibiotic-resistant bacteria (ARB) through co-selection of antibiotic-resistance genes (ARGs) and induced de novo evolution of resistance to antimicrobial coatings and materials.

Results: Two antimicrobial coatings were developed that show high efficacy against an array of common pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, with >3 log10 CFU reduction after only one hour of contact time (ISO 22195:2011(E)). Finger-mimicked transmission experiments on antimicrobial surfaces and controls demonstrate how real-life simulated conditions can be reproduced for harmonized tests and challenge current paradigms in the assessment of antimicrobial surfaces and surface disinfectants. Furthermore, selection experiments with libraries of antibiotic-resistant and antibiotic-susceptible strains, together with guided evolution experiments using antimicrobial materials, show how it is possible to predict whether an antimicrobial coating or material could contribute to select or develop antimicrobial resistance upon use.

Conclusions: We show how prevention of pathogen transfer through high-touch surfaces could be achieved with long-lasting antimicrobial coatings, and we provide a novel panel of innovative tests to be proposed as standards for studying this new category of products that expands the safety framework for their implementation in light of the ongoing burden of AMR.

WS01.05

Environmental transmission and infection control challenges during a carbapenem-resistant *Acinetobacter baumannii* outbreak in a university hospital

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Background: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is classified by the World Health Organization (WHO) as a critical priority pathogen and poses a major threat to hospitalized patients. Due to its ability to persist in the hospital environment and to rapidly spread in healthcare settings, CRAB outbreaks are particularly challenging to control. We report on the multilayered investigation of an outbreak with CRAB at a surgical ward in combination with an intensive care unit (ICU) at a university hospital in Northern Germany.

Methods: Between August and December 2025, 16 patients colonized and/or infected with CRAB were identified on a surgical ward and an intensive care unit (ICU). One of the 16 patients died in the context of CRAB respiratory infection and severe underlying pulmonary disease. Isolates underwent antimicrobial susceptibility testing, carbapenemase characterization and whole-genome sequencing (WGS). Environmental investigation included 128 environmental samples and 115 follow-up control samples. Outbreak investigation was adapted in a layered approach including enhanced environmental cleaning, active surveillance, and patient cohorting on the ward as well as environmental sampling aiming to identify the source of the outbreak and to interrupt transmission routes.

Results: WGS confirmed a monoclonal outbreak caused by an OXA-40-producing ST-2 strain. The median time from admission to CRAB detection was 28.5 days (IQR 18.3–31.3). Environmental sampling identified CRAB in 7/128 samples (5.5%), including NPWT equipment, mattresses and patient-room furniture. Although CRAB detection rates declined following implementation of intensified infection prevention and control measures, temporary closure of the surgical ward was required to facilitate intensified cleaning and disinfection. Following intensified infection prevention and control measures, all 115 follow-up environmental control samples remained CRAB-negative. In the ICU, a comprehensive infection prevention and control strategy was implemented and closely monitored, preventing unit closure.

Discussion: Active surveillance and enhanced infection control measures are mandatory to contain an outbreak with CRAB. Nevertheless, outbreak containment requires substantial personnel resources and strict adherence to routine processes and standard operating procedures. The identification of NPWT systems as a potential transmission vehicle revealed deficiencies in interpatient decontamination procedures and highlighted the importance of continuous evaluation of medical device reprocessing pathways. The prolonged interval between admission and CRAB detection together with contamination of patient-near surfaces

underscores the importance of active surveillance and rigorous environmental hygiene in high-risk hospital settings. As the prevalence of multidrug-resistant pathogens continues to rise, sustained investment in surveillance, environmental hygiene and medical device reprocessing is essential to maintain patient safety.

WS02.01

Function and spatio-temporal patterning of the Small basic protein (Sbp) in *Staphylococcus epidermidis* biofilm formation

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Background & Aims: *Staphylococcus epidermidis* is a common nosocomial pathogen causing implant-related infections. Pathogenicity essentially relies on biofilm formation, driven by the production of an extracellular matrix (Otto, 2009). The extracellular matrix provides structural stability, adhesion, and spatial heterogeneity, yet its molecular organization and functional interactions remain poorly understood (Rohde *et al*., 2010). Previously, a 18 kDa protein referred to as small basic protein (Sbp) was identified as a dominant biofilm matrix component (Decker *et al*., 2015). This study aimed to characterize the interactions and temporal-spatial distribution dynamics of Sbp in *S. epidermidis* biofilms.

Methods: Biofilm formation and Sbp localization in S. epidermidis 1457 were investigated using (Live-)CLSM, STED microscopy, and MINFLUX nanoscopy to resolve spatio-temporal protein distribution upon surface attachment. Surface topography and matrix architecture were analyzed by AFM. Phenotypic characterization of 1457 and the corresponding *sbp* knock-out mutant (1457Δ*sbp*) included growth curve analysis, biofilm adherence assays and comparative transcriptomic as well as proteomic profiling to assess stress response signatures. Extracellular reactive oxygen species (ROS) levels were quantified using colorimetric (NBT) assays. To identify Sbp interaction partners, proximity labeling and affinity-based pull-down assays were performed, followed by protein identification using proteomic analysis.

Results: Microscopy revealed that Sbp is produced immediately upon surface attachment of *S. epidermidis* 1457. Remarkably, Sbp spreads two-dimensionally across abiotic surfaces prior to bacterial colonization and is later incorporated into the intercellular matrix during three-dimensional biofilm development. AFM demonstrated that Sbp is essential for matrix architecture, indicating a Sbp-driven biofilm assembly process. Consistently, proximity-labelling and pull-down approaches identified multiple Sbp interaction partners (SIPs), supporting the concept of a superimposed biofilm matrix interactome. Enrichment of oxidoreductases among SIPs suggests a role in redox regulation, supported by increased ROS accumulation and growth defects in the sbp knockout mutant. Importantly, the growth defect of 1457Δ*sbp* could be rescued by antioxidant treatment, demonstrating that elevated ROS levels underlie the observed phenotype.

Conclusion: This study provides dynamic insights into biofilm matrix assembly in *S. epidermidis* at single molecule resolution. Control of ROS detoxification by biofilm matrix components, recruiting moonlighting cytosolic proteins to the biofilm matrix, puts a novel and so far undiscovered piece to the puzzle of how biofilms ensure bacterial homeostasis to support chronic biomaterial associated infections.

WS02.02

Positioning of effector proteins on the type VI secretion system apparatus is associated with the location of effector targets within the recipient cell

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The bacterial type VI secretion system (T6SS) translocates toxic effector proteins directly into prokaryotic and eukaryotic cells or the extracellular space. During translocation, effector proteins are located on the T6SS apparatus at various positions along a vertical axis from top to bottom. Why a particular effector is translocated at a given position is not fully understood. Here, we focus on the opportunistic pathogen *Pseudomonas aeruginosa* with multiple T6SSs and investigate the positioning of very diverse effector proteins for secretion. We found that effectors are not randomly distributed along the vertical axis of the T6SS. Across T6SSs and across bacterial strains with diverse effector sets, we observe effectors with targets in the recipient cells" cytoplasm positioned distal on the secretion apparatus to effectors with targets in the membrane and periplasm. We therefore identify a so far unknown association between the spatial organisation of the loaded effectors on the T6SS and the spatial organisation of the effector target in the recipient cell for both the H1-T6SS and the H2-T6SS. Intriguingly, this association applies in particular to effectors that target Gram-negative, prokaryotic cells that are compartmentalized into the periplasm and cytoplasm. These findings reveal new insights into the evolution of T6SS effectors and are of relevance for the rational design of effectors for therapeutic purposes.

WS02.03

Naturally occurring *rpoS* polymorphisms differentially modulate oxidative stress resistance in *E. coli* O104:H4

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Question: Stationary-phase sigma factor RpoS regulates adaptation of *Escherichia coli* (*E. coli*) and other Proteobacteria

to varying ecological conditions by gene polymorphism. We have recently identified two naturally occurring single nucleotide polymorphisms (SNPs) of *rpoS* that both resulted in significantly increased virulence in *E. coli* O104:H4, the causative agent of the outbreak of food-borne infections in Germany in 2011. Here, we explored the impact of these polymorphisms on the global gene expression in *E. coli* O104:H4 to further elucidate the RpoS regulatory role in pathogenic bacteria.

Methods: Naturally occuring *rpoS* alleles carrying the SNP 829C>T introducing a premature stop codon (*rpoS*_Stop), and the SNP 836G>T, leading to a non-synonymous amino acid change (*rpoS*_Cterm), as well as the wild type *rpoS* allele (*rpoS*_wt) were expressed heterologously in EHEC O104:H4 Δ*stx2* Δ*rpoS*. Total RNA was isolated from bacteria grown to early stationary phase, genomic DNA was removed and rRNA depleted, followed by directional library preparation and Illumina Sequencing. Differentially expressed genes were identified with DESeq2 and Gene Ontology (GO) Gene set enrichment (GSE) analysis using ClusterProfiler. To determine the oxidative stress resistance, we treated our strains at early stationary-phase with 60 mM H2O2 for 30 mins and the number of surviving cells was calculated by the ratio of colony-forming units per mL before and after treatment.

Results: DESeq2 analysis of our RNA sequencing data confirmed our previous observation of a significant increase in virulence gene expression in *E. coli* O104:H4 expressing *rpoS*_Stop and *rpoS*_Cterm, compared to *rpoS*_wt. GO GSE analysis revealed an additional characteristic of the *rpoS* mutants in comparison to *rpoS*_wt, i.e., a down-regulation of oxidative and pH stress response pathways. Interestingly, a closer investigation of the degree of gene expression revealed that *rpoS*_Stop was associated with stronger downregulation of oxidative stress-related genes than *rpoS*_Cterm. To further investigate these results, we subjected the strains carrying the different *rpoS* alleles and a *rpoS* knock-out strain (Δ*rpoS*) to oxidative stress with H_2O_2 and determined the percentage survival of bacteria. Whereas around 20% of the *rpoS*_wt strain survived, both *rpoS*_Stop and Δ*rpoS* reduced survival of bacteria to less than 1%. The *rpoS*_Cterm strain exhibited rather an intermediate resistance phenotype with 4% of bacteria surviving, lying between Δ*rpoS* and *rpoS*_wt.

Conclusions: Our findings demonstrate that naturally acquired *rpoS* mutations shape a specific genomic profile in *E. coli* O104:H4, in particular mediating differential resistance to oxidative stress. Together with our previous results on virulence, this highlights the importance of RpoS regulating the trade-off between hypervirulence and resistance to environmental factors in pathogenic bacteria.

WS02.04

Functional profiling of the Type III secretion system effector BipC from *Burkholderia thailandensis* E264 reveals a complex protein network involved in host cell membrane interactions

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Human and animal pathogenic bacteria of the *Burkholderia pseudomallei* group may cause severe infectious diseases such as melioidosis. These pathogens are characterised by multi-drug resistance and diverse virulence mechanisms, complicating effective treatment. A key driver of pathogenicity is their intracellular lifecycle, which involves host cell invasion, rapid phagosomal escape into the cytosol, and evasion of LC3-associated autophagy to enable survival and replication. The bacteria utilize actin-based motility to invade adjacent cells via membrane protrusions and subsequent membrane fusion, allowing the bacteria to infect neighbouring cells with minimal immune reaction. The Bsa Type III secretion system (T3SS), including *Burkholderia* invasion proteins (Bip), is a major virulence determinant. The translocator BipC, homologous to *Salmonella* SipC and *Shigella* IpaC, is thought to form a translocon pore within the host cell membrane. However, it may also act as a translocated effector, probably promoting intracellular survival through uncharacterised host cell interactions. To analyse the physiological role of BipC, a combination of bioinformatics, in vitro interaction screens, and infection models was used. Bioinformatics analyses were performed to predict protein structure. *In vitro* GST pulldown assays using recombinant BipC were conducted to identify novel interacting partners from primary human macrophage lysate. Furthermore, a CRISPRi-mediated gene knockdown strain of the model organism *B. thailandensis* was utilized in macrophage infection assays, follows by whole cell proteomics profiling. Bioinformatics revealed limited structural resemblance and ~40% sequence similarity between BipC and homologs, lacking clearly defined conserved motifs. *In vitro* pulldown assays and affinity purifications demonstrated that BipC interacts with F-actin alongside a complex network of host proteins. Interactors are clustered in pathways of vesicle trafficking and phagosome organisation, mitochondrial bioenergetics, and organelle membrane association, notably including membrane contact sites (MSC). Differential label-free proteomics confirmed inducible *bipC* gene knockdown at the protein level. Infections of primary human with the *B. thailandensis bipC* knockdown strains revealed a significantly impaired ability of the bacterium to escape from the phagosome. These findings indicate that BipC is essential for phagosomal escape, likely functioning within specialized microdomains to directly hijack host cell membrane. Furthermore, proteomic profiling underscores that BipC operates within a highly regulated T3SS virulence network. The limited sequence similarity between BipC and its homologs, as well as its unique host interactome, suggests a functionally distinct role. BipC may function not only as a potential component of the translocon, but also as a multi-target virulence factor probably reprogramming host cell metabolic pathways to secure cytosolic survival.

WS02.05

Precision over power: Engineering targeted antimicrobial peptides against mycobacteria

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Tuberculosis, caused by *Mycobacterium tuberculosis*, is the deadliest disease in the world caused by a single infectious agent. In 2024, 10.7 million people were infected with tuberculosis and 1.23 million people died. The emergence of multidrug-resistant strains has become a major global health concern, highlighting the urgent need for novel therapeutic interventions. Antimicrobial peptides (AMPs) are promising candidates for host-directed therapy due to their ability to disrupt bacterial membranes and modulate immune responses. However, the lipid-rich and rigid cell envelope significantly limits the binding and general efficiency of AMPs against mycobacteria compared to other Gram-negative and Gram-positive bacteria.

To overcome this limitation, we are developing a targeted antimicrobial strategy that combines AMPs with a mycobacteria-specific targeting (MST) motif designed to direct AMPs to the mycobacterial cell envelope. MST motifs are derived from proteins known to interact with mycobacteria and are synthesized as peptide conjugates. Initial studies are performed using *Mycobacterium marinum*, a well-established model that shares key genomic and physiological features with *M. tuberculosis*. Candidate targeting motifs are identified and optimized using flow cytometry-based binding assays to quantify bacterial association, complemented by advanced fluorescence microscopy to assess localization to the mycobacterial envelope. Selected candidate MST motifs show direct binding to mycobacteria including *M. tuberculosis*, supporting the feasibility of this targeting approach. Moreover, a prototype MST-AMP reduced intracellular growth of *M. marinum* in a *Dictyostelium discoideum* infection model, providing initial proof-of-concept for increased antimycobacterial activity. Future work will focus on the systematic optimization of additional MST-AMP conjugates including MIC determination and cytotoxicity assays. In addition, we will investigate their potential to act in combination with established first-line anti-tuberculosis drugs to identify synergistic effects and evaluate their suitability as adjunctive therapies.

WS02.06
Survival of *Streptococcus anginosus* in blood is linked to hydrogen peroxide production

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Streptococcus anginosus is an opportunistic pathogen causing bacteremia, respiratory tract infections, abscesses, odontogenic infections and has recently been associated with gastric cancer. While numerous genes encoding putative virulence factors have been detected in whole genome sequences, a detailed molecular characterization is missing for most of them.

To address putative virulence determinants, we investigated hydrogen peroxide production by *S. anginosus* and assessed its functional significance for survival in human blood. In *Streptococcus pneumoniae* the pyruvate oxidase, encoded by the *spxB* gene, is responsible for H_2O_2 production and represents a major virulence factor. Interestingly, *S. anginosus* harbors two genetic variants of the *spxB* gene, one with high homology to the *S. pneumoniae spxB* (98%) gene, while the other appears to be specific for *S. anginosus*. Strains carrying the *S. pneumoniae* variant demonstrate higher H_2O_2 production (10-20 µM) compared to strains harboring the *anginosus* variant (<1 µM). Deletion of the respective *spxB* genes, leads to a complete abolishment of H_2O_2 production, for both variants and decreased survival rates in whole blood. Assessing H_2O_2 production in an S. *anginosus* strain carrying a deletion of the CcpA regulator revealed the control of *spxB* and H_2O_2 production by carbon catabolite repression.

In conclusion, we found evidence for the presence of a major *S. pneumoniae* virulence factor in *S. anginosus*, and we were able to demonstrate that it increases the ability of S. *anginosus* to survive in human blood.

WS03.01
Breaking out: The role of the osmiophilic bodies in driving malaria gametocyte egress

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Malaria is a major vector-borne disease caused by *Plasmodium* parasites, which replicate within human erythrocytes and must exit the host cell to ensure transmission to the mosquito vector. Upon ingestion by a feeding *Anopheles* mosquito, gametocytes rapidly respond to environmental cues, thereby initiating gametogenesis and host cell exit. Gametocyte egress follows a tightly coordinated "inside-out" mechanism involving sequential rupture of the parasitophorous vacuole membrane (PVM) and the red blood cell membrane (RBCM). This process is mediated by two specialized secretory vesicles, the osmiophilic bodies (OBs) and PPLP2-positive egress vesicles (P-EVs), which orchestrate membrane destabilization and parasite release.

Using proximity-labeling proteomics, we previously identified various novel OB proteins, including the putative secreted ookinete protein 12 (PSOP12) and exported protein 3 (EXP3). Here, we investigate the localization and function of these candidate proteins during gametocyte egress. Transgenic parasite lines were generated using overexpression, inducible knockdown, and CRISPR/Cas9-based genome editing approaches. Protein localization and dynamics were analyzed by immunoblotting and high-resolution fluorescence microscopy, while functional relevance was assessed during gametogenesis and parasite transmission.

Our data demonstrate that PSOP12 localizes to OBs of female gametocytes and redistributes to the parasite periphery upon activation, consistent with secretion during egress. Although PSOP12-deficient parasites exhibit impaired erythrocyte egress, parasite transmission to the mosquito remains unaffected, suggesting functional redundancy within the vesicle machinery. In parallel, EXP3 exhibits partial colocalization with OB markers, indicating a previously unrecognized association with gametocyte egress vesicles.

Together, our findings expand the molecular repertoire of gametocyte egress vesicles and provide new insights into the secretory pathways that enable malaria parasite transmission.

WS03.02

Role of the Type VI secretion system in bacterial–fungal interactions between *Serratia marcescens* and *Candida albicans* in the murine gastrointestinal tract

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Interactions between microorganisms in the gastrointestinal tract play an important role in shaping microbial community structure and host homeostasis. Besides cooperative effects, competition between microbes is a major driving force within these ecosystems. One mechanism involved in such interactions is the Type VI secretion system (T6SS), a contact-dependent secretion apparatus used by many Gram-negative bacteria to deliver effector proteins into neighbouring cells. While the T6SS is well known for mediating bacterial competition, much less is understood about a potential contribution to bacterial –fungal interactions within the intestinal environment.

To investigate potential T6SS-mediated interkingdom interactions, germ-free mice were colonized with *Serratia marcescens* strains carrying either functional or deficient T6SS systems, alone or in combination with *Candida albicans*, over a period of 28 days. Colonization dynamics were analyzed by CFU-based quantification and qPCR. In parallel, host responses were assessed by measuring a panel of 48 cytokines in spleen and colon homogenates as well as serum samples. Immunohistochemical staining was additionally used to investigate microbial localization within intestinal tissues.

All tested microbial strains established stable colonization of the murine gastrointestinal tract independent of T6SS activity. During co-colonization experiments, comparable bacterial and fungal burdens were detected across experimental groups, and no clear effect of T6SS activity on *C. albicans* colonization was observed for either the *S. marcescens* wild-type strain Db10 or the clinical isolate SJC1036. In line with these findings, cytokine profiling revealed no substantial alterations in colon, spleen, or serum samples, indicating that neither mono- nor co-colonization induced a pronounced systemic or local inflammatory response under the investigated conditions.

Additional samples from ongoing experiments are currently being analyzed to further explore potential context-dependent effects of T6SS activity during intestinal colonization. Together, the generated datasets provide a valuable basis for future studies addressing bacterial–fungal interactions in the gastrointestinal tract and microbial community dynamics *in vivo*.

WS03.03

cgMLST of *Candidozyma auris*: a core genome typing scheme for outbreak surveillance

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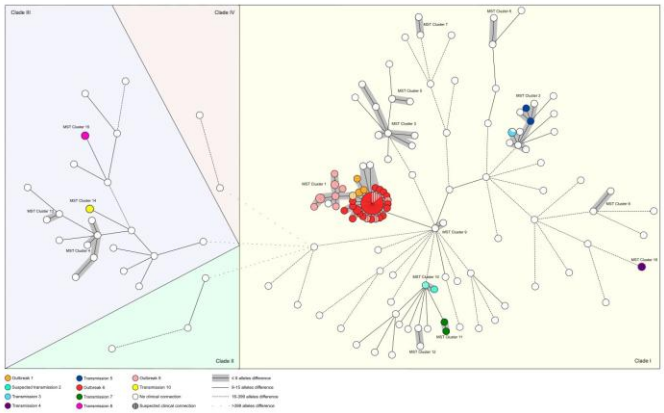
Background: *Candidozyma auris* is a WHO-critical-priority fungal pathogen linked to healthcare-associated outbreaks and multidrug resistance. Knowledge about non-outbreak-related spread remains limited. Current genotyping relies primarily on single nucleotide polymorphism (SNP) calling, which lacks standardization across labs and different institutions.

Methods: We analyzed 10years-epidemiological data from 258 German *C. auris* isolates - representing up to 91% of national cases - obtained from the National Reference Center for Invasive Fungal Infections (NRZMyk). A core genome multilocus sequence typing (cgMLST) scheme was developed and validated using 120 high-quality whole-genome sequences and the B11220 reference genome. Antifungal susceptibility testing was performed via Micronaut microdilution, with resistance confirmed by EUCAST reference methods.

Findings: The cgMLST scheme (4.231 target genes) demonstrated high reproducibility and comparability with SNP-based typing, validated using internal data and external outbreak datasets (n = 150 isolates from five outbreaks) and three non-outbreak-related clade-matched investigations. Analysis revealed importation of clades I–IV into Germany, with multiple clade I and four clade III transmission events, alongside recurrent clonal introductions from abroad. Follow-up isolates showed close genetic relatedness to index cases, but one patient harbored multiple genotypes. Resistance testing revealed high fluconazole resistance and emerging echinocandin resistance.

Interpretation: *C. auris* has been repeatedly imported into Germany, with limited sustained local transmission. A specific clade I genotype exhibits enhanced transmission potential and is responsible for all documented outbreaks. The novel cgMLST scheme enables high-resolution surveillance, supports cross-institutional data sharing, and identifies transmission-prone lineages and recurring import sources, which is critical for infection control and global monitoring.

Fig. 1



WS03.04
Drug susceptibility testing of axenic *Giardia duodenalis* isolates

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Introduction: Infections with the protozoan parasite *Giardia duodenalis* are widespread and typically lead to diarrhea and other gastrointestinal complaints. First line treatment with nitroimidazoles, such as metronidazole, is well established. However, anecdotal reports and monocentric studies suggest an increase in treatment refractory cases. These were particularly linked to returning travellers from South Asia. It is currently unknown whether treatment refractory cases are mechanistically linked to particular parasite genotypes or a defined tolerance mechanism.

Objectives: To assess the in vitro susceptibility of patient-derived axenic *G. duodenalis* isolates.

Materials and Methods: 20 selected axenic cultures of an in-house *G. duodenalis* collection were tested for their in vitro susceptibility against metronidazole. These included 11 assemblage AII and nine assemblage B genotypes and thus represented the two most widely found genotypes in humans. A standard bioluminescence viability assay has been used and IC50 values were calculated. Whole genome sequencing was performed to analyse sequence heterogeneity in genes predicted to be involved in metronidazole metabolism.

Results Preliminary: IC50 values of 20 clinical *G. duodenalis* isolates revealed for 19 isolates IC50 values between 2 and 10 µM metronidazole. One isolate showed a higher IC50 value of ~80 µM. The isolate belonged to *G. duodenalis* assemblage B and showed a high allelic sequence heterogeneity in its tetraploid genome. However, the case was not correlated with treatment failure. Whole genome sequencing and analysis of genes involved in metronidazole metabolic pathway revealed high sequence variation in nitroreductases, which are important for drug activation.

Conclusion: Our preliminary data suggests the existence of *G. duodenalis* isolates with higher intrinsic drug tolerance. We also observe high sequence variation in genes involved in metronidazole metabolism. Whether there is a mechanistic link between sequence variation of genes involved in metronidazole metabolism and drug susceptibility is currently under investigation.

WS03.05
The commensal functions of candidalysin

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The fungal pathobiont *Candida albicans* is both a commensal and an endogenous pathogen causing superficial and systemic infections under certain predisposing conditions. Key virulence attributes of *C. albicans* include the ability to transition from a yeast to a hyphal morphology and to secrete the hypha-associated peptide toxin candidalysin – the first peptide toxin discovered in a human pathogenic fungus - encoded by the gene *ECE1*. Recently it was shown that both hypha formation and the expression of *ECE1* play crucial roles in facilitating *C. albicans* gut colonization in the presence of a defined or undisturbed microbiota. In this study we dissect this proposed commensal function of candidalysin during gut colonization.

In vitro screening revealed that the growth of several potential co-colonizing bacteria is inhibited by candidalysin. To dissect the mechanism of candidalysin-mediated antibacterial activity, we focused on the model organism *Escherichia coli*. Using fluorescent labeled candidalysin, we showed that the toxin binds to the bacterial cell surface. Protoplastation experiments and treatment of synthetic bacterial-like membranes with candidalysin indicated that bacterial cell membranes are susceptible to candidalysin-mediated lysis. Screening of an *E. coli* deletion mutant library provided evidence that cell surface components, in particular lipopolysaccharide (LPS), are protective and prevent candidalysin-mediated bacterial killing. This view is in agreement with the transcriptional response of wildtype *E. coli* and an LPS-defective mutant strain upon candidalysin-treatment, indicating cell envelope stress. Furthermore, we propose that hypha-associated production of candidalysin in the gut environment can create a host-driven inflammatory and antibacterial environment, giving *C. albicans*

a competitive advantage during commensal colonization. In line with this proposal, we showed that the antibacterial activity of candidalysin is amplified through a synergistic effect with gut-associated antimicrobial peptides.

Our study provides mechanistic insights into the antibacterial activity of candidalysin and suggests that this multifunctional fungal factor likely evolved to improve fitness of *C. albicans* during gut colonization through inter-kingdom competition.

WS04.01
Characterization of the outer membrane protein HopZ in *Helicobacter pylori*

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Introduction: *Helicobacter pylori* is a Gram-negative pathogenic bacterium that localizes in the human stomach and causes severe infections such as ulcer disease or gastric cancer. Main virulence factors of *H. pylori* are the Cag type IV secretion system (CagT4SS) and outer membrane proteins (OMPs). *H. pylori* possesses a large family of about 30 OMPs. Some OMPs have been studied well, but not much is known about HopZ and its involvement in *H. pylori* infection. HopZ exists in two allelic variants and is regulated by a phase-variable dinucleotide repeat resulting in an ON or OFF status. *hopZ* exhibits high variability and within-host diversity. We recently discovered that HopZ interacts with proteins of the CagT4SS and the host receptors ceacam1 and integrin α5β1 and also plays a functional role in the context of the CagT4SS [1].

Aims. Our study aims to discover how HopZ is involved in host interaction, either alone or together with other virulence factors, and which function the switch and the two allelic variants have.

Methods and Results: In order to discover the function of HopZ during infection, we genetically engineered two *H. pylori* strains to switch the *hopZ* gene constitutively ON. During co-incubation with gastric epithelial cells, the constitutive ON mutants showed reduced induction of IL-8 production in infection assays and a reduced hummingbird phenotype in AGS cells, demonstrating a dampening function on the CagT4SS. Furthermore, we performed plate-binding assays with epithelial cells and purified proteins to investigate binding behavior of HopZ to proteins and cells under different conditions such as varying pH. HopZ binds to gastric epithelial cells in a concentration-dependent manner, with improved binding at pH 6 compared to pH 7. Despite our recent results that HopZ interacts with human ceacam1 and integrin α5β1 [1], our experiments suggest more binding partners. To identify them, we performed pulldowns of His-tagged HopZ with epithelial cell lysates and subsequent mass spectrometry. We are also performing expression and phenotyping assays in cells to identify novel functional factors in the context of HopZ. For this purpose, we are using a human ORFeome library to discover additional human interaction partners for HopZ.

Summary and Conclusion: In summary, HopZ exerts a reducing effect on host cell activation in different cell models. It interacts with various host receptors and Cag proteins. The mechanisms of HopZ activities and how different HopZ variants diverge in their functionalities are currently under investigation.

[1] Metz *et al*., 2025 Microlife

WS04.02
Structure function analysis of *Yersinia enterocolitica* YopQ

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Introduction: The type III secretion system (T3SS) effector YopQ/YopK plays an important role in macrophages infected with pathogenic *Yersinia* strains by regulating T3SS effector translocation and inhibition of T3SS-triggered inflammasome activation.

Goals: We aimed to characterise the structure-function relationship of YopQ/YopK and its N-terminus concerning its secretion, translocation, binding to the T3SS translocation pore and regulation of effector translocation into eukaryotic host cells. Further we aimed to decipher the influence of the N-terminal amino acid composition on secretion and translocation.

Materials and Methods: Small-angle X-ray scattering, X-ray crystallography and AlphaFold2 were employed for structural analysis. Truncated as well as mutated YopQ constructs, Western blot and SDS-Gel electrophoresis were employed for secretion and translocation assays (into target cells) and confocal immunofluorescence microscopy for localisation studies in host cells.

Results: X-ray and AlphaFold structures of *Y. enterocolitica* YopQ showed homology to cysteine proteases using Foldseek and revealed motifs that could represent the catalytic triad Cys-His-Asn/Asp characteristic for cysteine proteases. The N-terminal 25 amino acids of YopQ were disordered and sufficed for binding to the T3SS translocation pore within target cells, whereas the N-terminal 5 amino acids proved to be sufficient for T3SS-mediated secretion and translocation of heterologous proteins. Introducing single point mutations resulting in a less acidic N-terminal amino acid composition lead to drastic changes in secretion and translocation efficiency of YopQ. Finally, for regulation of T3SS-mediated translocation of Yop effector proteins into eukaryotic cells, the full length YopQ protein was essential.

Summary: Our study demonstrates that the disordered N-terminus of YopQ has multiple functions mediating secretion/translocation und localisation within target cells. Furthermore, it shows that secretion and translocation efficiency strongly depend on the N-terminal amino acid composition. The known activities of YopQ within host cells such as regulation of effector translocation depend on the complete protein and might be associated with cysteine protease activity.

WS04.03

Barrier dysfunction and immune activation in the colon mucosa during *Clostridioides difficile* infection

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Introduction: The antibiotic-associated *Clostridioides difficile* infection (CDI) is the most frequent cause of nosocomial diarrhea and colitis. Incidence rates and severity of CDI are increasing, which can be attributed to the emergence of hypervirulent strains that produce the binary toxin CDT as an additional toxin (to TcdA/B), which targets the tight junction by destabilizing the actin cytoskeleton.

Aim: The aim of this study was to elucidate mucosal impairment in hypervirulent CDI.

Material and Methods: The effects of three different isogenic mutant strains of *C. difficile* on the intestinal barrier and immune response were directly compared in a mouse model. Pre-treated ABx mice were infected orally with one of three different *C. difficile* strains: hypervirulent strain R20291 (TcdA/B+, CDT+); or virulent strain R20291ΔCDT (TcdA/B+, CDT-); or avirulent strain R20291ΔPaloc∆CDT (TcdA/B-, CDT-).

Results: Ussing chamber experiments revealed that virulent *C. difficile* induce a reduction in colonic transmural electrical resistance from 65 ± 3 Ω·cm² in avirulent infected control mice to 44 ± 2 Ω·cm² in virulent and to 22 ± 1 Ω·cm² in hypervirulent *C. difficile* infection. In impedance spectroscopy measurements, we determined that this was due to a reduction in epithelial resistance, while subepithelial resistances remained unchanged. Moreover, analysis of immune activation revealed a lesser activation in hypervirulent-infected mice compared to virulent-infected mice, although CDI symptoms were more severe in hypervirulent-infected mice. Interestingly, IL-22 was upregulated in virulent-infected mice but not in hypervirulent-infected mice. Furthermore, treatment of human PMBCs with *C. difficile* revealed the same pattern of IL-22-induction, indicating comparable mechanisms. These mechanisms were further analyzed on human epithelial cell monolayers to confirm the effects.

Conclusion: Hypervirulent *C. difficile* lead to epithelial damage with tight junction changes and to an altered immune response in the colon mucosa that differs from virulent *C. difficile* infection.

WS04.04

Macrophages require CD83 expression for the control of intestinal *Salmonella* Typhimurium infection

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Introduction: CD83, a member of the immunoglobulin receptor superfamily, modulates immune responses and inflammatory processes locally when expressed as membrane-bound isoform (mCD83) and at long distances when released as soluble isoform (sCD83). Lipopolysaccharid (LPS), a major cell wall component of Gram-negative bacteria, induces mCD83expression and sCD83 release by macrophages that contribute to the immune response against invading pathogens and the maintenance of tissue homeostasis. While CD83 has been associated with antigen uptake and presentation, as well as the resolution of inflammatory responses, its role in bacterial infections is less well understood.

Goals: Therefore, we investigated the influence of both CD83 isoforms on cell-specific immune responses against intestinal *Salmonella* Typhimurium infections.

Material and methods: We assessed the regulation of mCD83 expression and sCD83 release, the composition of intestinal microbiota, the intraluminal metabolome, *Salmonella* replication, and the severity of intestinal inflammation in mouse models of acute and chronic *Salmonella*-induced colitis using conditional CD83-knockout mice crossed to CD11c-Cre, Villin-Cre, and Cx3Cr1-Cre mice. We also treated these knockout strains and respective CD83-expressing littermate controls with sCD83.

Results: We observed that Cx3Cr1-Cre × CD83fl/fl mice, in particular, controlled *Salmonella* Typhimurium replication less efficiently and exhibited more severe colitis compared with CD83-expressing and Cx3Cr1-Cre littermates. Reduced neutrophil infiltration, enhanced TNF-alpha expression, and markedly reduced IL-10 induction accompanied this phenotype. Importantly, CD83-deficient macrophages exhibited a significantly decreased NOS2 and NADPH oxidase expression and diminished release of reactive nitrogen and oxygen species. The reduction of both genes likely accounted for the impaired antimicrobial activity of macrophages and the higher intracellular bacterial burden observed in the knockout setting. Gentamicin protection assays confirmed the defective elimination of intracellular bacteria by CD83-deficient macrophages *in vitro*. Vice versa, application of sCD83 to macrophages in gentamicin assays significantly reduced intracellular bacterial burden. Accordingly, therapeutic administration of sCD83 also reduced bacterial burden and suppressed inflammatory cytokine expression of infected mice *in vivo*.

Summary: Together, these findings identify mCD83 and sCD83 as crucial regulators of macrophage-mediated antibacterial immunity and as promising targets for therapeutic interventions urgently needed to combat *Salmonella* infections, which are increasingly resistant to commonly used frontline antibiotics. Furthermore, characterizing the molecular mechanisms following CD83-mediated macrophage activation during microbe-driven mucosal inflammation could improve understanding of the pathogenesis of inflammatory and infectious colitis.

WS04.05

DNA methylation influences *H. pylori* chromosome organization through the ParABS partitioning system

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Helicobacter pylori is a carcinogenic gastric pathogen with a large repertoire of restriction-modification systems, resulting in strain-specific sets of active methyltransferases and distinct methylomes. In bacteria, DNA methylation is involved in phage defense, transcriptional regulation, and other essential cellular processes. Still, the impact of methylation on chromosome organization remains largely unexplored. Therefore, we investigated the influence of methylation on the architecture of the *H. pylori* genome. The three-dimensional organization of the *H. pylori* chromosome was characterized using Hi-C, a sequencing-based method that maps physical interactions between genomic regions.

We found that *H. pylori* lacks known canonical SMC complexes but nevertheless achieves chromosome-arm alignment, as reflected by the secondary diagonal observed in Hi-C contact maps. In other species, chromosome arm alignment has been linked to SMC loading by the ParABS partitioning complex. Using purified proteins and *in vitro* binding assays, we show that ParB affinity for centromere-like *parS* sites is modulated by the number and methylation state of these sites. Consistently, variation in *parS* sites also influences the strength of secondary diagonals observed by Hi-C in distinct wild-type *H. pylori* strains. Together, these results define a novel methylation-dependent interaction within the ParABS system that supports SMC-independent chromosome-arm alignment and establish that methylation is essential for maintaining chromosome organization in *H. pylori*.

WS05.01

Performance of seven carbapenemase detection assays in Pseudomonas aeruginosa across different epidemiological settings: A multicenter cross-sectional study

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Background/Objective: Carbapenem resistance in *Pseudomonas aeruginosa* (PA) arises through multiple mechanisms, making reliable carbapenemase detection particularly challenging. While CLSI recommends the modified carbapenem inactivation method (mCIM) and Carba NP for phenotypic carbapenemase detection in PA, EUCAST currently endorses no specific method due to insufficient multicenter validation data. This study aimed to comparatively evaluate seven phenotypic carbapenemase detection assays across three epidemiological settings.

Methods: 320 PA isolates from three German centers with distinct carbapenemase-producing PA (CP-PA) prevalences (5.8–51.4%) underwent whole-genome sequencing as reference standard. Seven phenotypic assays were evaluated: modified carbapenem inactivation method (mCIM), modified zinc-supplemented CIM (mzCIM), simplified CIM (sCIM), Carba NP, imipenem–cloxacillin combined disk test (IC-4000), and two imipenem–EDTA disk assays (EDTA750 and EDTA930). Sensitivity, specificity, positive and negative predictive values (PPV/NPV), and the Youden index were calculated. For mzCIM, optimal cut-off thresholds were determined by ROC analysis, as no validated thresholds for PA existed prior to this study.

Results: Among 113 CP-PA isolates, metallo-β-lactamases predominated, with VIM-2 (n=58), NDM-1 (n=19), and GIM-1 (n=16) being most frequent. mzCIM and sCIM demonstrated the best overall balance of sensitivity and specificity (100%/94.2% and 99.1%/92.8%, respectively), followed by mCIM (93.8%/96.1%). Carba NP achieved the highest specificity (99.0%) but lowest sensitivity (85.8%). EDTA-based assays and IC-4000 were highly sensitive (96.5–100%) but less specific (79.7–87.0%). Importantly, false-positive IC-4000 results were strongly associated with larger baseline imipenem inhibition zones (AUC 0.893); application of a ≥14 mm imipenem zone cut-off improved IC-4000 specificity from 87.0% to 96.6%, albeit at the cost of potentially missing four true carbapenemase producers. NPVs remained consistently high across all assays (≥98–100%), while PPVs varied substantially across prevalence settings (72.5–98.0%), underscoring the dependency of positive result utility on local epidemiology. ROC analysis established a validated cut-off for mzCIM in PA (≤18 mm), with no overlap between CP-PA and nCP-PA above this threshold, and no indeterminate zone required.

Conclusion: mzCIM and sCIM demonstrated the most robust diagnostic performance across diverse epidemiological settings and represent the most suitable screening approach for carbapenemase detection in PA.

WS05.02

A new approach to an old problem: Specific detection of *Mycobacterium tuberculosis* using phage-derived receptor binding proteins

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Mycobacterium tuberculosis remains one of the most serious global threats to human health. The World Health Organization reported that approximately 10.8 million new tuberculosis cases were detected in 2023. Gold standard methods for diagnosis include the interferone-gamma release assay (IGRA), as well as nucleic acid amplification techniques (NAAT). Microbiological confirmation of cases is still required

but remains challenging due to the slow-growing state of *M. tuberculosis* (*Mtb*). There is a dire need for rapid and specific point-of-care (POC) tests capable of detecting the pathogen directly in patient samples. Such tests typically rely on antibodies to recognize bacterial antigens. However, antibody-based Mtb diagnostics have been largely unsuccessful, due to the bacterium's complex cell envelope immune evasion.

Phage receptor binding proteins (RBPs) represent a promising alternative to antibodies for directly detecting of *Mtb*. RBPs typically mediate the recognition of phages to their host bacteria and they have evolved to overcome bacterial surface barriers. Here, we utilize the specific binding of RBPs to develop novel tools for the detection of *Mtb*.

In silico structural analysis of phage proteins was used to identify RBPs with a high likelihood to bind to *Mtb*. These RBP candidates were then fused to a fluorescent reporter and heterologously expressed in *E. coli*. Purified proteins were subsequently used for binding analysis and diagnostic test development. Initial RBP analysis delivered 46 putative RBP candidates, most of which could be expressed as soluble recombinant fusion proteins with outstanding thermal stability. Initial screening using fluorescent microscopy-based analysis revealed binding of RBP-reporter fusions to under specific growth conditions of *Mtb* cells.

We show for the first time that recombinant phage RBPs can bind to *Mtb* cells, enabling rapid and specific pathogen detection. These results provide the basis for further applications including POC tests, e.g., later flow assays, or enrichment approaches for *Mtb* cells via coupling of RBPs to magnetic beads for use in complex clinical samples.

WS05.03

Automated discrimination of E. coli from Shigella spp. by Fourier-transform infrared spectroscopy (FTIR) and machine learning

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Question: Dependent of the chosen methods, differentiation of *E. coli* and *Shigella* spp. can be challenging for the diagnostic routine laboratory. Currently available methods either show insufficient discriminative power, or require time-consuming and labour-intensive workflows. Fourier-transform infrared spectroscopy (FTIR) was evaluated as a potential approach showing promising discriminatory potential for *E. coli* (Ec) and *Shigella* spp. isolates. In this study, a machine learning-based predictive model (classifier) was developed and tested for the automated FTIR-based discrimination of *E. coli* from *Shigella* spp.

Methods: 1005 well-characterized *E. coli* (830) and *Shigella* spp. (175) isolates, collected in different centres in Europe (Germany, France, Italy, the Netherlands) and Africa (Mali, Ghana) were included in this study. FTIR analysis was performed applying the IR Biotyper® system (IRBT - Bruker Daltonics, Germany). Spectra were acquired from overnight cultures on Columbia blood agar, TSA and TSA with 5% sheep blood. Machine learning was applied to create an automated classifier for the discrimination of *E. coli* from *Shigella* spp., using the algorithms included in the IRBT software. The training set included 228 isolates (23% of the total dataset, n=131 Ec and n=97 *Shigella* spp.), selected to cover biological, phenotypical, geographic and IR spectral variance. The remaining n=777 isolates were used as a testing set.

Results: The classifier built with the SVM (Radial Basis Function) algorithm showed the best performance, providing accuracy of 100% in a 4-fold cross-validation. Accuracy on the testing set was 100% for Ec (699/699) and 97.4% (76/78) for *Shigella* spp. The two observed errors involved n=1 *S. flexneri* svX and n=1 *S. dysenteriae* sv8. All *S. sonnei*, *S. boydii* and *S. dysenteriae* sv1 strains were correctly classified.

Conclusions: IR Biotyper proved to be a reliable method for the discrimination of *E. coli* and *Shigella* spp. The automated classifier provides an easy-to-use approach, leading to prompt results and allowing for a smooth implementation into routine workflows, after confirmation of its accuracy and robustness with extensive external validation.

WS05.04

Diagnostic performance of GenoType CMdirect for identification of *Mycobacterium tuberculosis* complex and non-tuberculous Mycobacteria from respiratory and non-respiratory clinical specimens

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Introduction: The *Mycobacterium tuberculosis* complex (MTBC) remains the leading cause of death from a single

infectious agent worldwide, while non-tuberculous mycobacteria (NTM) are increasingly recognized as clinically relevant pathogens in immunocompromised individuals. Rapid and accurate molecular diagnostics are essential for the timely detection and management of mycobacterial infections. In this study, we aimed to assess the diagnostic performance of a widely available molecular assay, the GenoType CMdirect VER 1.0 line-probe assay (CMD, Hain Lifesciences, Nehren, Germany, validated for sputum samples), for the identification of MTBC and NTM across various clinical specimens in a routine diagnostic setting.

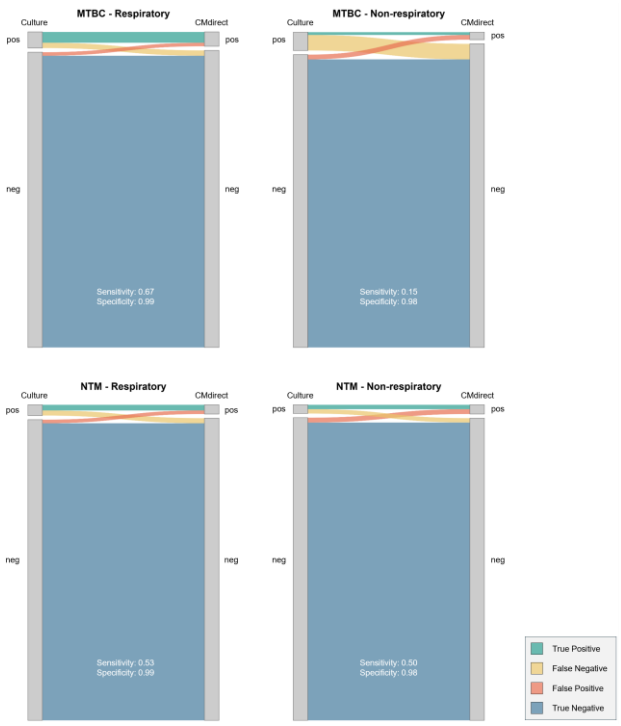
Methods: We conducted a retrospective diagnostic accuracy study including all clinical specimens with paired CMD and mycobacterial culture results collected between 2018 and 2025 at University Hospital Frankfurt, Germany. CMD was performed directly from clinical specimens as part of routine diagnostics. Mycobacterial culture (reference standard) was performed using both liquid and solid media. Data were extracted from the local laboratory information system, and diagnostic performance parameters were calculated.

Results: In this preliminary analysis, we included 1,775 clinical specimens with paired CMD and mycobacterial culture results, comprising 1,085 respiratory (sputum, bronchoalveolar lavage, bronchial secretions) and 690 non-respiratory specimens (e.g., biopsies, aspirates, cerebrospinal fluid). Overall, 148 (8%) cultures yielded mycobacterial growth, including 93 MTBC and 55 NTM isolates. Most culture-positive specimens were negative by Kinyoun and/or Auramin staining (87/130, 67%), indicating low mycobacterial burden. CMD showed a sensitivity (Sens) of 48% and specificity (Spec) of 99% for any mycobacterial species, with similar performance for MTBC (Sens: 45%, Spec: 99%) and NTM (Sens: 52%, Spec: 99%, Figure 1). Sensitivity was high in microscopy-positive specimens (98%) but markedly lower in microscopy-negative specimens (21%). Specificity remained >96% across all specimen types and species. However, sensitivity for MTBC was substantially higher in respiratory (67%) compared to non-respiratory specimens (15%), while sensitivity for NTM was comparable between respiratory and non-respiratory samples (53% vs. 50%).

Conclusion: CMD demonstrated moderate sensitivity and excellent specificity for the detection of both MTBC and NTM. However, sensitivity was poor for MTBC and NTM in microscopy-negative and for MTBC in non-respiratory specimens. The reduced sensitivity observed in non-respiratory specimens might be attributable to low MTBC burden in these sample types. While more sensitive molecular assays are established for MTBC detection, CMD may serve as an additional tool for NTM diagnosis, where rapid species identification is crucial for evaluating clinical relevance and guiding therapy.

Figure 1: Diagnostic performance of CMdirect

Fig. 1



WS05.05
Validation of microbiome-guided fluorescence in situ hybridization (MG-FISH) in clinical microbiology: Integrating next-generation sequencing with molecular microbiome imaging

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Question: Next-generation sequencing (NGS) is a powerful tool, which is broadly used for microbiome analysis. The implementation of NGS for diagnostic analysis of mixed bacterial infections is hampered by technical challenges, limited sensitivity, standardization issues, and risk of contamination with bacterial DNA-fragments, for example in reagents. Therefore, data might be difficult to interpret in the clinical setting. Whereas NGS allows the overall assessment of species involved, Fluorescence in situ hybridization (FISH) shows the abundance and localization of specific microbial populations in the tissue context. This way, key microbial species, which are directly associated with the host epithelial surfaces or are invasive within the host tissue, can be identified. However, targeted FISH probe selection requires prior knowledge of potential microbial candidates.

Methods: We developed MG-FISH (Microbiome-guided fluorescence in situ hybridization), which synergistically combines FISH with NGS, out of consecutive tissue sections. Consequently, microorganisms can be simultaneously identified, visualized and localized. We used an oral microbial biofilm sample for the analytical validation of intra- and inter-assay variability, sequencing depths, NGS sequencing methodology (Illumina or Nanopore sequencing) and overall

robustness of MG-FISH and different types of clinical samples for the clinical validation.

Results: In the oral biofilm sample used for the analytical validation of MG-FISH, we identified the nine most abundant species by NGS in different sample depths of the embedded sample. Using specific FISH probes for the respective target species, we visualized, quantified, and localized the microorganisms by FISH. The congruence of both NGS and FISH as well as intra- and inter-assay variability of MG-FISH were assessed. In clinical samples, NGS results were compared to culture results, if available, and FISH and Sanger sequencing results.

Conclusions: MG-FISH gives microbiome data a spatial dimension *in situ*: It allows quantification and distribution of the microorganisms involved, localization at and within the tissue, and identification of key pathogens. This will further the use of NGS for diagnostic applications.

WS06.01
Development of an AI-supported video-based hygiene training concept for parents in a perinatal center (Young talent)

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Introduction: Neonatal intensive care units are associated with a high risk of transmission events and outbreaks, making it essential to provide parents and relatives with hygiene-related knowledge to protect preterm and newborn infants. However, hygiene training in everyday clinical practice is often time- and resource-intensive, while parents themselves are frequently in a psychologically stressful situation.

Objectives: The aim of this project was the simple development and implementation of an AI-supported, resource-efficient, multilingual, and permanently accessible video-based hygiene training concept for parents in a perinatal center, focusing on the communication of basic hygiene and hand hygiene measures.

Materials and Methods: The video was developed as a combination of real-life sequences and AI-generated clips. The real-life recordings were produced during two filming days in the perinatal center, involving staff members from Department of Hospital Hygiene and Department of Strategic Innovation and Future Technologies. An iPhone 16 Pro and materials available on-site, such as hand disinfectant dispensers and milk bottles, were used for filming. The integration and blending of the real-life and AI-generated sequences were performed using a web application developed in-house by University Hospital Cologne. The AI generation was primarily based on the Gemini Veo 3.1 model. In addition, a fully AI-generated narration voice guides viewers through the individual sequences with explanatory commentary. The filming itself required approximately 3–4 hours on-site, while the overall conceptualization and implementation took approximately 20 hours.

Results: The development of the AI-supported video-based training concept enabled the establishment of a low-threshold, flexibly accessible, and resource-efficient educational tool for parents. The video is divided into seven chapters and can be used either as a complete training course or chapter by chapter, allowing targeted access to specific content. QR codes linking to the videos or individual video sequences are placed at hygiene-relevant locations on the ward. The videos are also available in multiple languages.

Production is comparatively cost-effective and requires only limited personnel resources. At the same time, the concept may help reduce the workload of clinical staff by providing standardized hygiene training. Additional advantages include multilingual accessibility and the ability to implement updates and modifications quickly and flexibly.

Conclusion: AI-supported video-based hygiene training modules can be developed cost-effectively by hygiene staff with manageable effort and offer a low-barrier, flexible, multilingual, and resource-efficient way to provide standardized instruction on hygiene measures for parents at the perinatal center.

WS06.02
"Ask Hilde." / "Frag Hilde." design of an on premise AI assistant as a low threshold first point of contact for hygiene related enquiries from clinical staff

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Introduction: Infection control teams receive a steady stream of recurring questions from ward staff on isolation, PPE and admission screening. Most answers are documented in institutional SOPs, KRINKO recommendations and RKI guidance [1,2,3]. Hygiene staff resources are limited, and timely bedside access to validated knowledge affects adherence to standard precautions and nosocomial infection rates [4]. Large language models could absorb part of this workload, but in German university hospitals cloud-based systems collide with data protection law, and unstructured retrieval produces hallucinations unacceptable in clinical use [5].

Aim: To develop the concept of an in-house AI assistant for hygiene enquiries from clinical staff, compliant with German data protection law and the EU AI Act [6] and operationally useful at the bedside.

Methods: HILDE (Hygiene Information and Learning Service Essen) is built around three principles. First, fully on-premise operation in a segregated hospital network without external APIs or cloud transfer, eliminating third country transfer risks under GDPR. Second, a structured knowledge base instead of full-text retrieval: rather than indexing PDFs, we maintain records with fixed fields (isolation, PPE, screening, reporting for pathogen-specific topics; indication, procedure, materials, frequency for general topics like hand hygiene or instrument reprocessing), each with source citation and version. This improves precision, enables granular approval, and reduces hallucination risk [5]. Third, a curated approval process: each response category is reviewed by a hygiene specialist before activation and documented with name, date and version.

Unclear, novel or outbreak-related queries are automatically escalated to the hygiene department. This meets EU AI Act requirements for human oversight and documentation in high-risk applications [6].

Concept: Implementation follows three stages. Stage I provides FAQ for all staff, Stage II adds ticket-based escalation, Stage III integrates internal surveillance data restricted to the hygiene team. A pilot is planned and will benchmark HILDE against a conventional cloud-based LLM to assess clinical accuracy and the added value of the curated knowledge base.

Conclusions: Generative AI can be applied to hospital hygiene under German regulatory conditions if architecture, content governance and oversight are aligned from the start. HILDE supports low-threshold knowledge transfer at the bedside and adherence to standard precautions, without questioning the hygiene department's professional responsibility. The concept is transferable; pilot results are expected next year.

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WS06.03

Targeted identification of nosocomial urinary tract infections using hospital routine data and machine learning: A retrospective point prevalence study in a German hospital

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Background: Despite high European standards, approximately 6% of hospitalised patients are affected by healthcare-associated infections (HAIs). Urinary tract infections (UTIs) account for 30% to 40% of all documented cases. Nevertheless, our retrospective surveillance indicates that strict diagnostic criteria (KISS) are often not met prior to the initiation of treatment. Such overdiagnosis artificially inflates hospital infection rates and places an additional burden on the infection prevention and control (IPC) teams responsible for HAI surveillance.

Aims: To alleviate these effects, we propose using a machine learning (ML) model to automate the detection of UTIs. The ML model is designed to distinguish between hospital- and community-acquired UTIs. This would mean that IPC teams would only need to manually evaluate a small number of cases with a high probability of being positive each day, while also enhancing the availability and quality of the infection surveillance data.

Methods: An ML model based on a Random Forest algorithm was trained using a non-random patient cohort (n = 326) with manually documented UTIs, differentiated as hospital-acquired (n = 154) or community-acquired (n = 169) by the IPC team. This model included 30 variables derived exclusively from anonymised hospital routine data. Following internal validation, the algorithm was applied to a hospital-wide, point-prevalence-like (PPL) cohort consisting of all patients hospitalised between 1 and 8 April from 2017 to 2025 (n = 8,353).

Results: Of the 30 variables considered, five – the number of procedures, ICU length of stay, total length of stay, ICU admission and DRG Major Diagnostic Category B (nervous system diseases) – were found to be the most predictive of hospital-acquired UTIs based on their SHapley Additive exPlanation (SHAP) values. The ML model predicted that 157 out of 326 UTIs were hospital-acquired and 169 were community-acquired (sensitivity: 84.9%; specificity: 85.1%; positive predictive value: 86.4%; negative predictive value: 83.4%; accuracy: 85%). In the PPI cohort, the model predicted that the neurosurgery (n = 959) and nephrology (n = 680) departments would have the most UTIs. However, there were proportional differences for hospital-acquired UTIs: 76% and 16%, respectively. The initial results of the manual evaluation of 133 high-probability neurosurgery cases were promising, with a hit rate for true hospital-acquired UTIs exceeding 60%.

Conclusion: Using an ML model on routine hospital data revealed five variables that greatly impact the differentiation between hospital- and community-acquired UTIs, with acceptable accuracy. However, further optimisation of the ML model is required to improve sensitivity. Detection based solely on administrative hospital data would be a significant advance in HAI surveillance, as the system could easily be implemented in any German hospital.

WS06.04

Integrating unsupervised and supervised machine learning to identify predictors of the length of hospital stay (LOS) in SARS-CoV-2-positive patients upon admission: A retrospective monocentric cohort study

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Question: With the emergence of the Omicron variant and widespread vaccination, hospitalizations primarily due to COVID-19 declined despite high infection rates, increasing the need to distinguish between patients admitted for COVID-19 and those with incidental SARS-CoV-2 positivity in terms of effective resource use. We therefore combined unsupervised and supervised machine learning to identify predictors of

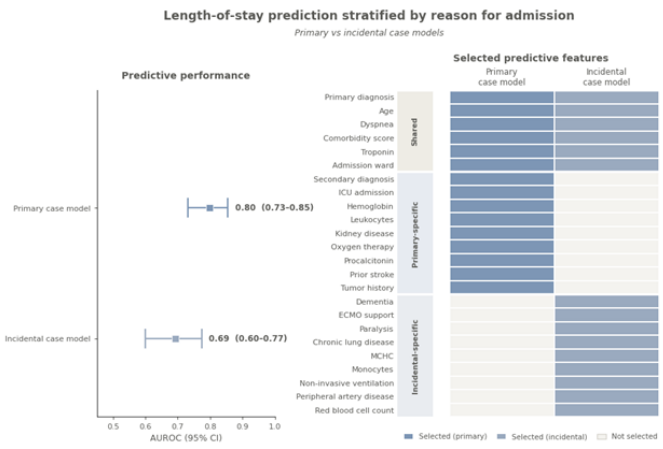
length of stay (LOS) in SARS-CoV-2-positive patients at admission and to assess the prediction performance and key predictors, with the aim of improving LOS estimation for administrative decision-making.

Methods: We analyzed hospitalized patients with a positive SARS-CoV-2 PCR at admission using 53 variables available. Patients were stratified into those admitted for COVID-19 (primary cases) and those with incidental SARS-CoV-2 positivity. LOS was converted into a binary outcome using K-means clustering on log-transformed LOS. We then combined three feature selection methods (mutual information, LASSO logistic regression, and recursive feature elimination) with three classifiers (logistic regression, random forest, and XGBoost) to identify the best-performing model. The area under the receiver operating characteristic (AUROC) was computed as a measure of a model's discriminative ability for each subpopulation.

Results: Of 345 patients, 194 (56%) were primary cases. K-means separated the cohort into short-stay (mean 2.9 days, n=187) and long-stay (mean 16.8 days, n=158) groups. When analysing the whole cohort, the LASSO-selected random forest achieved AUROC 0.76 with an accuracy of 0.71 using 22 features. Stratified analysis showed better prediction in primary than incidental cases (best AUROC 0.80 vs 0.70; accuracy 0.73 vs 0.66), each with 15 features. Six features were common to both groups (age, primary diagnosis, dyspnea, comorbidity score, troponin, admission ward); the remaining nine differed (Fig. 1). The additional primary-case predictors included ICU admission, need for oxygen, procalcitonin, tumor history, and kidney disease. In contrast, the additional incidental-case predictors included dementia, paralysis, chronic lung disease, peripheral artery disease, and ECMO therapy.

Conclusion: We demonstrate that LOS prediction can be operationalized at admission using routinely available data, with better performance for primary cases. The two groups were driven by largely non-overlapping predictors, and prediction accuracy differed by 0.10 AUROC, suggesting that separate models may be more appropriate than a pooled approach. This has practical value for early bed planning, resource allocation, and prioritization of patients at higher risk for prolonged hospitalization. Our framework is adaptable to other clinical settings with primary and incidental presentations, such as colonization versus infection in patients with multidrug-resistant organisms or influenza. However, prospective multicenter validation will be important to assess generalizability.

Fig. 1



WS07.01

Neisseria meningitidis induces the release of brain endothelial microvesicles that impair blood-brain barrier integrity

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Introduction: *Neisseria meningitidis* (*Nm*) is a human pathogen that penetrates brain endothelial cells (BECs) of the blood-brain barrier (BBB) and causes meningitis. Extracellular vesicles (EVs) have emerged as key mediators of communication at brain barriers, where they modulate barrier properties and transfer bioactive cargo. Recent studies have highlighted that *Nm* modulates sphingolipid (SL) metabolism in BECs, disrupting the balance of lipid species to promote cellular invasion. SLs, including ceramides (Cer) and dihydroceramides (dhCer), are critical for cell signaling, inflammation, and membrane stability. We observed that *Nm* infection significantly increased cellular and extracellular dhCer levels, suggesting that altered dhCer distribution within BECs is linked to EV release and contributes to BBB dysfunction.

Objectives: We aimed to identify the mechanisms underlying infection-induced dhCer accumulation and its intracellular redirection. Furthermore, we investigated how dhCer is released from infected BECs and affect BBB integrity.

Materials and Methods: BECs were infected with *Nm* and the activity of dhCer desaturase 1 (DEGS1), which converts dhCer to Cer, was measured in a substrate-conversion assay. To explore changes in subcellular SL distribution and transport, we combined confocal microscopy with LC-MS/MS to track SLs across cellular compartments. To characterize extracellular dhCer release, exosomes and microvesicles were isolated from infected and uninfected cells, and their SL profiles were analyzed by LC-MS/MS. The impact of microvesicles on barrier integrity in recipient BECs was measured by electric cell-substrate impedance sensing (ECIS).

Results: *Nm* infection significantly reduced DEGS1 activity, resulting in increased dhCer levels. Confocal microscopy with clickable SL analogs showed that infection redirected these lipids from the ER via the Golgi to the plasma membrane, which was corroborated by LC–MS/MS analysis of subcellular fractions. Notably, dhCer levels were increased in microvesicles derived from infected cells, whereas their levels in exosomes decreased. Treatment of recipient cells with microvesicles from infected cells caused a significant drop in endothelial resistance after approximately 20 h in ECIS measurements, indicating barrier disruption, whereas microvesicles from uninfected cells had no detectable effect.

Summary: Our findings indicate that dhCer increase in BECs after *Nm* infection is mainly caused by post-translational inhibition of DEGS1. We demonstrate that dhCer is transported from ER to the plasma membrane via the Golgi apparatus and subsequently released into the extracellular environment through microvesicles. Microvesicles derived from infected cells impair barrier integrity in recipient BECs, supporting the concept of an EV-mediated mechanism by which *Nm*-induced alterations in SL metabolism contribute to BBB dysfunction.

WS07.02

VREfm ST80 and ST117: Multicentre evidence for growth inhibition and enhanced translocation

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Background: Vancomycin-resistant *Enterococcus faecium* (VREfm) is a common cause of nosocomial infections. Certain subtypes of VREfm (Multilocus Sequence Typing Sequence Types, MLST ST), such as ST80 and ST117, are increasingly being detected in bloodstream infection isolates in Germany. The reasons for this rising prevalence of this high-prevalence VREfm are largely unknown. We tested two hypotheses *in vitro*: (i) VREfm have different abilities to suppress each other, resulting in increased translocation due to a quantitative overload; and (ii) certain VREfm harbour a higher translocation potential in a cellular (leaky) gut model *per se*, a potential that can be further increased by bacterial membrane vesicles (MV).

Methods: In total, 68 multicentre VREfm strains comprising 31 ST were used. Spot-on-lawn assays were conducted to investigate the phenotypic inhibition potential of the selected strains. To analyse the translocation potential, a transwell model using Caco-2 and Caco-2 *Dsg-2-/-* ("leaky gut") cells was employed. Cell barrier integrity was confirmed by measuring the transepithelial electrical resistance (TEER). The translocation potential of the bacteria and their MV was determined 6 hours post-infection. The number of translocated bacteria and MV was quantified using cfu/mL and nanoparticle tracking analysis (NTA), respectively. MV were isolated via ultracentrifugation of culture supernatants using a density gradient.

Results: In the spot-on-lawn assay, the inhibition rate was significantly higher for the high-prevalence VREfm ST80 and ST117 isolates than for the low-prevalence VREfm isolates when spotted (29 % vs. 15 %, $p<0.001$). All ST80 isolates inhibited one ST117 isolate (ST117 CT929). VREfm bacterial strains translocated less than susceptible strains on Caco-2 cells ($p=0.02$); this trend was not evident for MV ($p=0.5$). On Caco-2 *Dsg-2-/-* cells, which imitate reduced barrier integrity, higher translocation of VREfm MV was observed ($p=0.1$), with the highest values for ST80. Furthermore, MV significantly reduced TEER values by 10 % for ST80 and ST117 ($p<0.001$).

Conclusion: High-prevalence VREfm inhibits the growth of low-prevalence VREfm, which may result in quantitative overload and facilitate translocation. Due to their MV, high-prevalence VREfm translocate more easily through a damaged intestinal epithelial cell barrier *in vitro*, thereby further disrupting its integrity.

WS07.03

***Yersinia enterocolitica* YopP is phosphorylated by host kinases to modulate its activity**

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Introduction: The major virulence system of pathogenic *Yersinia* spp. is a type III secretion system that mediates the translocation of effector proteins (Yersinia outer proteins; Yops) into infected cells. These Yops interact with host proteins and exhibit manifold functions in modulating host cell immune responses. YopP is an acetyltransferase, which, via acetylation of NF-kB and MAPK pathway-related kinases, disrupts central innate immunity pathways within the host cell. IP6 is a crucial co-factor for YopP to exhibit its acetylation acitivity. Phosphorylations are common post-translational modifications, which may alter the function and activity of the modified protein. Our results show that YopP is phosphorylated at the N-terminus by host kinases and this may alters its activity.

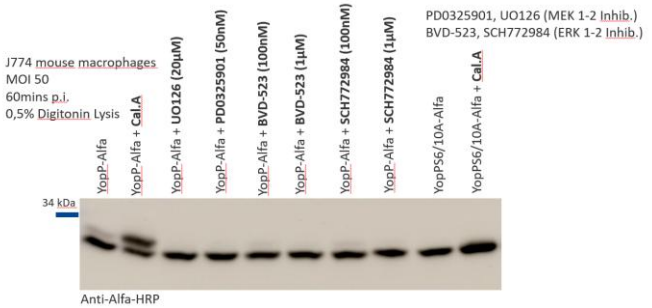
Materials and Methods: A fusion construct combining an ALFA-tag with YopP was generated and introduced into a *Yersinia* knockout lineage. J774 macrophages were infected, and cell lysates were analyzed by immunoblotting. ALFA-tagged YopP was immunoprecipitated and subsequently analyzed by mass spectrometry to identify phosphorylation sites. To assess the relevance of YopP phosphorylation during host cell infection, point mutations were introduced at the identified sites and investigated during infection. Recombinant YopP WT and mutant proteins were purified. Subsequently binding affinity and kinetics of IP6 were examined using a tryptophan fluorescence assay.

Results: Infection of J774 macrophages with *Yersinia*, followed by immunoblot analysis of cell lysates, revealed additional YopP bands with reduced electrophoretic mobility. Stabilization of these bands using the phosphatase inhibitor Calyculin A enabled subsequent MS analysis, which identified serine 6 and serine 10 of YopP as phosphorylation sites targeted by host cell kinases. To assess the functional

relevance of these modifications, phosphomimetic (S6/10D) and phospho-nil (S6/10A) mutants were generated, both of which displayed the expected shifts in electrophoretic mobility. Subsequently, ERK1/2 and GSK3β could be identified as the responsible host kinases. Finally, using recombinantly expressed YopP WT and mutants in a tryptophan assay the binding affinity and kinetics of IP6 to YopP was investigated, suggesting a slower kinetic in the S6/10D mutant.

Conclusion: Our results show that *Y. enterocolitica* YopP is phosphorylated at S6 and S10 by host cell kinases. Infection assays hint towards a lower acetylation acitivity of phosphorylated YopP. This might be due to an occlusion of the IP6 binding pocket by the phosphorylated N-terminus as our experiments suggest. Ultimately, this mechanism could exhibit a negative feedback loop between YopP and one of its targets, the MAPK-pathway, resulting in a specific downregulation of the activity of YopP upon *Yersinia* infection.

Fig. 1



WS07.04

Manipulation of VAP-mediated membrane contacts by pathogenic mycobacteria

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Tuberculosis, caused by *Mycobacterium tuberculosis*, remains one of the leading causes of death from bacterial infection worldwide. To establish a permissive intracellular niche, pathogenic mycobacteria manipulate host cell pathways to evade degradation and gain access to nutrients. Increasing evidence suggests that this involves membrane contact sites (MCS), specialized membrane interfaces that facilitate lipid transfer, membrane remodelling and signalling. MCS are stabilized by tether proteins such as the ER-resident VAMP-associated proteins (VAPs), which recruit lipid transfer proteins and additional effector proteins. Several intracellular pathogens, including *Chlamydia trachomatis* and *Rickettsia parkeri*, exploit the MCS machinery during infection. However, the role of MCS during mycobacterial infection is still poorly understood.

Using the *Dictyostelium discoideum*/*Mycobacterium marinum* infection model, we investigate the role of the host MCS machinery by combining fluorescence microscopy-based localization analyses with correlative and ultrastructural imaging, lipidomics, and intracellular growth assays. MCSs are emerging as important regulators of membrane repair, autophagy-related defence pathways and intracellular lipid trafficking, suggesting that they control access of mycobacteria to host-derived nutrients. Consistently, we detected GFP-VAP at MCV-associated membranes and in close proximity to cytosolic bacteria, indicating the formation of VAP-positive ER-contact sites at pathogen-associated membranes. In addition, VAP-deficient cells showed altered lipid profiles and reduced intracellular bacterial growth. These observations support a role for VAP-dependent MCSs in coordinating lipid homeostasis and host-pathogen membrane interactions during mycobacterial infection.

WS07.05

Not all toxins are created equal: Deciphering the strain- and host-specific cytotoxicity of E. coli *hlyA* variants

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Introduction: Alpha-hemolysin genes can vary in sequence, as confirmed by the 19 *hlyA* variants identified among 78 hemolytic *E. coli* isolates in our previously published study (Kolenda et al., 2021).

Objectives: An important step in colonization and infection is adhesion to host cell surfaces, which may enhance the cytotoxicity of alpha-hemolysin by increasing localized toxin concentrations at the cell membranes. It is hypothesized that the pathogenic effects of alpha-hemolysin depend not only on specific *hlyA* gene variants but also significantly influenced by other bacterial adhesins variants.

Methods: The cytotoxicity of all 78 strains was evaluated against various cell line (LoVo, 5637, and CaCo-2). Variants exhibiting the most and least exfoliation, as well as high prevalence and host-cell specificity, were selected to generate *hlyA* deletion mutants. Furthermore, specific variants of three primary adhesins (*fimH*, *csgA*, and *ecpD*) were selected to determine their role in hemolytic capability. All mutants will be complemented with their respective genes via reintroduction using pBAD33 or pACYC177 vectors. Additionally, *hlyCABD* operon expression is being analyzed by qPCR at multiple time points during the exfoliation of 5637 and LoVo cells.

Results: Cytotoxicity or exfoliation of *hlyA* variants is governed by complex interactions between specific variants, isolate"s genetic background, and the host cell sensitivity. Certain "generalist variants (like 4, 8, 9 and 12) are overwhelmingly

exfoliating across all cell lines irrespective of the strain encoding them. In contrast, other variants (such as 3,6 & 7) exhibit strain to strain exfoliating variability relying on the supportive virulence factors specific to each genetic background. Additionally, CaCo-2 are most vulnerable, and 5637 cells showed overall resilience, proving hlyA toxicity is host cell dependent. Highly cytotoxic *hlyA* variant 1 has been successfully deleted in two different isolates and both lost the ability to exfoliate cells as well as failed to induce hemolysis on the blood agar. Further generation of deletion mutants of both *hlyA* and adhesins is ongoing. Time based expression analysis has also showed differential expression of the operon of various variants against diverse host cells.

Summary: Hemolytic *E. coli* carry a broad variety of hemolysin gene variants along with diverse adhesin variants. The current data indicate that different *hlyA* variants have varying cytotoxic effects and the role of alpha hemolysin has been quantified in both exfoliation as well as hemolysis. Ongoing *hlyA* and adhesin deletion experiments will provide a comprehensive understanding of how specific variants and their interactions dictate pathogenesis.

Reference:

Kolenda, R. et. Al., 2021. Genome placement of alpha-hemolysin defines virulence-associated factor profile of *Escherichia coli*. Microbial Genomics 7, 000743. https://doi.org/10.1099/mgen.0.000743

Fig. 1

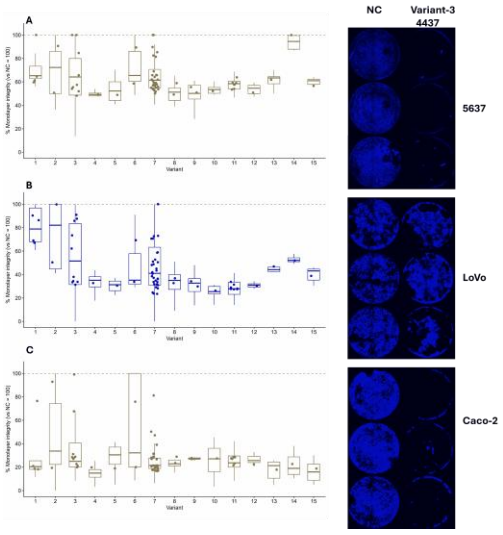
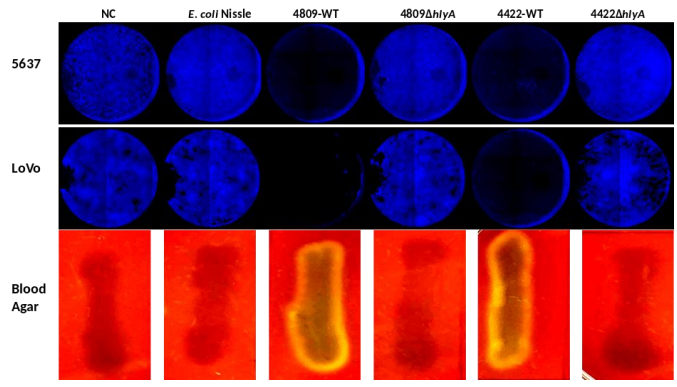


Fig. 2



WS07.06

Membrane lipid remodeling drives the non-lytic cellular exit of *Orientia tsutsugamushi*

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Objective: Intracellular bacteria establish a replicative niche within host cells but must exit to disseminate. *Orientia tsutsugamushi* (OT) employs a unique non-lytic cellular exit mechanism by budding from the plasma membrane. This study aimed to quantitatively assess budding efficiency of OT across human and murine host cell systems, using newly established qPCR and high-resolution microscopy approaches. Building on this, the roles of exosomal and membrane lipid remodeling pathways in OT exit were investigated via omics and inhibitor screening.

Methods: Human and murine cell lines were infected with OT at low and high multiplicities of infection (MOI). Intra- and extracellular bacterial loads at 3 to 9 days post-infection (dpi) were quantified by qPCR, and budding was visualized by transmission electron microscopy (TEM), with release events quantified per cell perimeter at 5 dpi. Small-molecule inhibitors were added at 6 dpi for 24 h, followed by qPCR: GW4869 (neutral sphingomyelinase [nSMase] inhibitor), U18666A (cholesterol transport inhibitor), Y-27632 (ROCK kinase inhibitor), and methyl-β-cyclodextrin (MβCD, cholesterol-depleting agent). Transcriptional and proteomic changes at 1 and 7 dpi were analyzed by RNA sequencing and LC–mass spectrometry, respectively.

Results: OT release efficiency varied across cell types, being most strongly induced at 5 dpi (high MOI) and 7 dpi (low MOI) in human MRC5 fibroblasts, while only a baseline release could be detected, e.g. in human HuH7 hepatocytes; thus, subsequent mechanistic analyses focused on 5–7 dpi in MRC5 cells.

Transcriptomics revealed OT-induced differential gene expression of >4,500 genes. Specifically, transcripts associated with ceramide-dependent membrane remodeling (e.g. *sphingosine kinase 1*, sphingolipid metabolism regulator), lipid homeostasis (e.g. *ABCA1,* cholesterol transport) and exocytic vesiculation (e.g. *RAB27B*, exosome secretion mediator) were significantly enriched at 7 dpi vs. 1 dpi. Proteomics at 7 dpi confirmed enrichment of ceramide pathway components, including nSMase and proteins associated with cholesterol-metabolism. In line with this, GW4869 and MβCD treatment reduced extracellular bacterial loads while increasing intracellular levels, indicating intracellular retention. TEM revealed distinct localization patterns: MβCD trapped bacteria in the cytosol, whereas GW4869 treatment resulted in accumulation of OT in tight association with the plasma membrane. These findings suggest that cholesterol depletion affects transport of OT to the membrane, whereas nSMase-generated ceramides contribute to membrane remodeling during the scission process. We thus identify nSMase as a novel host factor required for OT egress.

Summary: Efficient OT release in MRC5 cells involves both cholesterol metabolism and ceramide-dependent membrane

remodeling. The dependence on nSMase suggests that cellular exit of OT involves components of the exosomal vesiculation machinery.

WS08.01

Regulation of IFN-induced immune responses in Listeria infected cells by SUMOylation and ubiquitination

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Listeria monocytogenes (Lm) is a foodborne facultative intracellular pathogen that causes severe disease, particularly in immunocompromised individuals. Macrophages play a central role in the host's defence mechanisms by recognising extracellular and intracellular Lm via pattern recognition receptors (PRRs). Engagement of PRRs activates pro-inflammatory signalling cascades, including NF-κB, MAPK and interferon-dependent pathways, thereby inducing antimicrobial effector mechanisms. Pro-inflammatory signalling during listeriosis is tightly regulated by post-translational modifications (PTMs), such as phosphorylation and ubiquitination. However, the contribution of SUMOylation, another PTM, remains poorly understood. SUMOylation regulates various cellular processes, including DNA damage repair, chromatin remodelling and signal transduction. The promyelocytic leukaemia protein (PML) acts as a key scaffold for SUMOylation by forming PML nuclear bodies (PML-NBs).

We therefore hypothesised that PML-mediated SUMOylation might enhance intracellular Lm control. To address this, we generated myeloid cell-specific PML knockout mice (LysM-Cre PMLfl/fl). Upon Lm infection, PML-deficient bone marrow-derived macrophages exhibited reduced NF-κB activation, diminished anti-bacterial nitric oxide (NO) and reactive oxygen species (ROS) production, and impaired autophagy. This resulted in defective intracellular Lm control. However, IFN-γ, but not TNF or IL-6, restored bacterial clearance, indicating an IFN-γ dependent compensatory mechanism. Co-immunoprecipitation analyses revealed an interaction between PML and p65, supporting a role for PML in modulating NF-κB signalling. The translational relevance of this finding was confirmed in primary human macrophages using CRISPR/Cas9-mediated PML knockout; in these macrophages, we also observed reduced Lm control. Furthermore, IFN-γ receptor-deficient human induced pluripotent stem cell (iPSC)-derived macrophages (iMACs) displayed impaired intracellular Lm control and markedly reduced basal and infection-induced PML expression. Based on these findings, we hypothesise that, in iMACs, PML acts as a critical downstream effector of IFN-γ signalling in macrophage-mediated Lm control.

Ongoing studies employing mass spectrometry, nuclear–cytosolic fractionation, and PML knockout in IFNGR-deficient iMACs aim to elucidate the molecular mechanisms underlying this regulatory axis.

WS08.02

Transferring immunity: Intercellular communication in innate fungal immunity

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Histoplasma capsulatum (Hc) is a fungal pathogen causing pneumonia up to systemic mycoses in immunocompromised patients. Within the lungs of the host, Hc resides in alveolar macrophages, which form hypoxic granulomas. Herein, macrophages stabilize hypoxia-inducible factor-1α (HIF-1α). Previous data revealed, exaggerated HIF-1α abolished Hc-induced host autophagy and mitochondrial respiration, resulting in pathogen death. The mitochondrial metabolites succinate and fumarate were shown to be linked to pro-inflammatory priming of macrophages via HIF-1α. Beyond its intracellular effects, HIF-1α impacts intercellular communication by regulating the biogenesis and cargo of extracellular vesicles (EV). These EV promote anticandidal activity in recipient macrophages. Therefore, we hypothesized, HIF-1α alters the content of EV, thereby modulating the fungicidal ability of neighboring infected MΦ.

Human monocyte-derived macrophages (MΦ) were infected w/o chemical HIF-1α-stabilization (IOX2), diethyl fumarate (DEF) or dimethyl succinate (DMS) for 3 h. Further, EV from Hc-infected MΦ (MHc-EV) or Hc-infected MΦ treated with IOX2 (IOX2-EV) were isolated by ultracentrifugation 3 h post infection. Subsequently, infected MΦ were treated with MHc-EV or IOX2-EV for 3 h. Protein amounts of HIF-1α and LC3-II were assessed using Western blot. Expression of TNF-α, IL-1β, IL-10 and GLUT1 was analyzed by qRT-PCR. Metabolites fumarate and succinate were analyzed through LC-MS.

In infected MΦ, HIF-1α, succinate and fumarate were enhanced compared to uninfected controls ($p< 0.05$, n=4; $p< 0.05$, n=4; $p<0.0001$, n=3). Treatment of infected MΦ with DMS or DEF reduced HIF-1α protein stabilization ($p<0.05$, n=4; $p<0.01$, n=3). HIF-1α protein could be further elevated by chemical stabilizer IOX2 ($p<0.05$, n=4). Overstabilization of HIF-1α promoted accumulation of fumarate but not succinate ($p<0.0001$, n=3). Herein, expression of GLUT1, IL-1β and IL-10 was increased, while LC3-II protein was reduced ($p< 0.05$; $p< 0.05$; $p<0.01$; $p<0.05$, n=3).

infected MΦ treated with IOX2-EV, HIF-1α and IL-1β were enhanced, while LC3-II was diminished compared to infected MΦ treated with MHc-EV ($p<0.01$, n=4; $p<0.05$, n=3; $p<0.01$, n=4). Both, IOX2-EV and MHc-EV, enhanced GLUT1 ($p<0.05$; $p<0.05$, n=3) and TNF-α ($p<0.05$; $p<0.05$, n=3) in infected MΦ compared to infected controls.

Exaggerated HIF-1α decreased fungal survival by 69.41 ± 15.24% compared to infected controls ($p<0.01$, n=4). Infected MΦ treated with MHc-EV further decreased killing compared to IOX2-treated, infected MΦ by 55 +/- 10% ($p<0.05$). Furthermore, IOX2-EV decreased survival of Hc by 38 +/- 8% in infected MΦ compared to MHc-EV treated infected MΦ ($p<0.05$, n=3).

Our study revealed, EVs from infected MΦ produced a fungicidal state in recipient MΦ which was further boosted by HIF-dependent immunometabolic priming of the donor cells. In future, characterization of the content of EV and the signaling pathways in recipient MΦ will be further explored.

WS08.03

Intracellular *Staphylococcus aureus* induces isolate-specific host transcriptional responses alongside cell type-dependent TNF-R1-regulated cell fate decisions

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Background: *Staphylococcus aureus* (*S. aureus*) is increasingly recognized as a facultative intracellular pathogen, but infection outcomes vary depending on bacterial isolate and host cell type. Following uptake, *S. aureus* interferes with phagolysosomal trafficking, stress responses, inflammatory signaling, and regulated cell death or survival. The Tumor Necrosis Factor alpha (TNF) signaling axis is central to this balance, coordinating antimicrobial defense and inflammation, while excessive activation can promote host cell death and tissue damage.

Aims: To determine how intracellular *S. aureus* isolates from different clinical backgrounds modulate host signaling programs, cell death and TNF-R1-dependent regulation of infection outcomes in a human monocytic cell line. Infection-induced cell fates are compared to those of non-professional phagocytic cell lines.

Methods: Four *S. aureus* isolates (SH1000, EDCC 5055, 5458, 5464) were examined in U937 monocytes, A549 epithelial cells, and SaOS-2 osteoblast-like cells. Early intra- and subcellular localization was analyzed by transmission electron microscopy, structured illumination microscopy, serial block-face scanning electron microscopy, and imaging flow cytometry. Host transcriptional responses in U937 cells were assessed by mRNA sequencing at 4 h post infection. Infection outcomes and cell death were characterized using Luminex cytokine assays, flow cytometry, xCELLigence real-time cell analysis, and Western blotting in wild-type and TNF-R1-deficient cells.

Results: All tested isolates were internalized and localized to endolysosomal and cytosolic compartments early after infection, but also associated with the host nucleus in all three cell lines. In U937 monocytes, infection induced a conserved stress response together with isolate-specific transcriptional programs affecting inflammatory signaling, metabolism, and cell fate. These responses were markedly attenuated for the chronic-infection isolate EDCC 5464. Cell death was likewise isolate-dependent and involved intrinsic and extrinsic apoptosis, mitochondrial depolarization, and caspase-1 activation with distinct kinetics. TNF-R1 deficiency delayed early but increased late cytotoxicity independent of isolate, identifying TNF-R1 as a key regulator of monocyte infection outcome. Increased receptor surface expression was triggered in U937 monocytes, but not in A549 and SaOS-2 cells, and their viability was only marginally influenced by isolate or TNF-R1 loss.

Conclusions: Intracellular *S. aureus* infection outcomes are shaped by isolate-specific and host cell-specific factors. TNF/TNF-R1 signaling plays a critical, tissue-dependent role in regulating early host defense and cell survival. These findings highlight the importance of bacterial heterogeneity and suggest that infection risks should be considered when developing TNF-targeted or other host-directed therapies.

WS08.04

Immune dysregulation in human periprosthetic joint infection: Insights from synovial fluid analysis

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Background: A hallmark of periprosthetic joint infection (PJI) is the host"s inability to eradicate invading bacteria, which leads to chronic and persistent disease courses. Evidence from in vitro and animal models, particularly in the context of staphylococcal PJI, suggests that biofilm formation contributes to this persistence by driving an anti-inflammatory immune milieu. This shift is characterized by an expansion of myeloid-derived suppressor cells (MDSCs) and a predominance of M2-like macrophages, both of which impair effective bacterial clearance. However, it remains largely unclear whether these immunological mechanisms observed experimentally also play a significant role in human PJI.

Objective/Methods: The major aim of this study was to characterize innate and adaptive immune responses in synovial fluid (SF) from chronic PJI. Synovial fluid and peripheral blood were obtained from 20 patients (seven female, 13 male) undergoing revision arthroplasty for proven PJI and were analysed by flow cytometry.

Results: Infections were caused by coagulase-negative staphylococci (ConS; n=16) or anaerobic pathogens like C. acnes and F. magna (n=4). Flow cytometry-based immune profiling revealed higher frequencies of neutrophil granulocytes, conventional dendritic cells (type 1 and 2) and chronically activated CD4pos T cells in SF compared to the corresponding blood sample. The higher levels of classical monocytes, PD1pos CD4pos and CD127neg CD4pos T cells in SF may represent a state of chronic infection that cannot be cleared by immune cells due to biofilm formation by the pathogen. A comparative analysis of monocytes in SF and blood revealed in SF a higher proportion of M2-like macrophages, typically associated with anti-inflammatory and

tissue-repair functions. Consistent with this finding, the concentration of the anti-inflammatory cytokines interleukin-10 and arginase were significantly elevated in SF compared to plasma.

Conclusion: Our findings demonstrate that chronic PJI is characterized by a distinct locally immunosuppressive environment with chronically activated $CD4^+$ T cells, M2-like macrophages and elevated anti-inflammatory mediators such as IL-10 and arginase. A better understanding of these immune signatures may form future diagnostic and therapeutic strategies to improve the management of PJI.

WS08.05

Adipose–lung crosstalk and antiviral defense: IFITM as a mechanistic link between obesity and severe influenza infection

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Question: Obesity increases susceptibility to severe influenza A virus (IAV) infection (1). Own studies showed that obese mice had higher IAV loads with a more severe disease (2, 3). Bulk sequencing indicated downregulation of Interferon-Induced Transmembrane proteins (IFITM) 1 in obese mouse lungs, suggesting impaired IFITM-mediated antiviral defense contributes to obesity-related influenza susceptibility. We investigated how obesity affects adipose-lung crosstalk during IAV infection and the role of reduced IFITM1 expression.

Methods: We combined studies on adipocytes and pulmonary epithelial cells to explore the adipose–lung axis during IAV infection. IFITM1-knockout A549 cells were used to examine the role of IFITM1 during pulmonary infection. To address the adipose side of the axis, we analyzed the transcriptome of IAV-infected human primary adipocytes to determine how infection affects IFITM-associated responses. Conditioned media experiments were then performed to test whether adipocyte-derived factors alter IFITM expression and antiviral responses in lung cells during infection.

Results: Previous data showed that adipose tissue, adjacent to the lung, is not merely a passive bystander (2). In adipocytes, infection altered IFITM-related transcriptional responses, indicating that adipose cells themselves respond to IAV. In IFITM1-deficient pulmonary epithelial cells, antiviral responses during infection were altered, supporting a functional role for IFITM1 in restricting IAV at the lung barrier. Moreover, adipocyte-conditioned media (CM) affected lung cells and modulated IFITM expression during infection, supporting functional adipose–lung crosstalk. While CM did not significantly change viral titers, it induced cytokine expression in both infected and uninfected cells. During infection, CM downregulated interferon-related cytokines and chemokines, as well as IFITM1 expression, suggesting that adipose-derived signals contribute to suppression of the antiviral response.

Conclusion: Together, these findings support a model in which obesity reshapes the adipose–lung axis and compromises antiviral defense in the lung. IFITM1 emerges as one potential mechanistic link between obesity-associated adipose dysfunction and impaired control of IAV infection. These data strengthen the concept that adipose tissue-derived signals influence pulmonary immunity and may contribute to increased influenza susceptibility in obesity.

• (1) Hornung F, et al. *The Inflammatory Profile of Obesity and the Role on Pulmonary Bacterial and Viral Infections.*Int J Mol Sci. 2021;22(7):3456.

• (2) Hornung F, et al. *Thoracic adipose tissue contributes to severe virus infection of the lung.*Int J Obes (Lond). 2023;47(11):1088-1099.

• (3) Hornung F, et al. *High-fat diet impairs microbial metabolite production and aggravates influenza A infection*. Cell Commun Signal. 2025 Jul 31;23(1):359. doi: 10.1186/s12964-025-02367-w. PMID: 40745568; PMCID: PMC12312391.

WS08.06

Ca^{2+} influx downstream of inflammasome activation shapes pyroptotic cell death morphology in human macrophages during *Yersinia enterocolitica* infection

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Macrophages of the gut-associated lymphoid tissue represent a critical first line of defense against enteric pathogens such as *Yersinia enterocolitica*. A defining feature of pathogenic *Yersinia* species is the type III secretion system (T3SS), which injects effector proteins into host cells and can trigger or modulate inflammasome activation. Inflammasome assembly culminates in gasdermin D (GSDMD)-mediated pore formation and pyroptotic cell death. While Ca^{2+} signalling has been linked to both inflammasome activation and membrane repair mechanisms, the role of Ca^{2+} dynamics in shaping the phenomenology of pyroptotic cell death during bacterial infection remains incompletely understood.

Here, we investigated Ca^{2+} responses in human monocyte-derived macrophages (hMDMs) infected with a *Y. enterocolitica* strain expressing only the T3SS and lacking all Yop effectors. Infection with this strain induced a pronounced and sustained increase in cytosolic Ca^{2+} driven by extracellular Ca^{2+} influx. This Ca^{2+} signal occurred downstream of apoptosis-associated speck-like protein containing a CARD (ASC) speck formation and preceded loss of plasma membrane integrity, features consistent with pyroptotic cell death. Pharmacological inhibition of GSDMD demonstrated that GSDMD pores constitute the primary route for extracellular Ca^{2+} entry. Importantly, GSDMD-dependent Ca^{2+} influx was associated with a distinct pyroptotic morphology characterized by disruption of the subcortical F-actin network followed by localized membrane bulging. In contrast, depletion of extracellular Ca^{2+} abolished these morphological hallmarks and shifted cells toward a ballooning cell death phenotype, characterized by an extended lytic phase.

Our findings demonstrate that GSDMD-mediated Ca^{2+} influx following ASC speck formation is not merely a downstream consequence of inflammasome activation but actively contributes to the morphological execution of pyroptotic cell death. These results identify GSDMD-dependent Ca^{2+} entry as

a key determinant of pyroptotic cell death phenotypes in hMDMs during *Y. enterocolitica* infection.

WS09.01

Culturomics of human translocating gut bacteria in extraintestinal tissues of germ-free mice colonized with autoimmune patient microbiota

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Translocation of gut pathobionts into extraintestinal tissues is a potential mechanism of host-microbiota interactions in systemic autoimmunity. Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease of unknown etiology. Besides genetic predisposition and environmental factors, translocating enterococci and lactobacilli have been linked to the pathophysiology of this disease in murine models. We hypothesize that human SLE-derived gut bacteria colonize secondary lymphoid organs after translocation and initiate systemic autoimmune reactions.

To test this hypothesis, we established a culturomics approach to systematically isolate human translocating bacterial strains in a humanized, gnotobiotic model. To this end, specific-pathogen-free and germ-free mice were gavaged with human microbiota derived from SLE patients vs healthy controls. A subgroup was treated with imiquimod to promote gut barrier leakiness and mimic an SLE-like phenotype by inducing TLR7-driven immune responses. After euthanasia, tissue samples of Peyer`s patches, mesenteric lymph nodes, liver, spleen, kidney, heart and blood were homogenized under anaerobic conditions and analyzed via culturomics including multiple media, growth conditions, and extended incubation times. Species were identified by full-length 16S rDNA sequencing.

Multiple translocating species of human origin were isolated including known pathobionts, recently identified but poorly characterized species besides new species (e.g., members of the families *Bacteroidaceae*, *Eubacteriaceae*, *Tannerellaceae*, *Sutterellaceae* and *Streptococcaceae*).

Importantly, we introduced a curation procedure that allows the exclusion of potentially false-positive species. After curation, reliable bacterial translocation was observed in all analyzed tissues except blood. Unexpectedly, translocation was observed independent of imiquimod-treatment, donor origin (healthy vs. SLE), and mouse strain, suggesting more widespread translocation processes in vivo.

The isolated bacteria are currently being analyzed in-depth for host-microbiota interactions including molecular biological approaches and co-culture experiments (e.g., transposon-based mutagenesis, human organoids). These downstream experiments will provide a deeper understanding of fundamental molecular mechanisms of host-pathobiont interactions and thus result in new "bench-to-bedside" approaches to identify future treatment targets in autoimmune diseases.

Taken together, systematic culturomics of extraintestinal organs represents a powerful tool to identify immunologically relevant bacterial candidates that goes beyond conventional fecal 16S-rDNA-based and metagenomic comparisons of human microbiomes. This approach also revealed more prevalent gut bacterial translocation than anticipated, also with healthy donor microbiota, suggesting that the role of translocating gut bacteria is understudied also in other physiologic or pathophysiologic settings than autoimmunity.

WS09.02

The interplay between the microbiota and tissue damage during obesity

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Background: Obesity is associated with impaired wound healing and increased susceptibility to infections, yet the underlying mechanisms remain incompletely understood. Chronic low-grade inflammation and defective tissue regeneration may contribute to poor repair outcomes. Emerging evidence links these outcomes to obesity-induced gut dysbiosis, including the loss of beneficial microbes essential for mucosal barrier integrity and immune homeostasis. We hypothesize that obesity-induced dysbiosis disrupts intestinal permeability, thereby impairing tissue repair and altering immune responses.

Methods: In this study, we investigated the effects of a control diet (CD) versus a long-term high-fat diet (HFD) on gut microbiota composition and immune responses in mice. Following dietary intervention, mice were treated with broad-spectrum antibiotics or vancomycin for three days to induce dysbiosis, or supplemented with *Akkermansia muciniphila* for 10 days. Subsequently, the mice were infected with *Nippostrongylus brasiliensis* for five days to induce transient lung injury and a Th2-biased immune response. Gut microbiota composition was analysed from faecal samples using 16S rRNA sequencing.

Results: We observed significant obesity-associated microbiota alterations in the gut, alongside physiological changes including reduced colon length, decreased mucus production, and increased intestinal permeability. In the lungs, uninfected obese mice exhibited a downregulation of wound healing markers, whereas infected obese mice showed excessive tissue damage following *N. brasiliensis* infection. Interestingly, HFD-induced obesity delayed type 2 immunity and increased Th17 responses in the lungs of infected mice. In the gut, post-antibiotic microbiome recovery was impaired by obesity. Vancomycin treatment led to the enrichment of beneficial bacterial populations in the gut, such as *A. muciniphila*, and concurrently enhanced the pulmonary Th2 response. Notably, intestinal colonization with *A. muciniphila* counteracted the obesity-associated impairments. Finally, BugBase phenotype analysis demonstrated a functional restructuring of the gut microbiota across diet and treatment groups. Broad-spectrum antibiotics shifted the gut microbiota

toward a more pathogenic and stress-tolerant phenotype, an effect that was amplified in HFD-fed mice. Conversely, vancomycin treatment partially preserved a healthier, anaerobic microbial profile.

Conclusion: Overall, our findings demonstrate that obesity-associated gut dysbiosis disrupts barrier function, delays tissue repair, and skews pulmonary immune responses. Targeting the gut microbiota to restore microbial balance represents a promising therapeutic avenue to improve immune regulation, enhance wound healing, and mitigate obesity-related complications.

WS09.03

Transient nature of the lung microbiome and its alteration by *Aspergillus fumigatus*

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The existence of a resident lung microbiota and its role in health and disease remain debated. In particular, little is known about interactions between the lung microbiome and pulmonary diseases caused by fungal pathogens such as *Aspergillus fumigatus*, the major airborne fungal pathogen responsible for life-threatening invasive aspergillosis in immunocompromised patients.

Here, we investigated whether the lung microbiome represents a stable microbial community or a transient ecosystem shaped by continuous host-controlled microbial turnover. In addition, we assessed effects of immunosuppression, *A. fumigatus* infection, and antifungal treatment with voriconazole on the lung microbiome in a murine model of invasive aspergillosis.

To determine if the lung microbiome reflects persistent colonization, we genetically engineered the dominant murine lung bacterium *Ligilactobacillus murinus* to produce green fluorescent protein (GFP). Following intranasal administration of GFP-labeled *L. murinus* to healthy mice, bacteria were readily detectable in the lungs at early time points but rapidly declined over time. No viable GFP-positive bacteria remained detectable three days after administration of the highest permissive inoculum dose. Flow cytometry and fluorescence imaging demonstrated active phagocytosis of labeled bacteria by phagocyte-like cells, indicating rapid immune recognition and clearance. These findings demonstrate that even lung-adapted bacterial species fail to establish stable colonization and are efficiently eliminated by host immune mechanisms, highlighting the highly dynamic and transient nature of the lung microbiome.

A. fumigatus infection induced alterations in the lung microbiome composition which were partially restored by voriconazole treatment. Several anaerobic taxa, particularly *L. murinus*, significantly increased in abundance following *A. fumigatus* infection. Moreover, co-cultivation with *A. fumigatus in vitro* enhanced *L. murinus* growth while simultaneously reducing oxygen levels, suggesting that fungal hyphae create localized microaerophilic niches that favor the proliferation of anaerobic bacteria. The observed promotion of *L. murinus* growth both *in vivo* and *in vitro* suggests a potential direct interaction between *A. fumigatus* and pulmonary bacteria [1].

Together, these findings provide a basis for understanding the dynamic nature of the lung microbiome and its role in respiratory health and disease. Further investigation of microbiome alterations during invasive aspergillosis may reveal important insights into host-microbiota-pathogen interactions and their contribution to disease progression and immune regulation.

[1] Nikitashina L, Chen X, Radosa L, Li K, Straßburger M, Seelbinder B, et al. The murine lung microbiome is disbalanced by the human-pathogenic fungus *Aspergillus fumigatus* resulting in enrichment of anaerobic bacteria. Cell Rep. 2025;44:1–23. https://doi.org/10.1016/j.celrep.2025.115442.

WS09.04

Automated smart picking to expand gut microbial culturomics

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While microbiome research traditionally focuses on gut microbiota, cultivating isolated bacterial strains remains essential to unlock the mechanisms driving microbial interactions. However, standard isolation methods are labor intensive, difficult to scale, and lack integrated phenotypic and genotypic data. Consequently, they often overlook slow growing or rare taxa, despite the critical roles these organisms play in maintaining the ecological balance of complex microbial systems. This limitation restricts our understanding of overall community dynamics and undermines our ability to predict how microbiomes respond to environmental or therapeutic pressures.

Crucially, microbial species exhibit unique colony morphologies and growth phenotypes that allow for differentiation without immediate genotyping. To exploit this phenotypic diversity, we are developing an AI driven automated microbial isolation platform at the Microbial Automation and Culturomics Core

Facility (MACCF) at EMBL. This system builds upon similar technology developed by the Harris Wang lab at Columbia University (1) to dramatically increase the recovery of diverse, slow growing, and rare gut microbes for downstream mechanistic studies.

Our approach first utilizes an intelligent imaging algorithm capable of distinguishing roughly 2000 distinct phenotypic features, including colony size, shape, color, and depth. We coupled this with custom plate imaging systems that capture transilluminated images to measure colony height, radius, and circularity, alongside epi illuminated images to reveal color and complex morphological features like wrinkling. This imaging setup is fully integrated with an automated colony picking robot for high throughput isolation and arraying inside an anaerobic chamber. Currently, the AI algorithm is being trained on colony images from all known gut microbial species to enable the selective targeting, genotyping, and biobanking of previously uncultured, rare taxa. Ultimately, this platform establishes an automated, image guided smart picking strategy to systematically expand the diversity of cultivable microbes.

WS09.05

Dynamics of the gut and oral resistome and virulome in returning travelers receiving Atovaquone/Proguanil for Malaria prophylaxis

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Question. International travel to malaria-endemic regions is a well-established risk factor for acquiring antimicrobial resistance genes (ARG) and transient gut microbiome perturbations. However, potential microbiome specificities linked to anti-malarial chemoprophylaxis have not yet been assessed. Atovaquone/proguanil (A/P) is a first-line regimen in Western travel medicine. Proguanil is bioactivated by hepatic CYP2C19 to cycloguanil, a dihydrofolate-reductase inhibitor reaching the gut lumen, a plausible selective pressure on folate-dependent microbes. A/P tolerability varies widely, and the oral microbiome, the initial drug-contact site, remains uncharacterized.

We aimed to characterize, in paired oral and gut microbiomes of A/P-receiving patients traveling to malaria-endemic settings:

i. travel-associated changes of the resistome and virulome,

ii. abundance and variability of microbial folate biosynthesis pathways as a functional surrogate for gut-luminal cycloguanil exposure, and

iii. the relationship of these readouts to reported adverse events.

Methods. Adult travelers receiving A/P for ≥14 days provided paired saliva and stool samples pre-departure and within one week post-prophylaxis (n=22). Questionnaires captured itinerary, exposures, intercurrent illness, and adverse events. Illumina metagenomic sequencing was performed. Processing included host depletion against GRCh38 with phiX spike-in, Kraken2/Bracken profiling, MEGAHIT assembly, HUMAnN3/MetaPhlAn4 functional profiling, and resistome calling on reads (RGI/CARD) and contigs (Abricate vs. CARD/VFDB; AMRFinderPlus). Within-subject comparisons used paired Wilcoxon tests. Beta diversity used Aitchison distances (CLR) with PERMANOVA stratified by subject.

Results. Preliminary analyses demonstrate a post-travel expansion of the gut resistome, with most participants showing higher ARG abundance in stool. Virulence-factor profiles showed a concordant directional shift. Saliva exhibited similar but attenuated trends. These changes coincided with increased relative abundance of Enterobacterales, most pronounced for *Escherichia* spp. Alpha and beta diversity remained largely stable, suggesting travel rearranges gene content more than community structure. Functional profiling recovered folate pathways relevant to cycloguanil action: PWY-3841 and 1CMET2-PWY were stable across subjects, whereas FOLSYN-PWY and PWY-6612 varied between individuals in a pattern co-segregating with reported adverse events.

Conclusion. In travelers taking A/P prophylaxis, we observed a consistent expansion of the gut and oral resistome and virulome without major compositional restructuring. Inter-individual variability in microbial folate biosynthesis pathways, a functional proxy for cycloguanil exposure, may provide a mechanistic link to heterogeneity in A/P tolerability. Future studies should analyze travelers without A/P to disentangle travel-associated from A/P-related microbiota changes.

WS09.06

Elucidation of mobile genetic element-defence system interactions in human gut bacteria

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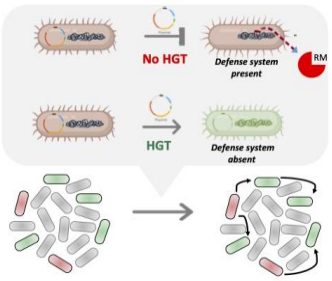
The spread of antibiotic resistance in bacterial pathogens is driven by horizontal gene transfer mediated by mobile genetic elements (MGEs). The successful transfer of resistance-carrying MGEs depends on complex interactions with bacterial defence systems that restrict the establishment of incoming foreign DNA. This has implications in identifying the environmental sources of resistance carrying MGEs in pathogenic bacteria, a major challenge in the field, since defence systems shape the movement of resistance genes across bacterial communities.

To address this gap, we developed a computational framework that integrates state-of-the-art methods to identify MGEs, defence systems, and their specific targets within the genomes and metagenomes of human gut bacteria, enabling the systematic characterization of MGE–defence system interactions. Applying this framework to restriction–modification (RM) and CRISPR-cas systems, we found that a substantial fraction, up to 50% of defence systems are themselves carried by MGEs, that the distribution of defence system varies between closely related strains. Furthermore, for RM systems, shorter MGEs exhibit stronger avoidance of their target motifs than longer MGEs, suggesting selection to evade host immunity. Together, these observations point to a complex network of host microbe - MGE and MGE - MGE interactions that influences the spread of mobile elements and, consequently, the dissemination of antibiotic resistance genes they carry. Beyond these interactions, knowledge of defence

system targets can inform the design of cloning and genome-engineering strategies by identifying sequence features that are likely to restrict the introduction of genetic constructs into human gut bacterial species.

Our study demonstrates how interactions between MGEs and defense systems may generate strain-level variation. Our framework provides a means to predict which MGEs are likely to successfully establish in particular bacterial hosts, and thus lays the foundation for identifying environmental donor species that contribute resistance-carrying MGEs to clinically relevant pathogens.

Fig. 1



WS10.01
Clinical Case 1

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Introduction: *Neoehrlichia mikurensis* has gained increasing attention as an emerging tick-borne zoonosis, especially in immunocompromised patients in Europe. We report a case of human neoehrlichiosis in Germany, which occurred in a 66-year-old woman with a history of granulomatosis with polyangiitis (GPA), B-cell depletion therapy, and secondary antiphospholipid syndrome. While undergoing long-term treatment for GPA with the monoclonal anti-CD20 antibody rituximab, the patient was hospitalized because of a prolonged fever of unknown origin (FUO) and hyperinflammatory syndrome. In addition to a systemic infection, the patient also suffered from complicating myocarditis (Fig.1).

Methods: DNA was extracted from whole blood using a automated MagNA Pure system based on magnetic class particle technology. The detection was archived by a broad range 16s RNA PCR followed by amplicon sequencing to confirm species identity. To confirm the molecular diagnosis, we performed a species-specific real-time PCR that targeted the groEL-gene.

Results: After several negative blood cultures, molecular analyses showed a positive result for *N. mikurensis,* leading to targeted antibiotic treatment with doxycycline. A follow-up PCR revealed that the neoehrlichia DNA was finally cleared from her blood.

Conclusion: Our findings provide further evidence that human neoehrlichiosis is endemic in Germany and suggest that *N. mikurensis* can also cause myocarditis. Consequently, in potential areas of endemicity, *N. mikurensis* should be considered in immunocompromised individuals with FUO and signs of infection.

Fig. 1

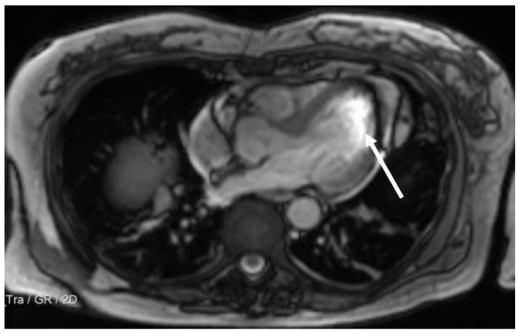

Fig 1. MRI demonstrating active myocarditis (arrow) with preserved systolic left ventricular function and intrapulmonary consolidations

WS10.02
Clinical case 2 (Young talent)

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Introduction: Diarrheal disease represents a frequent clinical problem in travelers and returnees from tropical and subtropical regions, with Non-cholera vibrios (NCV) as a probable underdiagnosed pathogen.

Case report: We report a case of a previously well 53-year-old female returning traveller from Madagascar with sudden onset of progressive diarrhea (20–25 bowel movements per day), abdominal pain, fever and vomiting. During her stay in Madagascar, she undertook excursions to the countryside and consumed local food from street markets. After initial presentation at the emergency department of the University Hospital Halle (Saale), she was admitted and isolated on the ward. Initial treatment included intravenous fluid replacement and empiric antimicrobial therapy with ampicillin/sulbactam. The patient's symptoms improved within one week.

Results: Cultivation of the stool sample on selective media for *Clostridia*, *Salmonella*, *Yersinia*, *Shigella* and *Campylobacter* remained negative. Diagnostic investigation for protozoa and helminthic pathogens was unremarkable. However, three different types of enteropathogenic *Escherichia coli* (EPEC, ETEC, EAEC) were detected using NAT. The ETEC strain was successfully cultured on Columbia blood agar, and Vibrio cholerae was surprisingly found and isolated in addition. This isolate was sent to the Center for highly pathogenic Microorganisms, where typical morphological and biochemical features of *Vibrio cholerae* were confirmed. The sodB marker was detected via PCR. Further analysis excluded O:1 or O:139 serogroups, cholera toxin genes (ctxA and ctxB) were not detected. Thus, the strain was classified as non-O:1/non-O:139 Non-cholera vibrio.

Discussion: NCV can cause gastroenteritis with a relatively mild course in immunocompetent individuals. In this patient, it

is likely that the severity of the illness was due to a coinfection with multiple enteropathogenic *E. coli* strains. Contaminated food intake in Madagascar seemed to be the most likely source of infection in this case.

Conclusion: NCV might represent a frequently underdiagnosed cause for gastroenteritis in returning travelers from tropical countries. Travel history should always prompt clinicians to utilize the full range of diagnostic options available for pathogen detection and consecutive treatment.

WS11.01

Prospective AMR culturomics of hospital wastewater systems: Understanding transmission events and AMR plasmid evolution

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Objectives: Causative pathogens of hospital outbreaks are often detected in wastewater compartments. However, the question of whether wastewater is the source or pathogen detection is a consequence of the presence of affected patients can often not be answered. Mass identification of multidrug-resistant pathogens (AMR culturomics) in the sewage system outside of the outbreak situation can therefore help to clarify this question.

Methods: In an outbreak-free situation, representative siphons of washbasin and showers as well as toilet waste water in patient bath rooms from two buildings were analysed by culture on on chromID® ESBL agar (bioMérieux, Marcy l'Etoile, France) selective for 3rd generation cephalosporin-resistant *Enterobacterales* (3GCRE). Wastewater as well as patient isolates displaying carbapenem-resistance (CRE) were further characterized and compared using whole genome sequencing and subsequent analysis using MBioSeq Ridom Typer (Bruker, Bremen, Germany).

Results: Growth of 3GCRE was observed in 44.3 % of showers (191/431), 10.6 % of washbasins (21/198) and in 47.6 % of toilet water samples (214/450). *Citrobacter freundii* (n=244), isolates of the *Enterobacter cloacae* complex (n=171) and *Escherichia coli* (n=65) were dominating in the investigated samples, whereas *Klebsiella pneumoniae* (n=18) and other *Enterobacterales* were rarely observed. CRE were observed in 52.6 % of the samples positive for 3GCRE with gens of the $bla_{\text{OXA-48}}$-group and $bla_{\text{VIM-1}}$ were observed most frequently. Several months after the last investigation a total of five patients carrying $bla_{\text{OXA-48}}$-positive *K. pneumoniae* could be linked to a single siphon isolate observed during the study, confirming wastewater to patient transmission. Detailed analysis of plasmids revealed several genetic events (deletions + insertions) during the observation period. Despite the high prevalence of CR *C. freundii*, isolates of the *E. cloacae* complex and *E. coli* no transmission event for theses species was detected during the study period.

Conclusions: AMR culturomics with subsequent molecular epidemiology outside of an outbreak situation provided the basis for elucidating the transmission of a $bla_{\text{OXA-48}}$-positive *K. pneumoniae*. However, despite the massive contamination of the building's sewage system, clinically relevant transmission events are very rare, and additional direct transmission between patients might occur after individual wastewater to patient transmissions.

WS11.02

Comparison of culture-based and culture-independent approaches for wastewater surveillance of carbapenem-resistant *Enterobacterale*s in four German cities

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Wastewater-based surveillance is increasingly used to monitor antimicrobial resistance (AMR) at the population level, and under the European Urban Wastewater Directive, wastewater-based AMR monitoring will become mandatory from 2027 onwards. However, methodological differences between culture-based and culture-independent approaches may influence the detection and interpretation of resistance patterns, particularly for carbapenem-resistant *Enterobacterales* (CRE). Therefore, this study aimed to compare the detection of carbapenem resistance and microbial community composition across four German cities using culture-based and culture-independent methods.

Wastewater samples were collected biweekly from July 2025 to February 2026 from four urban wastewater treatment plants in Germany within the framework of the AMELAG project. Samples were analyzed using both culture-based and culture-independent approaches. Culture-based methods included selective plating for CRE, colony isolation, species identification by MALDI-TOF MS, and PCR screening targeting *bla*NDM, *bla*OXA-48, *bla*VIM-1, *bla*KPC, and *bla*GES. Culture-independent analyses comprised DNA extraction directly from wastewater samples, dPCR quantification of the same resistance determinants together with a 16S rRNA gene marker to estimate total bacterial load, and full-length 16S rRNA gene sequencing using Oxford Nanopore Technologies to characterize microbial community composition. Comparisons were performed across sites and between methodological approaches.

Culture-based analysis revealed distinct city-specific patterns in the distribution of carbapenemase genes. For example, *bla*GES predominated in one city, while *bla*KPC and *bla*OXA-48 were more prominent in other cities. Gene-genus associations also varied between sites, with differing dominant carbapenemase gene and genus combinations observed across cities. For example, 97% of *Raoultella* isolates from one city carried *bla*GES genes, whereas the same gene was detected in only 6% of *Raoultella* isolates from another city.

In contrast, dPCR-based detection identified carbapenemase genes across all sites but did not reproduce the spatial patterns observed in culture-based analysis. For example, *bla*GES genes were prominent primarily in one city by culture and subsequent PCR but were detected more equally across all four cities with dPCR. Trends in gene prevalence detected by isolate-based PCR were not reflected in dPCR results, indicating discordance between methods.

Wastewater-based surveillance of carbapenem resistance is highly dependent on the methodological approach. Discrepancies between culture-based and culture-independent methods highlight challenges in interpreting AMR trends and underscore the need for method standardization in surveillance systems. These findings have important implications for the design and comparability of AMR monitoring strategies in urban settings.

WS11.03
Presence and expression of virulence factors in environmental *Pseudomonas aeruginosa* isolates
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Pseudomonas aeruginosa is considered a major opportunistic pathogen that is widespread in the environment due to its ability to form biofilms and its low environmental requirements. The microorganism can cause infections of the skin, blood, lungs, gastrointestinal tract, and other parts of the body, with symptoms varying depending on the site of infection. Exposure primarily occurs through contaminated water, moist surfaces, or aerosols generated by environmental, industrial or domestic water systems.

The risk assessment of a *P. aeruginosa* infection considers both the likelihood of exposure and the severity of potential health effects. The minimum infectious dose is not known, and the risk of infection depends on the route of exposure, the health status of the exposed person and the virulence of the strain.

Virulence is determined by genetic factors and phenotypic expression. In this study we characterized *P. aeruginosa* isolated from the environment and house installations with respect to the phenotypic expression and the molecular virulence factor profiles to assess the pathogenicity potential.

A total of 135 *P. aeruginosa*, isolated from the environment and water-bearing systems, were evaluated genotypically for the presence of ten *P. aeruginosa*-specific virulence genes (*lasB*, *toxA, plcH, phzM, exoS, exoU, ecfX, exoY, plcN, exoT*) using RT-PCR assays. The isolates were characterized phenotypically in terms of pigmentation, morphology, fluorescence property, hemolysis, exoenzymatic and swarming and twitching activities after cultivation on various agar plates. In addition, the ability of biofilm formation in 96-well plates determined by crystal violet staining.

Genotypically, 52.2 % of the strains carried nine and 47.8 % eight of the ten virulence genes analyzed. All isolates carried the genes *toxA, plcH* and *lasB*. The gene *lasB* encodes the enzyme elastase, and high activity was observed at 75% and lower activity at 13% of the isolates. Nevertheless, 16 isolates (12%) showed no activity. A particularly large number of isolates exhibited pronounced hemolysis (78%), exoenzyme activity (83%), and twitching activity (98%) and swarming activity (16%) on agar plates with a diameter of > 20 mm. All isolates demonstrated intense biofilm formation.

Our results show that *P. aeruginosa*, isolated from the environment and water-bearing systems, carry and express numerous pathogenicity factors, and, therefore, such isolates should not be neglected in terms of pathogenicity. The risk of transmission and infection can be minimized through measures such as regular monitoring of water systems, prevention of water stagnation, control of biofilm formation and implementation of appropriate hygiene practices.

WS11.04
A multi-clonal outbreak of VIM-1-producing *Enterobacter hormaechei*: Shower drains as potential environmental reservoirs
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Question: Carbapenemase-producing Enterobacterales (CPE) are an increasing healthcare threat due to limited treatment options and nosocomial transmission potential. *Enterobacter (E.) hormaechei*, an emerging member of the *E. cloacae* complex, is particularly relevant in vulnerable hospital populations. Hospital wastewater systems may serve as persistent reservoirs and potential sources of transmission for CPE. We investigated an outbreak of VIM-1-producing *E. hormaechei* in a German tertiary-care hospital, focusing on sustained transmission within a bone marrow transplant unit and potential environmental reservoirs.

Methods: Cases were defined as inpatients colonized or infected with VIM-1-producing *E. hormaechei*. Case identification was performed through retrospective and prospective microbiological data analysis and prospective culture-based rectal screening combining CPE-detection and carbapenemase identification. Epidemiological and clinical data were subsequently analyzed. Patient and environmental isolates underwent molecular carbapenemase confirmation and whole-genome sequencing (WGS). Relatedness was assessed by core-genome multilocus sequence typing (cgMLST), with ≤12 alleles defining close relatedness.

Results: The investigation was initiated in July 2025 following the detection of three patients with nosocomial colonization by VIM-1-producing *E. hormaechei* on a surgical transplant ward, all detected through repeated inpatient screening. A retrospective analysis back to August 2024, combined with prospective surveillance until January 2026 identified 28 cases across eight wards. Most cases (24/28; 85.7%) were first detected through rectal screening rather than clinical samples. 12/28 isolates (42.9%) were susceptible to both meropenem and imipenem according to EUCAST. Only 16/28 (57.1%) showed elevated MICs and were categorized as susceptible at increased exposure or resistant to at least one of the two carbapenems. cgMLST revealed six sequence types (ST), indicating a multi-clonal outbreak. *E. hormaechei*-ST200 was detected in two patients on a bone marrow transplant unit in February and April 2025 and subsequently reappeared from November 2025 through January 2026 (N=6). Closely related ST200 isolates were recovered from three patient-room shower drains.

Conclusion: Our analyses highlight the complex transmission dynamics of VIM-1-producing *E. hormaechei* in high-risk hospital settings. Repeated inpatient screening and molecular carbapenemase testing were crucial for case detection, while cgMLST revealed a multi-clonal transmission event. The prolonged persistence of ST200 on the bone marrow transplant unit and the identification of closely related environmental isolates, suggests shower-drain wastewater reservoirs as a plausible source of persistence and recurrent transmission.

WS13.01

Validation of an endopeptidase-suspension immunoassay (Endopep-SIA) for *in vitro* diagnostics of botulism in humans

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Introduction: Botulism is a severe, potentially fatal disease caused by botulinum neurotoxins (BoNTs), produced by bacteria of the genus *Clostridium*. Accurate laboratory detection of BoNTs in patient samples is critical for confirming clinical diagnoses and determining the specific serotype involved. Five of eight BoNT serotypes are known to naturally cause botulism in humans (BoNT/A, B, E, F and H). Due to their exceptional toxicity, diagnostic assays must reliably detect minute concentrations across more than 40 subtypes within complex samples. Despite advances in analytical methods, the mouse bioassay (MBA) continues to serve as the gold standard for botulism diagnostics. Given the significant ethical concerns associated with severe animal suffering, as well as the intrinsic technical and analytical limitations of the assay, the development of alternative, *in vitro*-based detection strategies has long been recognised as a critical objective in the field.

Aims: To develop and validate an *in-vitro* diagnostic assay to replace the burdensome MBA in human botulism diagnostics.

Methods: We established an alternative *in vitro* multiplex assay, the endopeptidase-suspension immunoassay (Endopep-SIA), utilising Luminex® Multi-Analyte Profiling technology. The assay integrates antibody-mediated extraction of BoNTs from complex matrices with broad subtype coverage, followed by detection of serotype-specific cleavage events through neoepitope-targeting monoclonal antibodies (Neo-mAbs). First, we validated our prototype assay for the simultaneous detection and quantitation of BoNT/A and B, thereby accounting for 90% of human cases in Europe. We proceeded with development of a kit prototype while developing and validating Endopep-SIAs for serotypes BoNT/E, F and H. Analytical assay performance was evaluated in buffer solution using recombinant toxins as calibration standards to determine limit of detection (LoD) and quantitation limits. Matrix effects and detection ranges were assessed in human sera and food matrices.

Results: We generated extraction antibodies with broad subtype recognition as well as a complete panel of Neo-mAbs and show that their employment in our Endopep-SIA enables detection of BoNT in buffer solution in the low pg/ml-range. Moreover, BoNTs could be detected with high sensitivity in individual human sera and food matrices. The BoNT/A and B assays showed transferability to other laboratories in two European-wide transfer studies. Kit optimisation and standardisation are ongoing. At RKI, we now have started to routinely apply the Endopep-SIA for samples received within our capacity as German national *consultant laboratory for neurotoxin-producing Clostridia.*

Conclusion: Our Endopep-SIAs detect all human-pathogenic BoNT serotypes with sensitivity comparable to the MBA, supporting their potential as an animal-free diagnostic. Efforts continue to increase visibility and optimise the prototype assay kit for potential commercialisation.

WS13.02

Accelerated identification of patients colonized with carbapenem-resistant *Acinetobacter baumannii* from screening samples using the RESIST ACINETO lateral flow assay

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Introduction: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a major nosocomial threat, particularly during outbreaks. Standard culture-based screening requires 48-72 h, delaying infection control. In Germany, 95% of CRAB harbour carbapenemase genes, making carbapenemase detection a reliable surrogate marker for rapid CRAB recognition.

Objectives: To evaluate the carbapenemase-targeting lateral flow assay (LFA) RESIST ACINETO (Coris BioConcept) applied directly to screening swabs without prior culture during a suspected CRAB outbreak, and to compare its performance with conventional culture-based diagnostics.

Materials and Methods: Between 6–16 December 2025, 106 screening samples from 35 patients across four wards were analysed. Routine diagnostics performed carbapenemase detection after culture, species identification (MALDI-TOF-MS) and antimicrobial susceptibility testing (VITEK 2; EUCAST v15.0). The LFA (targeting OXA-23, OXA-40/58 and NDM) was applied directly to the eSwab transport medium. Concordant positives were classified as true positives. Discordant LFA-positive/culture-negative results were resolved by meropenem-supplemented enrichment culture.

Results: Routine diagnostics detected CRAB in 6/106 samples (5.7%) from two patients. The LFA was positive in 11/106 samples (10.4%) from five patients and identified all routine-positive samples. Enrichment culture confirmed 3 additional CRAB, yielding 9/106 (8.5%) true positives. The LFA showed 100% sensitivity (9/9) and 97.9% specificity (95/97). One false positive result was due to an NDM-producing *Klebsiella pneumoniae* in the same rectal swab, while the other remained inconclusive. Excluding NDM, specificity rose to 99.0%. One CRAB-colonised patient would have been missed by routine

diagnostics alone. LFA results were available at least 24 h earlier than routine diagnostics.

Summary: Direct application of the RESIST ACINETO LFA to screening swabs enables rapid, highly sensitive CRAB detection and may facilitate earlier infection control interventions during outbreaks.

WS13.03

Penicillin susceptibility testing of *S. aureus* using EUCAST rapid AST from blood culture bottles

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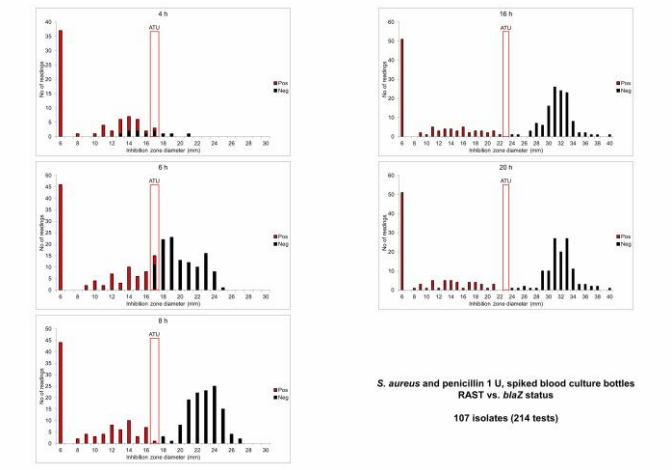
Staphylococcus aureus remains a major cause of severe invasive diseases such as bloodstream infections (BSI). Data from the *Staphylococcus aureus* Network Adaptive Platform (SNAP) Trial suggests similar efficacy of penicillin compared to cefazolin in the treatment of BSI and with fewer side effects than flucloxacillin. However, the ability to swiftly, reliably, and cost-effectively identify susceptibility is paramount to achieving clinical benefits. The gold standard for the detection of penicillin resistance in *S. aureus* is molecular testing for penicillinase *blaZ*. Here, we tested the performance of rapid antimicrobial susceptibility testing (RAST) on the detection of penicillin resistance directly from spiked positive blood culture bottles.

The EUCAST RAST methodology was adapted to test penicillin disk diffusion on a strain collection of 107 clinical *S. aureus* isolates consisting of *blaZ*-negative (n = 61) and *blaZ*-positive (n = 46) strains. We prepared a 1:1,000,000 serial dilution of a McFarland standard of 0.5, and inoculated 1 mL into aerobic blood culture bottles (BD, Heidelberg, Germany) which were prefilled with 5 mL of defibrinated sheep blood (Oxoid, Wesel Germany). Bottles were incubated in BACTEC FX (BD) blood culture systems and processed within 18 hours after being flagged positive. Penicillin disks (1 U) were placed onto cation-adjusted Mueller-Hinton (MH; BD) and Mueller-Hinton Fastidious (MH-F; BD) plates previously inoculated with 125 ±25 µL. Evaluation of inhibition zone diameters (IZD) was conducted at 4 hours, 6 hours, 8 hours, 16 hours, and 20 hours after incubation at 35 ±1 °C in ambient air.

The proportions of readable IZD were 34% (n = 72/214) after 4 hours, 97% (n = 208/214) after 6 hours, and 100% (n = 214/214) after >8 hours. Correct identification was found in 100% of readings when IZD cutoffs of <24 mm and ≥24 mm were applied, respectively, at 16 h and 20 h. A similar outcome was observed when IZD cutoffs of <18 mm and ≥18 mm were used after 8 hours of incubation. This resulted in 100% sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the detection of penicillin resistance at these three time points. After 6 hours of incubation, 11 *blaZ*-negative readings out of a total of 208 below an IZD of 18 mm resulted in a major error (ME) of 11% (n = 11/103), while sensitivity and PPV for the detection of penicillin resistance were still 91% and 89%, respectively. Meanwhile, the specificity and negative predictive value (NPV) were determined to be 100%. While the sensitivity for detecting penicillin resistance was 27% (n = 3/11) after 4 hours of incubation with an IZD of ≥18 mm, the PPV was already at 88% (n = 61/69) albeit with an ME of 12% (n = 8/69) but also showing 100% specificity and NPV.

We have demonstrated that reliable detection of penicillin resistance using RAST methodology is feasible after 6 hours of incubation from positive blood culture bottles.

Fig. 1



WS13.04

Comparative evaluation of Illumina and Oxford Nanopore sequencing for bacterial outbreak investigations (Young talent)

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Whole-genome sequencing (WGS) has become an essential tool for genomic surveillance and outbreak investigation in clinical microbiology. While Illumina short-read sequencing remains the gold standard in terms of accuracy, Oxford Nanopore Technologies (ONT) long-read sequencing offers rapid turnaround times, scalability, lower costs, and superior resolution of repetitive regions and plasmids. In this retrospective investigation, we evaluated the suitability of ONT for hospital outbreak analysis in comparison with Illumina sequencing.

504 clinical isolates (17 genera and 35 species), predominantly multidrug-resistant Gram-negative bacteria (MRGN), were analyzed using both sequencing platforms. Following standardized DNA extraction and library preparation, Illumina reads were analyzed using MBioSEQ Ridom Typer, whereas ONT data were processed using custom bioinformatic workflows. Two ONT assembly strategies, Flye and the multi-assembler Autocycler, were evaluated across varying sequencing depths (50×, 100×, and 120×). The analytical frameworks assessed sequencing quality, core-genome multilocus sequence typing (cgMLST), single-nucleotide polymorphism (SNP) analysis, and plasmid reconstruction. Reproducibility was examined using technical replicates and interlaboratory validation with identical analysis pipelines.

Illumina and ONT demonstrated over 90 % exact concordance in cgMLST-based typing across all assemblies. Discrepancies

were observed primarily in SNP analyses and were associated with ONT workflow-derived errors in majority in non-core genomic regions as well as assembly fragmentation. The latter increased the proportion of assemblies with more than 10 variations compared with the Illumina data from 7.75 % to 52.45 % on average. Plasmid reconstruction differed substantially between platforms: ONT assemblies, particular those generated with Autocycler, predicted mostly complete plasmids represented by a single contig, whereas Illumina assemblies resulted in more predicted and smaller but highly fragmented plasmids, comprising an average of 5 contigs. Increased ONT sequencing depth consistently improved concordance in downstream analyses. Generally, Flye outperformed Autocycler, demonstrating greater robustness at lower sequencing depths, although isolate-specific variability remained.

Both platforms are suitable for genomic surveillance and provide complementary strengths. However, ONT performance remained highly dependent on sequencing depth, basecalling accuracy, and bioinformatic workflows. For certain isolates, ONT introduced analytical deviations in high-resolution SNP analyses that may obscure transmission links. These limitations likely reflect persistent ONT-specific challenges in complex genomic regions, including the influence of non-standard DNA methylation patterns. Despite substantial technological advances, ONT sequencing cannot yet fully replace short-read sequencing when maximal phylogenetic resolution is required.

WS13.05

Comparative evaluation of VITEK REVEAL and QuickMIC for rapid direct antimicrobial susceptibility testing from positive blood cultures in gram-negative bloodstream infections

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Background: Bloodstream infections (BSI) require rapid pathogen identification and antimicrobial susceptibility testing (AST) to guide targeted therapy. Standard AST workflows usually provide results within 24–48 h. The increasing prevalence of multidrug-resistant Gram-negative bacteria highlights the need for rapid AST to optimize antimicrobial therapy. Direct phenotypic AST from positive blood cultures (BCs) can provide actionable results within 2–6 h and shorten time to appropriate antimicrobial treatment. Gradientech QuickMIC and bioMérieux VITEK REVEAL are two such systems: QuickMIC uses a microfluidic antibiotic gradient to determine minimal inhibitory concentrations (MICs), whereas VITEK REVEAL detects volatile organic compounds as a marker of bacterial growth.

Objective: To compare the performance and time-to-result (TTR) of QuickMIC and VITEK REVEAL for rapid AST directly from positive blood cultures.

Methods: Between 12/2025 and 05/2026, a total of 121 positive BC bottles, including 77 spiked and 44 clinical samples, were analyzed using the QuickMIC Gram-negative panel and the VITEK REVEAL Gram-negative BC panel (GN-01 AST). Testing was performed according to the manufacturers" instructions. Overall, 11 Gram-negative species were included: *Escherichia coli* (26.4%), *Klebsiella pneumoniae* (23.1%), *Pseudomonas aeruginosa* (11.6%), *Acinetobacter baumannii* (3.3%), and other Enterobacterales (35.5%). Among the isolates, 58 were classified as 3MRGN or 4MRGN. Broth microdilution (BMD) using the Micronaut-S Pseudomonas MIC plate (Bruker) served as the reference method and was performed in triplicate for all isolates. MIC interpretation was based on EUCAST clinical breakpoints version 16.0.

Results: The overall essential agreement (EA) compared with reference BMD was 91.85% for QuickMIC and 92.2% for VITEK REVEAL. For QuickMIC, categorical agreement (CA) exceeded 90% for most antibiotics, except cefepime (87.3%), ceftazidime (89.0%), and meropenem (87.5%) in Enterobacterales, as well as cefepime (85.7%), ceftazidime (84.6%), ceftazidime/avibactam (84.6%), meropenem (76.9%), and piperacillin/tazobactam (81.8%) in *P. aeruginosa*. For VITEK REVEAL, CA was above 90% for most agents, except meropenem (89.3%) and tobramycin (88.9%) in Enterobacterales, and ceftazidime (76.9%), cefepime (84.6%), and piperacillin/tazobactam (84.6%) in *P. aeruginosa*. The median TTR for QuickMIC and VITEK REVEAL was 3 h 27 min (range 2 h 21 min–3 h 58 min) and 6 h 00 min (range 3 h 00 min–8 h :00 min), respectively. The median TTR for piperacillin/tazobactam was 3 h 47 min (range 2 h 47 min–3 h 58 min) and 6 h 30 min (range 3 h 00 min–8 h 00 min) for QuickMIC and VITEK REVEAL, respectively.

Conclusion: Both QuickMIC and VITEK REVEAL demonstrated high overall agreement with reference BMD for rapid AST directly from positive BCs in Gram-negative BSI. Although QuickMIC showed lower CA for selected antibiotics compared with VITEK REVEAL, it provided shorter time-to-result.

WS14.01

Extracellular vesicle binding to *Candida albicans* modulates neutrophil phagocytosis

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Introduction: *Candida albicans* is a major cause of bloodstream infections, particularly in immunocompromised patients. Although neutrophils are essential for fungal clearance through phagocytosis, a subset of *C. albicans* cells escapes uptake and persists extracellularly in human blood. The mechanisms underlying this immune evasion remain unclear.

Objectives: We investigated host-pathogen interactions enabling extracellular persistence of *C. albicans* during blood infection and whether neutrophil-derived extracellular vesicles (EVs) contribute to fungal immune evasion.

Methods: Interactions between neutrophils and *C. albicans* were analyzed using *ex vivo* whole-blood infection assays and purified neutrophil confrontation models. Neutrophil-derived

EVs were characterized by nanoparticle tracking analysis, cryo-TEM, Western blotting, and proteomics. EV-fungus interactions and their effects on neutrophil phagocytosis were examined, supported by mathematical modeling.

Results: A subpopulation of *C. albicans* cells remained viable and extracellular in human blood despite ongoing neutrophil-mediated antifungal responses. Extracellular fungal cells acquired neutrophil-associated markers including CD66b, CD45, CD11b, and CD63, together with Annexin V positivity, suggesting deposition of host-derived membrane material. Confrontation with *C. albicans* increased the release of neutrophil-derived EVs. Isolated EVs displayed characteristic vesicular morphology and expressed established neutrophil EV markers, including CD11b, CD63, and Annexin A1. While spontaneous and infection-induced EVs shared a conserved, predominantly ectosome-like core proteome enriched in adhesion molecules, complement receptors, cytoskeletal regulators, and antimicrobial proteins, fungal challenge induced pronounced quantitative and qualitative remodeling of EV cargo toward enhanced antimicrobial and inflammatory effector functions. Purified EVs bound to the surface of *C. albicans* and transferred host-cell markers onto fungal cells. EV binding preferentially occurred on complement-opsonized fungi and was reduced following CD11b blockade, implicating CR3-mediated interactions. Despite carrying antimicrobial cargo, EVs did not impair fungal viability. Instead, EV decoration reduced phagocytic uptake of *C. albicans* by neutrophils in a purified cell system and whole blood. Mathematical modeling quantified a 1.6-fold reduction in phagocytosis of EV-coated fungal cells.

Summary: Our findings uncover a previously unrecognized host-driven immune evasion mechanism during blood infection. Neutrophil-derived EVs bind to complement-opsonized *C. albicans*, decorate the fungal surface with host-derived molecules, and impair phagocytic uptake, promoting fungal persistence in blood. These results reveal an unexpected role for neutrophil EVs in antifungal immunity and broaden understanding of host-pathogen interactions during systemic candidiasis.

WS14.02

3D lung infection models to study *A. fumigatus* infection and screen antifungal compounds

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Aspergillus fumigatus is an opportunistic fungal pathogen and one of the most common causes of invasive pulmonary aspergillosis (IPA) in solid-organ transplant and allogenic hematopoietic stem cell recipients as well as leukemia patients. The prognosis is poor with mortality rates of 40-50 %. The main causes of the high mortality are the immunocompromised state of the patient and the challenging diagnostic of IPA. However, in recent years azole resistance has emerged as another aggravating factor. This highlights the need for more research of antifungal substances. The investigation of antifungal substances is hampered by our lack of understanding of the interaction of *A. fumigatus* with the lung epithelium. The reason for this is the use of 2D cell culture approaches, which do not recapitulate several important characteristics of the lung, like cell-to-cell interactions, the heterogeneity of cell types or proximal to distal patterning. Additionally, the use of animal models suffers from species-specific differences, ethical concerns and low throughput. We attempt to address these challenges in a two-pronged approach: (I) To increase the throughput of animal experiments while reducing the number of required animals (3R), we developed a compound screening assay based on murine precision-cut lung slices (PCLS). These slices contain many mature lung cell types and retain the ultrastructure of the lung. PCLS were infected with a fluorescent *A. fumigatus* strain and treated with antifungal compounds, automatically imaged and subjected to an image analysis pipeline to determine fungal survival based on the fluorescence intensity of infected slices. (II) To better investigate the interaction of *A. fumigatus* and lung epithelium, we are currently establishing an induced pluripotent stem cell (iPSC)-derived lung organoid model. In this three-step protocol, iPSCs are differentiated to endoderm, anteriorized to anterior foregut and finally differentiated to lung epithelial cells. The PCLS-based high-throughput assay accurately showed the antifungal effect of voriconazole on *A. fumigatus*. Furthermore, the innate resistance of *A. fumigatus* to fluconazole could be detected in the assay, demonstrating the reliability of the approach. The differentiation of iPSCs to lung organoids has been successfully performed and both anterior foregut and lung progenitor cells could be detected. However, the protocol still lacks consistency in the later steps.

WS14.03

Not all chromosomes are equal: Chromosome-specific aneuploidy stability in *Candida albicans*

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Candida albicans exhibits high genome plasticity, with aneuploidy - an imbalance in chromosome copy numbers - being well tolerated. Aneuploidy has been linked to the emergence of drug evasion mechanisms like resistance and tolerance, but the dynamics and mechanisms of aneuploidy adaptation remain poorly understood. Here, we leveraged a collection of 200 natural *C. albicans* strains isolated from diverse clinical and environmental sources, each containing whole-chromosome copy number alterations, to study aneuploidy stability. We propagated isolates under non-stressed conditions for 150 generations and profiled both fitness and proteomes of evolved and ancestral strains using high-throughput methods. Using proteomic data, we inferred relative chromosome copy numbers of each isolate by exploiting the proportional scaling of protein abundance with chromosomal dosage, thus tracking karyotype stability across clades. Surprisingly, we find that in ~20% of aneuploid strains, aneuploidy can be stably maintained. Rare aneuploidies (e.g. Chr6, Chr7) often persist, whereas more common ones (e.g.

Chr4) tend to be unstable and revert to euploidy, suggesting chromosome-specific cellular constraints on the ability of cells to tolerate aneuploidy. Furthermore, we observe that stable aneuploids exhibit significantly increased minimum inhibitory concentrations against multiple classes of antifungals. Global proteome comparisons reveal adaptations to aneuploidy, with stable isolates upregulating cell wall components and amino acid metabolism, whereas reverting isolates show higher translational activity. We also observe increased fitness of stable aneuploids in response to caffeine exposure, consistent with a role of cell wall integrity in the maintenance of aneuploidy and, potentially, associated resistance to antifungals. Together, these results reveal chromosome-specific patterns of aneuploidy stability across diverse genetic backgrounds and link stable aneuploidy to proteome remodeling and reduced antifungal susceptibility.

WS14.04

The *Plasmodium falciparum* Bromodomain 4 (PfBDP4) regulates essential processes at the host-parasite interface

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Epigenetic processes play an important role in the regulation of parasite differentiation and virulence during the *Plasmodium falciparum* life cycle. Bromodomain proteins bind acetylated histones and contribute to transcriptional regulation; however, their precise roles in malaria parasites remain poorly characterized. Here, we investigated the molecular function of the *P. falciparum* bromodomain protein 4 (PfBDP4), which lacks an orthologue in higher eukaryotes. Using a conditional knockout system, we show that PfBDP4 serves as a crucial regulator of two critical stage transitions in the life cycle. Deletion of PfBDP4 in ring-stage parasites caused a failure to progress from the ring to the trophozoite stage in the subsequent parasite generation and was accompanied by abnormal morphology, whereas deletion in trophozoites allowed normal schizogony in the next cycle but led to impaired merozoite invasion thereafter. Genome-wide mapping of PfBDP4 binding sites by chromatin immunoprecipitation revealed that PfBDP4 is predominantly enriched in intergenic regions of highly expressed genes in a stage-specific manner. In ring stage parasites, PfBDP4-regulated genes were involved in nutrient uptake, food vacuole function, and haemoglobin metabolism as well as in protein trafficking. Consistent with a function of PfBDP4 in directly regulating these processes, PfBDP4 KO parasites exhibited impaired haemoglobin metabolism and a marked decrease in protein biosynthesis as measured by SUnSET (Surface sensing of Translation) assays. Further, the PfBDP4 KO parasites demonstrated decreased levels of components of the *Plasmodium* translocon (PTEX), parasitophorous vacuole, and Maurer"s clefts, suggesting that protein export and import processes between the parasite and its host cell were disturbed. Thus, our results identify PfBDP4 as a crucial epigenetic regulator of essential processes at the host-parasite interface that enable *P. falciparum* to exploit the erythrocyte as its host cell.

WS14.05

Histone lactylation acts as a microenvironment-sensitive epigenetic control mechanism in the malaria parasite *P. falciparum*

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In severe malaria, high parasite density and inflammation often cause elevated lactate levels, contributing to lactic acidosis. During human infection, Plasmodium falciparum is exposed to a dynamic host environment shaped by immune responses and metabolic changes, and adapts through epigenetic mechanisms that reprogram genes involved in virulence and differentiation. Because lactate was recently identified as a histone post-translational modification, we investigated its role in the epigenetic regulation of malaria parasites.

We applied a multi-omics approach combining RNA-seq, chromatin immunoprecipitation, and metabolomics to study histone lactylation in P. falciparum. High culture density induced histone lactylation and led to prolonged asexual maturation and reduced reinvasion. Pharmacological inhibition of the lactate transporter PfFNT increased intracellular lactate and histone lactylation in low-density cultures, thereby mimicking high-density conditions.

To define the genome-wide distribution of histone lactylation, we performed chromatin immunoprecipitation using lactylation-specific antibodies and found lactylation at both activated and repressed genes. Activated genes showed sharp enrichment of lactylation in promoter regions, whereas repressed genes were associated with broader peaks extending into coding regions, suggesting that histone lactylation can either enhance or repress gene expression depending on its genomic position. Inhibition of lactate dehydrogenase with oxamate reduced intracellular lactate and histone lactylation and partially reversed lactate-driven transcriptional changes under high-density conditions, further supporting the impact of lactate as a driver of the observed trasncriptional adaptations.

Mechanistically, knockout of the histone acetyltransferase PfGCN5 decreased histone lactylation, while deletion of the histone deacetylases PfSir2A and PfSir2B increased both acetylation and lactylation. Together, these data identify histone lactylation as a metabolically responsive epigenetic mark that links intracellular lactate levels to gene regulation and parasite adaptation.

WS15.01

Wastewater-based surveillance for early detection of toxigenic *Corynebacterium diphtheriae* circulation in an urban metropolitan region

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Inroduction: Diphtheria is re-emerging as a public health concern in Europe, with recent fatal cases highlighting the need for enhanced surveillance strategies. In highly vaccinated populations, reduced clinical suspicion and limited routine testing may obscure low-level circulation of toxigenic strains, increasing the risk of delayed detection and silent transmission. Beyond classical *Corynebacterium diphtheriae*

infections, the mobility of the diphtheria toxin (*tox*) gene among *Corynebacterium* species represents an additional epidemiological concern.

Methods: A total of 160 wastewater samples were collected between 2022 and 2024 from wastewater treatment plants, municipal sewer systems and hospital effluents in the Ruhr metropolitan area, Germany. After shotgun metagenomics, Hidden Markov Models (HMMs) were applied for screening conserved tox domains. Positive findings were validated using PCR targeting the *tox* gene and the Corynebacterium *rpo*B gene for species discrimination. Amplicons were sequenced using Oxford Nanopore Technologies, and metagenomic *rpo*B-containing contigs were further analyzed to identify potential tox gene host species.

Results: HMM-based screening identified tox-associated sequences in 18/160 wastewater samples. PCR validation confirmed 15 of these samples, while no tox signal was detected in tested HMM-negative controls, indicating high specificity of the approach. Sequencing confirmed the presence of *tox*-related sequences. *rpo*B PCR excluded *Corynebacterium diphtheriae* as host in all positive samples, while nine samples were consistent with *Corynebacterium ulcerans*/*Corynebacterium pseudotuberculosis*. Metagenomic *rpo*B profiling identified a broad diversity of human-associated *Corynebacterium* spp., including known and potential novel t*ox* gene hosts. A clinically confirmed tox-positive *C. ulceran*s case at University Medicine Essen temporally coincided with detection of *tox* signals in hospital wastewater and the downstream municipal wastewater treatment plant influent, demonstrating the sensitivity of wastewater surveillance.

Conclusions: Untargeted metagenomic wastewater surveillance combined with HMM-based screening and targeted molecular validation enables sensitive detection of Corynebacterium spp. in complex wastewater samples. The approach supports early warning of diphtheria circulation independent of healthcare-seeking behavior and may help identify emerging or atypical tox gene hosts. These findings support the integration of diphtheria surveillance into wastewater-based epidemiology frameworks to strengthen preparedness and public health response to re-emerging vaccine-preventable diseases.

WS15.02

The Pharynx as major location for antimicrobial resistance development in *Neisseria gonorrhoeae*

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Background: *Neisseria gonorrhoeae* (NG), the causative agent of gonorrhoea, has developed resistance against all antibiotic classes currently used for treatment. The pharynx is considered to play a major role in antimicrobial resistance development in NG as infections at this location often remain asymptomatic and antibiotic effectiveness is reduced. Additionally, horizontal gene transfer of extracellular DNA or resistance plasmids from the surrounding microbiome can further contribute to resistance development. Since culture of pharyngeal NG is challenging due to low bacterial load and the surrounding microbiome, data on the prevalence of NG-resistance determinants in the pharynx remain limited.

Methods: Partner clinics in Berlin recruited participants that either presented with a symptomatic NG infection or that tested positive for NG in an STI screening. Clinicians took two pharyngeal swabs from each participant, one of which was send to a partner laboratory for confirmatory NG-testing. The second swab was sent to the Robert Koch Institute (RKI) for culture. Culture was only pursued for NG-positive samples. After successful isolation, phenotypic AMR-testing and whole genome sequencing were performed. Bioinformatic analyses were conducted to determine the molecular relationship of isolates and identify resistance determinants.

Results: During the study period, throat swabs were collected from a total of 463 participants, 259 of which were confirmed positive for pharyngeal NG by the partner laboratory. For 106 of these NG-positive samples, culture was successful at the RKI, corresponding to a culture rate of 41 %. While phenotypic antimicrobial resistance analyses showed a 15,1 % rate of resistance to azithromycin and no resistance to ceftriaxone and cefixime, 28 % of isolates exhibited reduced minimal inhibitor concentrations (MIC) for cefixime with values ranging from 0,032 mg/L to 0,094 mg/L. Molecular analyses revealed that all isolates exhibiting elevated MIC values against cefixime contain mosaic variants of the *penA* gene, which encodes the penicillin-binding protein 2.

Conclusion:

A substantial proportion of pharyngeal NG isolates in the Berlin study population exhibited elevated cefixime MICs associated with mosaic *penA* variants. As mosaic *penA* alleles are established markers of reduced β-lactam susceptibility and emerging cephalosporin resistance, these findings may signal early resistance development. The results reinforce the role of the pharynx as a potential niche for the emergence and persistence of resistant NG strains and support the need for enhanced molecular surveillance.

WS15.03

Association between genomic virulence scores and clinical outcomes in carbapenem-resistant *Klebsiella pneumoniae* infections in Germany, 2023-2025

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Question: Carbapenem-resistant *Klebsiella pneumoniae* strains carrying virulence-associated markers represent a growing public health concern, as the combination of antimicrobial resistance and enhanced virulence may complicate infection control and clinical management. Genomic virulence scores evaluating the presence of specific genetic markers have been proposed to identify potentially hypervirulent isolates. However, their association with severe clinical outcomes in routine surveillance settings remains

unclear. We therefore assessed whether genomic virulence scores were associated with severe clinical outcome among patients with carbapenem-resistant *Klebsiella pneumoniae* infections in Germany between 2023 and 2025.

Methods: We conducted a retrospective observational study using data from the German Integrated Genomic Surveillance system. Carbapenem-resistant *Klebsiella pneumoniae* isolates with available whole genome sequencing data were linked to mandatory notification data from patients with infection reported between January 2023 and August 2025. Genomic virulence scores were determined using Kleborate v2.3.2 and the virulence marker set proposed by Russo et al., consisting of *rmpA, rmpA2, iucA, iroB,* and *peg-344*. Severe clinical outcome was defined as at least one of the following: invasive infection (bloodstream or cerebrospinal fluid), ICU admission, or death. Associations between genomic virulence scores and severe clinical outcome were assessed using multivariable logistic regression adjusted for age, sex, and acquisition setting (adjusted odds ratio [aOR]).

Results: A total of 845 matched carbapenem-resistant *Klebsiella pneumoniae* cases were included. Median patient age was 68 years, and 568/845 (67%) were male. Overall, 302 cases (36%) fulfilled the definition of severe clinical outcome. Invasive infection occurred in 160 cases (19%), ICU admission in 131 (16%), and death in 74 (9%). Kleborate virulence scores of 5 and 4 were identified in 6 isolates (0.7%) and 232 isolates (27%), respectively. No isolate reached a Russo score of 5, while 42 isolates (5%) had a score of 4. In the adjusted analyses, neither the Kleborate score (aOR 1.02 per point; 95% CI 0.92–1.12) nor the Russo score (aOR 1.04 per point; 95% CI 0.91–1.20) was significantly associated with severe clinical outcome. No statistically significant associations were observed for invasive infection, ICU admission, or death when analysed separately.

Conclusions: In our surveillance data, genomic virulence scores were not significantly associated with severe clinical outcomes among carbapenem-resistant *Klebsiella pneumoniae* infections. These findings suggest limited predictive value of current genomic virulence scores for clinical outcomes and indicate that convergent *Klebsiella pneumoniae* strains remain relatively uncommon in Germany. Further research is needed to better understand how combinations of virulence-associated traits contribute to severe disease manifestations.

Fig. 1

WS15.04

Beyond resistance genes: Sequence-aware machine learning improves antibiotic resistance prediction

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Introduction: Predicting antibiotic resistance from genomic sequence data is increasingly important for clinical microbiology and surveillance. Many approaches rely on resistance determinants from curated databases such as the Comprehensive Antibiotic Resistance Database (CARD). However, for some antibiotics, genomic information beyond the presence or absence of known resistance genes may contribute substantially to resistance phenotypes.

Objectives: This study aimed to develop and evaluate a machine learning approach for predicting phenotypic antibiotic resistance from genomic data and to investigate whether raw sequence-derived features improve prediction performance for specific antibiotics.

Materials and Methods: Nearly 2,500 clinical isolates of *Escherichia coli, Klebsiella pneumoniae,* and *Acinetobacter baumannii* from the PATRIC dataset were analyzed. Phenotypic susceptibility classifications (S/I/R) for 18 antibiotics, determined using the bioMérieux VITEK antimicrobial susceptibility testing (AST) system, served as labels. First a two-layer stacked random forest model was trained using CARD-derived resistance gene features. The first layer consisted of one random forest classifier per antibiotic. In the second layer, predictions from all 18 classifiers were combined to capture cross-resistance patterns. Because cefotaxime prediction performance was markedly lower than for all other antibiotics, a sequence-based approach was added to the first layer of the model. Contigs were segmented into k-mers and embedded using Word2Vec. Absolute positional embeddings were incorporated to retain positional information within genomic sequences. Figure 1 illustrates the workflow.

Figure 1: Overview of the stacked random forest framework integrating CARD-based resistance gene mapping and sequence-derived features. Sequence data were transformed into sample-level mean vectors using Word2Vec and positional embeddings and incorporated into the first model layer. Predicted probabilities from antibiotic-specific models were combined in a second layer to capture cross-resistance patterns.

Results: The CARD-based stacked random forest achieved high predictive performance for most antibiotics. A notable exception was cefotaxime, for which the model achieved an accuracy of 0.585. Incorporation of sequence-derived k-mer embeddings with positional information increased cefotaxime

prediction accuracy to 0.907. Figure 2 shows the results for each antibiotic.

Figure 2: Accuracy, ROC AUC, F1-score, very major error and major error for all antibiotics using CARD and for cefotaxime using CARD and sequence data.

Conclusion: Resistance prediction for most antibiotics could largely be explained by known resistance genes. In contrast, cefotaxime resistance appeared to depend on additional sequence-level features not captured by standard resistance gene annotations. Sequence-aware embedding approaches may therefore provide added value for antibiotics with complex, context-dependent resistance mechanisms.

Fig. 1

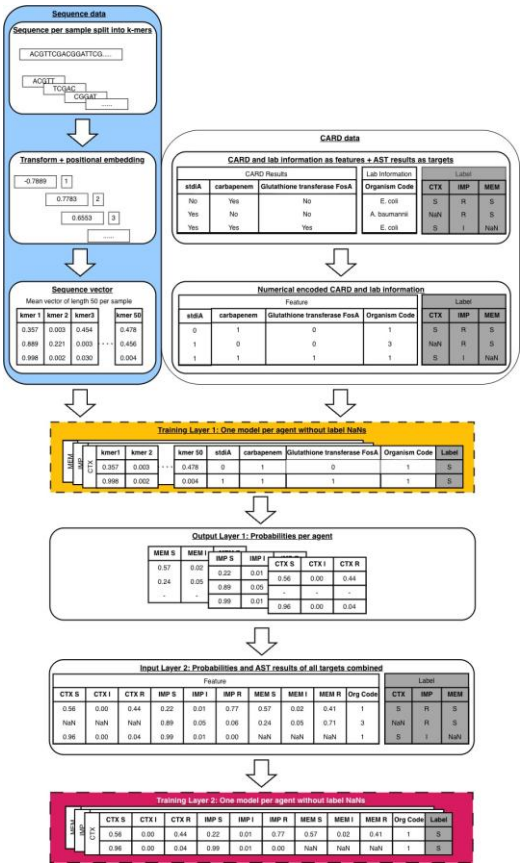

Fig. 2

	Accuracy	ROC AUC	F1 Score	VME	ME
Amikacin (CARD)	0.94	0.97	0.88	4.41	1.44
Ampicillin (CARD)	0.95	0.96	0.69	0.17	4.35
Aztreonam (CARD)	0.96	0.97	0.91	0.51	3.18
Cefazolin (CARD)	0.97	0.99	0.96	0.15	2.62
Cefepime (CARD)	0.90	0.93	0.83	1.59	7.85
Cefotaxime (CARD)	0.59	0.99	0.37	0	41.48
Cefotaxime (Sequence)	0.91	0.99	0.90	0.27	9.07
Ceftazidime (CARD)	0.97	0.98	0.94	0.86	2.10
Ceftriaxone (CARD)	0.98	0.99	0.97	2.37	0
Cefuroxime (CARD)	0.99	0.97	0.98	0	1.37
Ciprofloxacin (CARD)	0.93	0.97	0.63	0.91	5.82
Ertapenem (CARD)	0.74	0.89	0.66	1.07	25.17
Gentamicin (CARD)	0.95	0.97	0.94	2.31	3.09
Imipenem (CARD)	0.88	0.90	0.56	1.35	10.56
Levofloxacin (CARD)	0.93	0.97	0.85	1.84	4.76
Meropenem (CARD)	0.90	0.97	0.71	1.31	7.21
Norfloxacin (CARD)	0.97	0.98	0.96	0	2.70
Tobramycin (CARD)	0.93	0.96	0.93	4.27	2.29
Trimethoprim (CARD)	0.88	0.95	0.84	6.96	5.22

WS15.05

Conserved plasmid proteins enable machine learning-based detection of pOXA-48-carrying *Enterobacterales* from routine MALDI-TOF mass spectra

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Introduction: OXA-48 carbapenemases are clinically highly relevant resistance mechanisms in *Enterobacterales*. However, their detection typically requires screening via antimicrobial susceptibility testing plus phenotypic or molecular confirmation once pure cultures are available. We hypothesized that conserved proteins encoded on the endemic IncL plasmid pOXA-48 generate reproducible signatures detectable in routine MALDI-TOF species-identification spectra, and, therefore, developed and biologically validated a machine learning classifier for pOXA-48 detection across species, acquisition protocols, and instrument platforms.

Materials and Methods: A total of 153 clinical OXA-48-producing *Enterobacterales* isolates from Germany, Switzerland, and Israel with whole-genome sequencing-confirmed IncL plasmids were included alongside 153 species- and country-matched OXA-48-negative controls. MALDI-TOF spectra acquired under five conditions were used to train and validate a LightGBM classifier. External validation used independent isolate collections from Munich (n = 42, Bruker Biotyper) and Frankfurt (n = 89, bioMérieux VITEK MS). Biological validation included plasmid conjugation, curing experiments, and label-free proteomics.

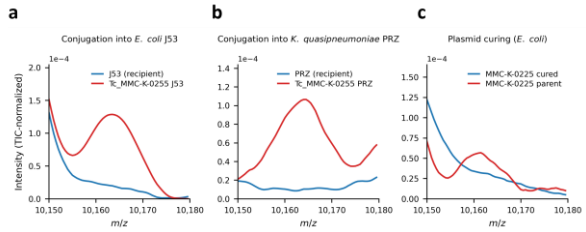
Results: The classifier achieved AUROCs of 0.951–0.975 for pOXA-48 detection across acquisition conditions and generalized to external datasets from Munich (AUROC 0.978) and Frankfurt (AUROC 0.951). At the default decision threshold, external sensitivities/specificities were 95.0%/90.9% (Munich) and 83.8%/90.4% (Frankfurt). Feature importance analysis identified a compact 58-feature spectral signature dominated by a peak at m/z 10,163. Conjugation of pOXA-48 into plasmid-free recipient strains shifted predictions from negative to positive, whereas plasmid curing reversed predictions, with the peak at m/z 10,163 appearing or disappearing, respectively (Figure 1). Proteomics linked discriminative spectral features to expressed pOXA-48-encoded proteins, particularly a DUF1496 protein

corresponding to the dominant m/z 10,163 feature. This association was further supported by the absence of the peak in a clinical isolate with a pOXA-48 deletion variant lacking the DUF1496-encoding region. Analysis of 10,626 publicly available *bla*OXA-48/*bla*OXA-162 genome assemblies demonstrated worldwide presence of the DUF1496-encoding gene in 70.9% of isolates.

Conclusion: Biology-informed machine learning enables rapid and biologically interpretable inference of pOXA-48-positive *Enterobacterales* directly from routine MALDI-TOF mass spectra, potentially providing resistance alerts more than 24 hours earlier than conventional workflows. More broadly, conserved plasmid-encoded proteins represent promising targets for MALDI-TOF-based antimicrobial resistance inference.

Figure 1. MALDI-TOF spectra at m/z 10,163: The peak appears after pOXA-48 conjugation into plasmid-free recipients (a and b) and disappears after plasmid curing (c).

Fig. 1



WS16.01

Molecular characterization of carbapenemase-producing *Serratia* spp. in Germany

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Background/Objective: While epidemiology of carbapenemases is well characterized for many Enterobacterales, data for *Serratia* spp. This study investigated the molecular epidemiology of carbapenemase-producing *Serratia* spp. (CPSER) in Germany and evaluated the antimicrobial susceptibility including newer beta-lactam/beta-lactamase inhibitor combinations.

Methods: A total of 95 CPSER isolates collected between 2021 and 2024 from four university hospitals and the German National Reference Center for Multidrug-Resistant Gram-Negative Bacteria were analyzed. All isolates had initially been identified as *Serratia marcescens* by MALDI-TOF and showed evidence of carbapenemase production (PCR or immunochromatographic methods). Whole genome sequencing was performed for molecular characterization, including species assignment via Type (Strain) Genome Server (TYGS), *in silico* MLST and ad-hoc core genome MLST (cgMLST). Antimicrobial susceptibility to 15 antibiotics was assessed by broth microdilution (BMD), with MIC interpretation according to EUCAST breakpoints v16.0.

Results: WGS-based species assignment revealed that the large majority of isolates belonged to the recently described species *Serratia sarumanii* (n=78; 82%), followed by *S. bockelmannii* (n=13; 14%). *S. marcescens* sensu stricto and *S. ureilytica* were detected only sporadically (n=2 each). MLST analysis demonstrated high clonal diversity with no dominant sequence type. One-third of isolates, predominantly among novel species, could not be assigned to established ST, reflecting the limited coverage of current typing schemes. Ad-hoc cgMLST revealed a highly diverse population structure (median pairwise distance: 3,617 alleles), while simultaneously identifying small clonal clusters with distances as low as 1–5 alleles. OXA-48-like carbapenemases predominated, accounting for 86% of all isolates, with OXA-48 being the most frequent variant (n=77; 81%), whereas KPC (7%), IMP-type (4%), and VIM-type enzymes (2%) were detected infrequently. Co-production of an ESBL was observed in 20% of isolates, predominantly CTX-M-15. Despite carbapenemase production, 37 isolates (39%) remained susceptible to meropenem. All isolates had meropenem MICs above the EUCAST screening breakpoint for carbapenemase detection (0.125 mg/L). Aztreonam-avibactam showed the highest in vitro activity (98% susceptible), followed by ceftazidime-avibactam (90%). Ceftazidime alone retained susceptibility in 68% of OXA-48-producing isolates, whereas no activity was observed against isolates producing other carbapenemase types.

Conclusion: This study provides the first comprehensive characterization of CPSER in Germany, demonstrating that the recently described *S. sarumanii* accounts for the majority of isolates. The predominance of OXA-48-like enzymes in Germany contrasts with global datasets dominated by KPC and NDM, likely reflecting national epidemiological characteristics which are underrepresented in international surveillance.

WS16.02

When antimicrobial resistance meets virulence: Convergence and fitness adaptation in clinical *Klebsiella pneumoniae*

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Klebsiella pneumoniae has become one of the most concerning bacterial pathogens in both healthcare and community settings. Its success is driven by remarkable genomic plasticity, allowing the acquisition of antimicrobial resistance (AMR) determinants alongside virulence-associated

traits. Particular concern is caused by convergent strains that combine multidrug resistance with enhanced virulence, as these may challenge both treatment and infection control.

To move beyond genotype-based assumptions, we established a robust *Galleria mellonella* infection model to functionally differentiate *K. pneumoniae* pathotypes. Using a previously sequenced and genomically characterized non-clonal isolate collection, selected to represent distinct resistance and virulence architectures, the model reliably separated classical (*cKp*), hypervirulent (*hvKp*), and convergent isolates based on their pathogenicity profiles.

These findings are embedded in our broader genomic work showing that global *K. pneumoniae* populations are shaped by internationally disseminated high-risk clones, some of which are also detected across One Health compartments, including wildlife-associated settings. Importantly, several of these globally disseminated lineages are increasingly associated with convergent resistance and virulence traits. This was exemplified by the outbreak-associated ST307 lineage in Western Pomerania, in which we identified a hybrid virulence-resistance plasmid carrying both virulence-associated loci and clinically relevant AMR determinants.

To better understand the evolutionary consequences of convergence, we combined experimental evolution and genome-wide functional screening. Experimental evolution under ceftazidime-avibactam exposure led to resistance development, while subsequent evolution revealed compensatory adaptation mitigating associated fitness costs. In addition, Transposon Directed Insertion-site Sequencing under cefiderocol exposure identified genetic determinants contributing to resistance. Notably, selected knockouts were not only associated with increased cefiderocol MICs, but also with enhanced virulence in the infection model, suggesting unexpected links between resistance mechanisms, fitness, and pathogenicity.

Together, our data highlight the dynamic interplay between clonal success, AMR evolution, and virulence in *K. pneumoniae*. They further demonstrate the value of integrating functional pathotyping, comparative genomics, experimental evolution, and genome-wide screening to understand the emergence and stabilization of convergent high-risk clones across clinical and One Health settings.

WS16.03

Whole genome sequencing (WGS) and improved diagnostics uncover a putative multispecies plasmid-borne GES-5/OXA-917 carbapenemase outbreak driven by the *Citrobacter freundii* complex

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Background: The introduction of EUCAST screening breakpoints in 2018 and their implementation at our tertiary care university hospital in Germany (UHoR) led to frequent detection of multidrug-resistant (MDR) GES-5-positive *Citrobacter freundii*.

Aim: To investigate infection control implications of rising numbers of GES-5 positive isolates using whole genome sequencing (WGS).

Methods: GES-5-positive *C. freundii* isolates (2020-2025, n=34) were sequenced (long-reads: Oxford Nanopore Technologies, short-reads: Illumina NextSeq) and hybrid assemblies were generated. Further MDR, GES-5- and/or OXA-917-positive GNB identified during routine WGS-based surveillance were retrieved from our database.

Environmental investigations were done at five departments (n=118, three rounds, sinks and shower drains). The samples were screened using an in-house specific GES-5 PCR and, if positive, cultured on selective media. Five additional isolates were available from routine investigations (toilets). All isolates were also sequenced.

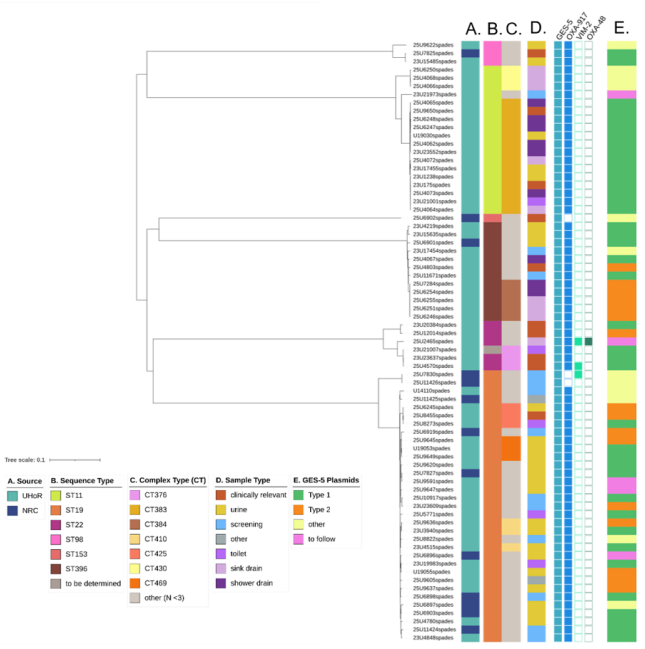
Further GES-5-positive isolates from Germany (not Regensburg) collected between 2011-2025 were provided for WGS by the National Reference Centre (NRC; 15 of 31 *C. freundii*, 11 of 26 other enterobacterales).

Results: Of 34 MDR *C. freundii* diagnosed at UHoR, 30 met the screening breakpoint and were confirmed with GES-5 carbapenemase. Four isolates were only diagnosed during WGS-based surveillance. Environmental PCR-based investigations revealed 42/118 positive samples. GES-5-positive strains (all *C. freundii)* grew from only 23/42 samples, totaling 28 environmental isolates.

WGS divided UHoR *C. freundii* isolates – human and environmental - into nine genotypes. Three sequence type [ST] predominated in both patient and environmental samples: ST19 (19/3), ST11 (8/9) und ST396 (8/1). Among NRC isolates 13/15 *C. freundii* and 2/11 Enterobacterales were GES-5/OXA-917 positive, and *C. freundii* isolates clustered those from UHoR. All isolates were confirmed with plasmid-born GES-5 and OXA-917 carbapenemases using plasmid visualization. Notably, two plasmid types of incompatibility class N (size: 26.8 kb and 25.5 kb) that only differ by one insertion sequence (IS26) were identified in a majority of *C. freundii* isolates (Regensburg: n=27, NRC: n=6, environmental: n=20) and several other enterobacterales (Regensburg: n=6, Germany: n=4) from Regensburg and Germany. Epidemiological and clinical data rarely suggest nosocomial transmission.

Conclusion: Implementation of EUCAST screening breakpoints revealed high prevalence of GES-5 and OXA-917 in Regensburg. WGS confirmed that the antimicrobial resistance is determined by highly conserved plasmids with national distribution, and the resuts do not indicate a clinical outbreak at UHoR. Further investigations are ongoing.

Fig. 1



WS16.04

Co-dissemination of *mcr-9* and carbapenemase genes via conserved IncHI2A plasmids in carbapenem-resistant *Citrobacter* spp. and *Enterobacter cloacae* complex isolates in a German tertiary-care hospital, 2018–2022

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Introduction: Colistin is a last-line option against multidrug-resistant Gram-negative bacteria, but mobile colistin resistance (*mcr*) genes threaten its efficacy. Unlike other variants, *mcr-9* often does not confer phenotypic resistance, is inducible under colistin exposure, and may therefore escape routine susceptibility testing. Data on *mcr-9* in clinical isolates from hospitalised patients in Germany remain sparse.

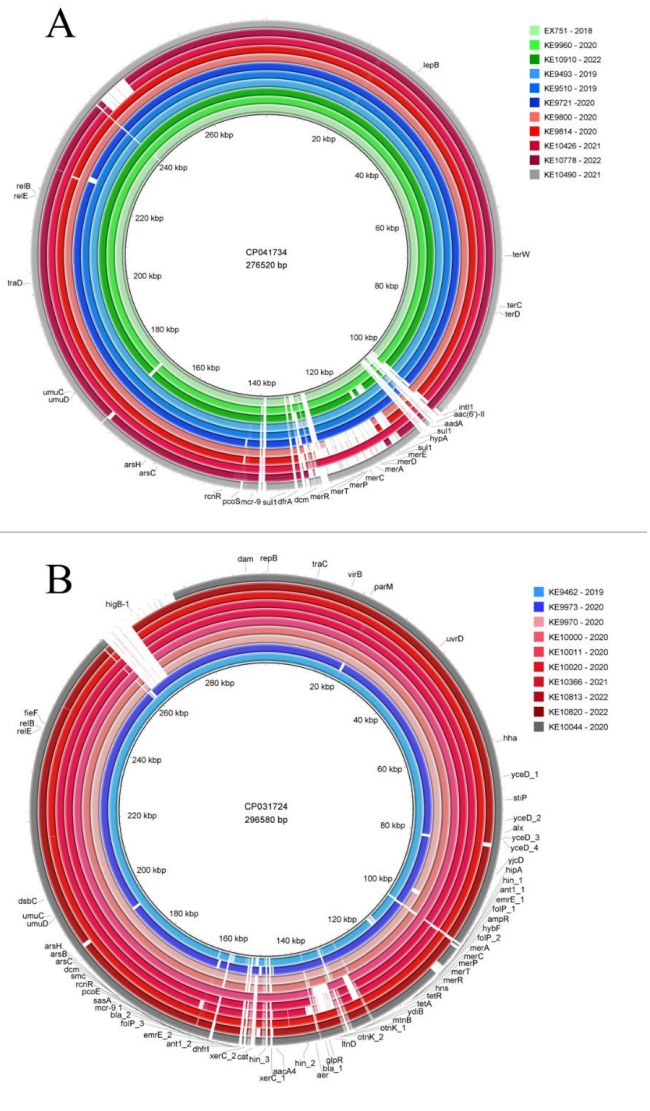
Objectives: To investigate the occurrence, genetic localization and phenotypic colistin susceptibility of *mcr-9* in carbapenem-resistant *Citrobacter* spp. and *Enterobacter cloacae* complex isolates from a German tertiary-care hospital.

Materials and Methods: 181 carbapenem-resistant isolates from 175 patients, collected from rectal screening samples between 2018 and 2022, were analysed. Whole-genome sequencing (Illumina MiSeq) was used to characterise resistance determinants and plasmids (MOB-suite, AMRFinderPlus, ANIclustermap, BRIG). Colistin MICs were determined by broth microdilution and interpreted per EUCAST v15.0.

Results: *mcr-9* was detected in 35/181 isolates (19.3%), including *mcr-9.2* (24/35) and *mcr-9.1* (11/35). The proportion rose from 9.1% (2018) to 31.3% (2020) and remained high thereafter. Most *mcr-9*-positive isolates also carried carbapenemase genes (33/35; 94.3%), and 11 were dual carbapenemase producers, predominantly *E. roggenkampii* ST96. *mcr-9* was plasmid-borne in 33/35 isolates, located on IncHI2A plasmids belonging to a single primary cluster, indicating a shared, conjugative backbone circulating across species and years. In nine isolates, *mcr-9* was co-located with carbapenemase genes (*bla*$_{KPC-2}$, *bla*$_{OXA-48}$, *bla*$_{VIM-1}$) on the same plasmid. Despite carriage of *mcr-9*, most isolates remained colistin-susceptible; resistance (>2 mg/L) occurred in 7/35.

Summary: A conserved IncHI2A plasmid backbone repeatedly disseminated *mcr-9* across multiple species and years, with frequent co-location of *mcr-9* and carbapenemase genes. The genotype-phenotype discordance favours silent spread, highlighting the need for molecular surveillance of last-resort resistance determinants.

Fig. 1



WS16.05

Method comparison between whole genome sequencing and fourier transform infrared spectroscopy for carbapenemase-producing *A. baumannii* strains

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Introduction: Infection with carbapenem-resistant *Acinetobacter baumannii* is associated with high mortality rates globally. Rapid and reliable typing methods are crucial for the prompt identification and tracking of strain transmission in the wake of a potential clinical outbreak. Whole genome sequencing (WGS) and Fourier Transform Infrared Spectroscopy (FTIR) have emerged as effective methods for bacterial typing in this context.

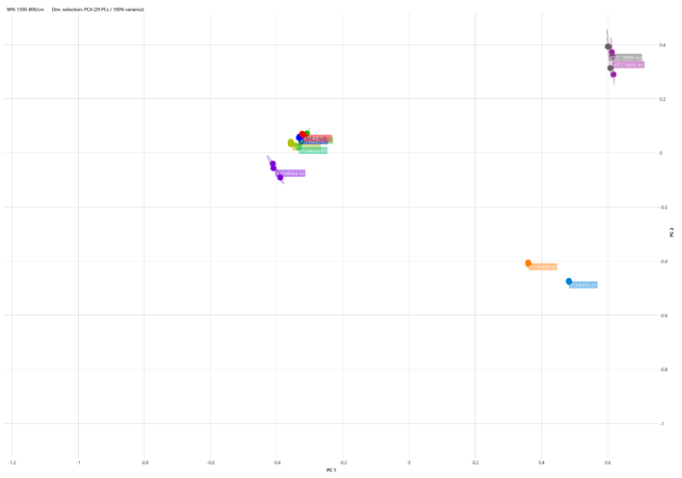
Objective: This study aimed to compare the reliability of WGS and FTIR (IR Biotyper®, Bruker Daltonics, Bremen, Germany) for characterizing a dataset of eight Carbapenem-resistant *A. baumannii* strains involved in a university hospital outbreak and to assess its potential for enhancing infection control and tracking transmission dynamics.

Methods: A total of eight carbapenemase-producing *A. baumannii* isolates were included in the study. Isolates were cultured for 24 hours on three different media (Columbia blood agar, Müller-Hinton agar and MacConkey agar. Identification was done using Vitek-MS (BioMerieux, France), AST results were obtained through the Vitek system (BioMerieux, France). Spectra acquisition, processing and analysis were performed using the FTIR Biotyper® system (IRBT; Bruker Daltonics, Germany) using ATCC19606 strain as control. WGS was performed at the German National Reference Centre for multidrug-resistant gram-negative bacteria.

Results: WGS was able to detect three different sequence types (ST), each of them defining one cluster (ST2, ST19, ST2826). IRBT showed similar results, confirming 3 distinct and different clustering differences between the analyzed *A. baumannii* strains corresponding to the distinct outbreak (Fig. 1).

Conclusions: Even though both methods were able to identify all outbreak-associated strains, the cgMLST analysis revealed allelic differences of up to 28 within clusters formed by FTIR. Considering the commonly used threshold of about 9 alleles for *A. baumannii,* these isolates would genomically not be classified as part of the same transmission cluster. In this study, FTIR was able to reproduce these WGS results. FTIR might thus represent a rapid screening and preliminary typing method for poential outbreak investigation. Although WGS continues to provide significantly higher resolution, FTIR could be considered as a faster, cheaper and more user-friendly identification for Carbapenemase-producing *A. baumannii* strains.

Fig. 1



WS16.06
Molecular epidemiology of vancomycin-resistant *Enterococcus faecium* in southwestern Germany

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Introduction: Vancomycin-resistant *Enterococcus faecium* (VREfm) is a major nosocomial pathogen with increasing prevalence in Germany. Clinical VREfm isolates show a high degree of clonality, with sequence types (STs) 117 and 80 predominating nationwide. This study investigated the VREfm clones circulating in southwestern Germany.

Material and Methods: A total of 24 bacterial isolates were recovered in 2025 from rectal swabs collected from a hospital in the federal state of Rhineland-Palatinate, Germany. Species identification and antimicrobial susceptibility testing were performed using the BIOMERIEUX VITEK 2 system. DNA extraction was conducted with the Promega Maxwell platform, and whole-genome sequencing was performed on an Illumina NextSeq 1000 platform. Analysis included cgMLST, species confirmation (Mash Distance), *van* gene detection (AMRFinder), ST, and complex type (CT) assignment using the MBioSEQ Ridom Typer (Bruker). Isolates were additionally compared with a database of >3,000 *E. faecium* isolates from different regions of Germany.

Results: All isolates were identified as *E. faecium* and were tested resistant to vancomycin, ampicillin and susceptible to linezolid. Thirteen isolates carried *vanA* and were resistant to teicoplanin, while 10 isolates carried *vanB* and remained susceptible to teicoplanin. One isolate harboured both *vanA* and *vanB* and was resistant to teicoplanin. The predominant lineage was ST80 (n = 20), assigned to CT1470 (n = 9), CT6650 (n = 4), CT6045 (n = 2), CT6028, CT9408, CT10075, CT10359, and CT10360 (n = 1 each). Two ST117 isolates belonged to CT71 and CT9733, respectively. One isolate belonged to ST1299 (CT10358), and one represented a novel

ST. cgMLST analysis using a threshold of ≤3 allelic differences identified three clusters, each consisting of two closely related ST80/CT1470 isolates, while all remaining isolates were unrelated. Comparison with isolates from Germany showed that three ST80/CT1470 isolates differed by <2 alleles from isolates recovered in western Germany, and one ST80/CT10075 isolate was identical to an isolate from the same region. The ST117/CT71 isolate differed by 7 alleles from an isolate recovered in west-central Germany in 2017, whereas the closest related isolate to ST117/CT9733 differed by 29 alleles. The ST1299 isolate showed no close relation to previously reported German ST1299 isolates.

Conclusion: The investigated VREfm population from Rhineland-Palatinate was dominated by the ST80/CT1470/*vanA* clone, highlighting the ongoing dissemination of this lineage in Germany. cgMLST analysis demonstrated both local transmission clusters and close genetic relationships to strains previously identified in other German regions. These findings underline the importance of continuous genomic surveillance to monitor the spread and evolution of epidemic VREfm clones in healthcare settings.

WS17.01

Staphylococcus aureus exploits the lung environment to cause persistent infections

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Staphylococcus aureus is a bacterial pathogen associated with one million annual deaths. The pathogen's sophisticated virulence strategies enable it to cause severe infections in versatile tissues and organ systems including skin and soft tissue infections, endocarditis, septicemia and pneumonia. *S. aureus* pulmonary infections are especially dangerous for people suffering from cystic fibrosis (CF). Hallmarks of CF are thick lung mucus and a decreased mucociliary clearance, which predispose people with CF to chronic lung inflammation and infection. In affected lungs, *S. aureus* small colony variants (SCVs) frequently emerge, which are characterized by a reduced growth rate and higher resistance against antibiotic treatment. Despite their widespread and manifold auxotrophies, these SCVs cause persistent and recurring infections. Here, we investigated a refined mechanism that enables *S. aureus* SCVs to cause relapsing pulmonary infections. Using biochemical and growth analyses, we particularly identified bacterial key factors required to overcome host-shaped stresses encountered in pulmonary environments. Supported by tissue culture experiments and a murine pneumonia model, we discovered a crucial resilience strategy utilized by *S. aureus* SCVs. Overall, our findings may refine antibacterial therapy in CF and aid in the development of novel antibacterial treatments specifically targeting staphylococcal SCVs in pulmonary infections.

WS17.02

Comparative analysis of two membrane-based methods for the differentiation of viable and dead *Listeria monocytogenes*

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Listeriosis is a foodborne infectious disease with high relevance for public health. The common route of infection is ingestion of food contaminated with *Listeria monocytogenes* (*Lm*) that enters the food chain via contaminated raw material. This ubiquitous pathogen is known to survive a broad range of conditions, facilitating its persistence in food-processing environments. Adverse environmental stressors such as nutrient deprivation, cold stress or disinfectant use have been shown to promote the induction of the viable but non-culturable (VBNC) state. VBNC cells have lost their ability to grow on culture media, but maintain their metabolic activity and consequently, can contaminate foodstuffs.

According to DIN 10516, the effectiveness of cleaning and disinfection in food-processing environments is monitored using culture-based analysis of surface samples. However, as VBNC cells are not detected by these standard methods, viable cell numbers may be underestimated. Therefore, viability-based methods independent of bacterial growth may improve detection of viable cells. These include staining methods that discriminate between viable and dead cells due to differences in membrane integrity.

In this study, two membrane-based methods, namely LIVE/DEAD™*Bac*Light™ staining with subsequent fluorescence microscopy and viability real-time PCR using propidium monoazide (PMA-qPCR), were compared for their power to discriminate between viable and dead *Lm*. Cell mixtures (~108 cfu/ml) containing viable and isopropanol-inactivated cells (20 min, 21 °C) at ratios from 1:1 to 1:100,000 were analyzed. Furthermore, their ability to detect VBNC cells after long- and short-term induction was assessed. VBNC induction was achieved by starvation in mineral water (up to eleven weeks) or detergent exposure (1 h), respectively.

We were able to show that both methods are reliable tools for differentiating viable and dead *Lm*. The dual staining of the *Bac*Light™ kit allows to directly visualize the two physiological

states. Combined with automated image-based cell counting, both viable and dead cells can be quantified. This method detected viable cells at ratios up to 1:1000 within mixtures of dead cells.

For PMA-qPCR, specific primer and probe sets were used for detection of *Lm* and the dead-cell control *Listeria grayi*. The death control was included to verify PMA treatment efficiency. qPCR of the samples treated with and without PMA quantified genomic equivalents of viable and total cells, respectively. PMA-qPCR detected viable cells at ratios up to 1:10,000 within mixtures of dead cells. As PMA did not completely inhibit amplification of DNA from dead cells, viable cell numbers may have been overestimated.

Both methods detected VBNC cells after long- and short-term induction. While *Bac*Light™ staining and PMA-qPCR detected similar cell numbers after short-term induction, starved VBNC cells were underestimated by PMA-qPCR.

WS17.03
Differential gene expression of *Listeria monocytogenes* in the viable but non-culturable (VBNC) stage

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Introduction: Bacterial pathogens such as *Listeria monocytogenes*, which are also found in food, can enter a viable but non-culturable (VBNC) state. In this state, they are no longer detectable in food using established cultivation techniques, but can still lead to outbreaks. It has also been shown that VBNC cells are more resistant to decontamination attempts and therefore survive in biofilms. The changes that occur in the metabolism of *L. monocytogenes* strains in the VBNC state have not yet been studied in detail. However, this knowledge is essential to understand the development of the VBNC stage of *L. monocytogenes.* Moreover, it can contribute to improve detection systems for VBNC cells of *L. monocytogenes* in food or develop methods to reduce the persistence of these pathogens in biofilms.

Aims: The focus of this examination was to investigate the transcriptional activity of the *L. monocytogenes* strain L234, an outbreak-associated isolate from pork, by comparing gene expression level of VBNC cells to control cells at cold temperatures.

Material and Methods: The VBNC cells were prepared by starvation in sterile water. The control cells were grown in BHI medium, both at 12 °C. After harvesting of the cells, the RNA was extracted and sequenced using next generation sequencing technologies (Illumina). Differential expression analysis was performed using Deseq2.

Results: Differential gene expression (DGE) analysis showed distinct gene expression pattern of control and VBNC cells, with more than 2.200 genes being differentially expressed (p-value < 0.05). KEGG enrichment analysis of upregulated genes of VBNC cells (> 1 Log2FC) revealed a significant enrichment of genes for ABC transporters, for peptidoglycan biosynthesis and histidine metabolism. KEGG enrichment analysis of downregulated genes (< -1 Log2FC) of VBNC cells, showed enrichment of genes for the phosphotransferase system, several sugar metabolism pathways, lipoic acid metabolism and the folate biosynthesis. Virulence gene expression of VBNC *L. monocytogenes* showed an upregulation of genes for adherence, stress survival, invasion, posttranslational modification and several nutritional and metabolic factors as well as several genes for metal uptake.

Conclusion: The results of DGE and KEGG enrichment analysis show that *L. monocytogenes* L234 cells undergo extensive changes in gene expression across a wide range of metabolic pathways upon entering the VBNC stage, enabling the cells to adapt their membrane transport systems and the composition of their cell wall, as well as to utilize histidine as an alternative source of carbon, energy and nitrogen. Further, some virulence genes of *L. monocytogenes* L234 cells were upregulated in the VBNC stage, which possibly support the survival in this stage or maintains infectivity.

WS17.04
***Klebsiella pneumoniae* evolution of heterovirulence in the urinary tract**

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Infections caused by classical *Klebsiella pneumoniae* (cKp) strains are a major burden of mortality and morbidity in the clinic and are becoming increasingly difficult to treat due to rising multidrug-resistance. Traditionally, research on cKp has focused mostly on the acquisition and evolution of antibiotic resistance factors which has also revealed a potential role of heteroresistant bacterial populations in complicating the treatment of infections. Heteroresistant populations include antibiotic resistant subpopulations that are difficult to detect in diagnostic settings due to a fitness cost in the absence of the antibiotic and serve as a steppingstone in the evolution to high level antibiotic resistance. Meanwhile studies on the pathogenesis of cKp have been limited by low virulence in animal infection models despite clearly documented morbidity and mortality in the clinic. Despite these limitations, we combined clinical observations, epidemiology and mechanistic investigations to study the within-patient evolution of virulence of cKp. Our studies highlighted important pathogenesis principles of cKp in UTIs[1] and led to the discovery of heterovirulence in the urinary tract[1,2]. In analogy to heteroresistance, heterovirulent populations have increasingly virulent subpopulations that do not take over the population due to a fitness cost affecting biofilm formation and invasion of bladder epithelial cells, as well as slower growth in axenic medium which may complicate the detection of heterovirulence in the clinic.

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the Urinary Tract. New England Journal of Medicine (accepted for publication)

WS17.05

Molecular detection of dormant *Listeria monocytogenes* driving persistent contamination in food production settings

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Questions: Bacterial dormancy enables survival under fluctuating or lethal conditions through reversible phenotypes that vary in metabolic activity and antimicrobial tolerance without genetic change (1). Dormant states are widespread among pathogens and may drive persistence in hygiene-critical settings (2 *Listeria monocytogenes* can persist in food production environments for months to years, with irregular recurrence of clonal isolates at the same sites without evidence of reintroduction. Dormancy states may explain these patterns and enable survival despite intensive cleaning and disinfection measures (3)

In this study we investigated the contribution of dormancy to persistent *L. monocytogenes* contamination in three Austrian food production facilities by combining microbiological and molecular biological techniques. In addition, we developed and evaluated diagnostic strategies for the detection of dormant cells including combinatorial approaches or viability PCRs.

Methods: Over 3–6 months, we sampled three independent Austrian food production facilities, two with known persistent *L. monocytogenes* contamination, collecting more than 300 samples. All samples were tested for *L. monocytogenes* using the ISO 11290-1, a routine PCR performed after the first enrichment, and a direct qPCR to obtain quantitative information on non-growing cells. Culture-positive, confirmed *L. monocytogenes* isolates underwent whole-genome sequencing, and core-genome MLST was performed following Ruppitsch et al. (4).

In parallel, we developed and evaluated a vPCR using the PMAxx viability dye and the PMA-Lite 2.0 LED Photolysis Device. Seven primer sets were screened to identify those suitable for routine vPCR use. We also assessed the impact of different disinfection techniques and commercial disinfectants to identify potential biases in live/dead discrimination. Finally, we tested the protocol"s applicability in food processing environments via spike-in experiments using natural matrices with relevant background flora.

Results & Conclusions: Analysis of >300 samples from three food production facilitiesindicated the presumptive presence of dormant Listeria monocytogenes that may contribute to persistent contamination under varying scenarios. Our results show that it is possible to detect and quantify intact, non-cultivable (presumptively dormant) cells and clearly distinguish them from compromised (dead) cells and free DNA. However, we also identified technical hurdles and potential interfering factors, such as certain disinfectants, highlighting the need for careful interpretation of vPCR results. Additionally, under high-contamination scenarios, the practical utility of molecular methods can be limited by the interpretability of the results.

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4. Ruppitsch, W. et al. J. Clin. Microbiol. 53, 2869–2876, 2015

WS18.01

Accuracies of multimodal large language models used in a zero-shot-fashion for detecting bacterial growth on agar media

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Question: Advances in computer vision and deep learning have promoted the creation and commercial distribution of models able to classify images of agar plates regarding the growth of bacteria into "positive" (i.e. growth present) and "negative" (i.e. growth absent). These models require testing and validation on many thousands of pictures. Recent multi-modal large language models (LLMs) could potentially yield comparable performances without the need for training. The current project explores this idea using a small sample of images of three media types.

Methods: Images of to-be-discarded agar plates from routine requests processed in a microbiology laboratory were acquired using an iPhone 14 (Fig. 1A) and subsequently segmented, de-skewed, and cropped using custom-made software "arucutter", version 0.1.3 (https://github.com/joheli/arucutter, Fig 1B). The following media types were used: Chromid® VRE, Chromid® ESBL (bioMérieux), and BBLTM CHROMagarTM MRSAII/SA bi-plates (BD). Processed images were submitted together with custom prompts to LLMs Gemma 3 (4b), Gemma 4 (e4b), Qwen 3.5 (4b), and Qwen 3-VL (8b) served over "Ollama" (Figure 1C). Calls to LLMs were relayed in a "zero-shot" fashion using custom library "oneshot", version 0.1.5 (https://github.com/joheli/oneshot).

Results: An image sample of 83 bi-plates, 79 ESBL plates, and 36 VRE plates was labelled. Proportions of media without growth (labelled "negative") were 50.6%, 29.1%, and 55.6%, respectively. Accuracies of LLMs ranged from 0.458 to 0.975 and were lowest for classification of entire bi-plates and model Gemma 3. In contrast, accuracy was higher when the two bi-plate halves were evaluated separately against the ground-truth labels (*S. aureus*, 0.578 to 0.819; MRSA, 0.651 to 0.940), rather than when both halves were compared jointly (Figure 2). The highest accuracy of 0.975 was achieved in ESBL-plates by both models Gemma 4 and Qwen 3.5. All LLMs yielded identical accuracies for the VRE subset (33 correct classifications out of 36) but failed on different images. Classification time was longer for the two "Qwen" compared to the two "Gemma" models.

Conclusions: LLMs called in a zero-shot fashion achieve remarkable accuracies regarding the classification of agar media into "positive" (i.e. specific bacterial growth present) and

"negative" (i.e. specific bacterial growth absent) on routine media used for the screening of multi-resistant bacteria. Classification of bipartite bi-plates (containing separate halves for *S. aureus* and MRSA) seems more challenging than assessment of entire ESBL or VRE agar media. Further studies are necessary to explore whether few-shot approaches can further improve accuracies.

Fig. 1

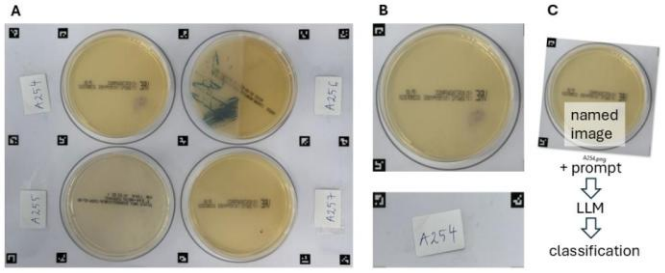

Fig. 2

LMM	Biplate: both halves	Biplate: *S. aureus*	Biplate: MRSA	ESBL	VRE
Gemma3 (4b)	0.458 (38 of 83)	0.578 (48 of 83)	0.651 (54 of 83)	0.886 (70 of 79)	0.917 (33 of 36)
Gemma 4 (e4b)	0.759 (63 of 83)	0.819 (68 of 83)	0.940 (78 of 83)	0.975 (77 of 79)	0.917 (33 of 36)
Qwen 3.5 (4b)	0.663 (55 of 83)	0.675 (56 of 83)	0.795 (66 of 83)	0.975 (77 of 79)	0.917 (33 of 36)
Qwen 3 VL (8b)	0.711 (59 of 83)	0.735 (61 of 83)	0.904 (75 of 83)	0.899 (71 of 79)	0.917 (33 of 36)

WS18.02

CAP-NGS: Advantage of molecular diagnostic in community-acquired pneumoniae with new generation sequencing

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Community-acquired pneumonia (CAP) is a respiratory illness which can progress to severe complications and even death. Diagnosis of CAP mostly depends on clinical presentation, radiology, and culture. As a result, the causative pathogen is not definitively identified in up to 60% of the cases, leading to sub-optimal therapy. Diagnosis of CAP is further complicated by the diverse aetiology of the disease, in terms of the presence and distribution of CAP-causing pathogens in different regions. Metagenomic sequencing (mNGS) is an emerging solution to unbiased CAP diagnosis. However, its application remains challenging due to the cost, turnaround time, and the need for skilled bioinformaticians. The study aims to compare two different next-generation sequencing technologies: the targeted RPIP panel from Illumina and the untargeted metagenomic sequencing (mNGS) to the gold-standard diagnostic based on culture and targeted PCR. We included 200 nasopharyngeal swabs for CAP patients as well as 100 matching bronchial lavage samples. For qPCR, samples were extracted with alphaClean RNA/DNA extraction kit and analyzed with Mikrogen's ampli Cube Respiratory Bacterial Panels 1, 3, and 4. For sequencing, samples were extracted using ZymoBIOMICS™ DNA/RNA Miniprep Kit before library preparation with Illumina's Respiratory Pathogen ID/AMR Enrichment Panel Kit. The enriched factions were analyzed with the DRAGEN Microbial Enrichment Plus workflow on Illumina's BaseSpace, while the untargeted factions were analyzed with an in-house pipeline based on Kraken2. Compared to the gold standard qPCR assays targeting seven common CAP pathogens (Staphylococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis, Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae), RPIP panel showed a low rate of detection of 13.54%. On the other hand, mNGS achieved a detection rate of 52.94%. Of note, combining qPCR and mNGS allowed pathogen detection in 66.44% of the samples, compared to 41.86% when using qPCR alone. Metagenomic sequencing also detected other CAP-relevant bacteria (Pseudomonas aeruginosa, Acinetobacter baumannii, Coxiella burnetii), fungi (Aspergillus spp., Fusarium spp., ...), and viruses (herpes simplex virus, betacoronavirus, ...) in 80 samples. The results of our study indicate that the combination of qPCR and mNGS would increase the rate of detection of the CAP pathogen, leading to better diagnostics. Of course, our study suffers some limitations, especially regarding viral detection as the usage of cryo-preserved samples reduces the quality of the RNA. Furthermore, currently our results support a combined usage of the two methods, either simultaneously or sequentially. Therefore, further studies are required to evaluate the effects of different extraction protocols on the performance of this assay, which may explain the discordance between mNGS and traditional qPCR.

WS18.03

Comparison of sample worklow processes of explanted prosthesis for sonication – A prospective diagnostic study

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Background: Sonication is a key component in the diagnosis of device associated infections. Especially in periprothetic joint infections (PJI) the quantified results of culture from sonicate fluid can be used to define an infection according to the unified definition of PJI. However, the ideal sample processing for have not been fully identified.

The goal of this study was to identify the impact of an modified workflow process for the sonication and culturing compared to the standard process.

Methods: The standard process includes the use of 0.9% saline solution with 30 seconds vortexing of the sample followed by 5 minutes of sonication with subsequent additional

vortexing for 30 seconds. 500µl Sonication fluid were plated onto each agar plate (Columbia, Chocolate, UTI, Schaedler, Schaedler KV) and 4ml were inoculated into BMX Bactalert PED bloodculture bottles. In the modified workflow samples were sonicated for 1 minute without vortexing. Sonicate fluid was centrifuged at 3000 RPM for 15 minutes. The supernatant was removed and the pellet resuspended. Agarplates as well as Thioglycolate broth were inoculated with 30µl of the resuspended pellet.

Detection of growth an quantification of CFU/ml were interpreted according to the EBJIS/MSIS/ESGIAI definition for periprosthetic joint infection /Cutoff of >=50CFU/ml in the standard process and >=200 CFU/ml in the modified process.

Results: We included a total of 129 routine samples into the study. Overall a total of 91 samples showed no growth at all. Any growth of potential pathogens was detected in 43 samples for the standard samples and 38 samples in the modified process. Relevant CFU/ml proofing a PJI according to EBJIS criteria were detected in 16 samples processed with the standard process and 21 samples with the modified process respectively. In 8 samples the modified process lead to a additional PJI defining CFU count compared to the standard process (major Error). In 2 samples the modified process led to a CFU count of <200CFU/ml compared to a CFU >50CFU/ml in the standard process (very major error). Staphylococcus *epidermidis* was identified in 9 samples (18.8%) followed by Klebsiella *oxytoca* and Staphylococcus *capitis* in 4 samples each (8.3%) followed by Staphylococcus haemolyticus in 3 samples (6.2%).

Conclusion: Sonication is a relevant tool in the diagnostic process of PJI. The modified sample process does not appear to reduce detection of pathogens or relevant CFU count. Our data suggest an even increased detection of PJI proving CFU counts in sonicate fluid.

WS18.04

A simple sonication protocol for improved microbiological diagnosis of infective endocarditis

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Background: Infective endocarditis (IE) is associated with high morbidity and mortality, while microbiological diagnosis is often limited by prior antibiotic therapy, fastidious organisms and biofilm formation, particularly in prosthetic valve endocarditis. Sonication may improve pathogen detection by disrupting biofilms. This study evaluated a simplified sonication protocol for routine IE diagnostics.

Methods: 32 explanted native and prosthetic heart valves from 27 patients with suspected IE were analyzed using conventional culture, sonication-assisted culture and molecular diagnostics. 56 control samples from patients without IE served as negative controls. Sonication involved mechanical homogenization, 1-minute ultrasonic treatment (BactoSonic), vortexing and culture on standard media. Molecular testing included broad-range 16S rRNA PCR and multiplex PCR.

Results/Discussion: Conventional culture identified microorganisms in 17/32 samples (53%), with 15/32 (47%) considered true pathogens. Sonication detected growth in 18/32 samples (56%), with 17 true pathogens, representing an ~6% increase in diagnostic yield and faster detection.

In native valves (n=13), conventional culture was positive in 9 cases, while sonication detected 8 positives with earlier growth (often day 1). One culture-positive late-growing viridans streptococcus was missed by sonication. Detected pathogens included viridans group streptococci, *Staphylococcus aureus, Streptococcus pyogenes* and *Enterococcus faecalis.*

In prosthetic valves (n=19), conventional culture identified 8 positives (including 2 contaminants), while sonication detected three additional true pathogens and one contaminant. Sonication also accelerated detection in several cases (growth on day 1–2 vs up to day 7). Pathogens included coagulase-negative staphylococci (including *S. lugdunensis*), *E. faecalis*, viridans streptococci, *S. aureus* and *Proteus* mirabilis.

Molecular diagnostics (n=25) identified pathogens in 23 cases (92%) and showed good concordance with culture. Some organisms detected by sonication, including *S. lugdunensis*, were not identified by PCR but supported by positive blood cultures. Combined use of all methods enabled pathogen detection in all clinically suspected IE cases. The most frequent pathogens were *S. aureus*, viridans streptococci, coagulase-negative staphylococci, *E. faecalis* and β-hemolytic streptococci.

Among 56 controls, one conventional culture grew *S. epidermidis*. Sonication detected three contaminants (*Cutibacterium acnes, Staphylococcus hominis, S. capitis*).

Conclusion: A simplified sonication protocol improved pathogen detection from explanted heart valves, increased diagnostic yield, and shortened time to positivity while maintaining low contamination rates. The method is easy to implement and cost-effective. In combination with molecular diagnostics, sonication could enhance diagnostic accuracy in IE and support earlier targeted antimicrobial therapy.

WS18.05

Expanding molecular TB diagnostics beyond sputum: Performance assessment across diverse pulmonary and extrapulmonary sample materials for molecular detection of *M. tuberculosis* complex and first-line drug resistance

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Detection of *Mycobacterium tuberculosis* in non-sputum specimens is crucial for patients who are unable to produce sputum. However, diagnosing extrapulmonary tuberculosis remains challenging due to its varied presentations and the limited availability of molecular methods. In this study we evaluated the diagnostic accuracy of the FluoroType® MTBDR VER 2.0 for detection of *M. tuberculosis* complex (MTBC) and first-line drug resistance in non-sputum specimens including

bronchoalveolar lavage (BAL), tracheobronchial secretions (TBS), pleural and pericardial effusions, and tissue biopsies from the lung, liver and lymph nodes. The performance of FluoroType® MTBDR on the FluoroCycler® XT was assessed in an observational, non-interventional diagnostic accuracy study using anonymized left-overspecimens. The specimens underwent NALC-NaOH decontamination and tissues were homogenized and treated with Proteinase K. DNA extraction was performed using either the manual FluoroLyse method or the automated procedure using GenoXtract® with X2 Cartridge. The results were compared to a composite reference standard comprising MGIT culture (confirmed by GenoType MTBDRplus or GenoType MTBC), phenotypic drug susceptibility testing, and sequencing analyses. A total of 532 samples were analysed in this study, comprising of 32 % MTBC positive specimens according to the reference standard. The samples were divided into approximately 24 % BAL, 29 % TBS, 17 % effusions and 30 % tissue. The assay demonstrated high diagnostic accuracy across all specimen types, with strong specificities (from 93 % to 100 %) and sensitivities (from 82 % to 100 %) for MTBC detection in the combined smear-positive and smear-negative sample groups. Agreement for resistance detection was very high (from 98 % to 100 %), with reliable identification of mutations associated with isoniazid and rifampicin resistance. Both extraction workflows demonstrated robust and comparable performance, even with challenging specimen matrices such as tissues. We found that the FluoroType® MTBDR assay is suitable for detecting MTBC and resistance-conferring mutations in non-sputum specimens. This has the potential to improve diagnostic coverage for extrapulmonary tuberculosis and for patients who are unable to produce sputum. These results suggest that the use of the assay could be expanded beyond sputum-based testing to include a broader range of clinically relevant specimen types.

WS19.01

Salmonella SopB suppresses post-transcriptionally regulated cytokine release to reduce early tissue inflammation and delay disease progression in neonates

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Salmonella enterica subsp. *enterica* serovar Typhimurium (*S.* Typhimurium) is a major cause of childhood mortality, particularly in low-resource settings. The *Salmonella* pathogenicity island 1 (SPI-1)–encoded type 3 secretion system (T3SS) effector SopB is known to influence bacterial internalization, host cell survival and trafficking, and signalling pathways. In our previous work (Zhang K *et al.*, 2018), we made the unexpected observation that neonatal mice infected with a SopB-deficient (Δ*sopB*) *S.* Typhimurium strain developed a markedly accelerated disease course with premature mortality— an phenomenon that was strongly age-dependent.

To investigate this further, we employed our recently established neonatal murine infection model and identified a mechanistic basis for SopB-mediated modulation of early epithelial immunity. We found that SopB inhibits activation and plasma-membrane translocation of the metalloprotease ADAM17, thereby limiting the shedding of membrane-bound TNFα from enterocytes. Also, SopB reduced epithelial IL-18 secretion through mTOR-regulated secretory autophagy. Together, these activities suppress the early epithelial transcriptional response, diminish immune-cell recruitment, and mitigate programmed cell death–driven mucosal barrier disruption, ultimately delaying disease progression.

Importantly, this immunosuppressive function of SopB is independent of its C-terminal phosphatidylinositol phosphatase activities and instead requires an intact N-terminal domain.

Overall, our findings reveal that SopB dampens early post-transcriptional epithelial cytokine release in an inositol-phosphatase–independent manner, a strategy that likely enhances *S.* Typhimurium transmission during neonatal infection.

Zhang K, Riba A, Nietschke M, Torow N, Repnik U, Pütz A, Fulde M, Dupont A, Hensel M, Hornef MW. Minimal SPI1-T3SS effector requirement for *Salmonella* enterocyte invasion and intracellular proliferation *in vivo*. PLOS Pathogens (2018), (3): e1006925. IF: 6.463. Cited: 86. DOI: 10.1371/journal.ppat.1006925

WS19.02

Ascaris suum-induced immunomodulation exacerbates local and systemic _Staphylococcus aureus_ infections _in vivo_

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Introduction: Neglected tropical diseases (NTDs) affect more than 1 billion people around the world, with ascariasis alone accounting for roughly 800 million cases globally. The migration of *Ascaris suum* larvae through the host's organs, primarily the liver and lungs, has been linked to a lasting inflammation and immunomodulation that can persist for more than 100 days post infection. While these effects on the local and systemic immune response have been studied, the further consequences on the host's anti-bacterial defense remain largely unexplored. Considering the disproportionate mortality associated with *Staphylococcus aureus* infections in tropical and subtropical regions, where parasitic diseases are also largely present, the unexplored interactions between parasite-induced immune modulation and bacterial pathogenesis warrant further study.

Objectives: Evaluate how *A. suum*-induced immunomodulation alters host responses and disease severity *in vivo* in murine *S. aureus* skin and soft tissue (SSTI) and bloodstream infection models.

Materials and Methods: A complex murine infection model, combining a primary *A. suum* infection, followed by a short recovery period and a secondary infection with methicillin-resistant *S. aureus* (MRSA), was developed. Post euthanasia of the animals, lungs, livers, kidneys and skin were recovered for bacterial load evaluation. For the SSTI - abscess sizes were recorded during the 3-day bacterial infection period. For the bloodstream infection – bronchoalveolar lavage was performed to assess the effects of infection on the lung. Blood was collected and a full blood count was performed. A gentamycin-protection assay allowed for the evaluation of the intracellular bacterial load in the murine kidney.

Results: In our SSTI model, animals consecutively infected with *A. suum* and MRSA had significantly larger skin abscess sizes compared to the solely MRSA infected group during all 3 days of the bacterial infection, despite having no difference in the final bacterial load. The systemic infection showed a significant increase in the bacterial burden in the lungs of animals that had recovered from a previous *A. suum* infection. Furthermore, a significant increase in the intracellular bacterial population was discovered in the kidneys of the *A. suum*/MRSA group. Finally, a notable increase in the total amounts of leucocytes in both blood and spleen was observed for the consecutively infected group.

Summary: Both local and systemic MRSA infection models show an increase in disease severity in animals that had previously been exposed to *A. suum*. The higher bacterial loads in internal organs, as well as the enlarged cutaneous abscess sizes throughout the infection suggest an impairment in anti-bacterial defense. Further studies on the exact alterations of local and systemic anti-bacterial cytokine levels and leucocyte subpopulation are needed to determine the definite cause behind the observed changes.

WS19.03

Exploiting HIF-1α-dependent macrophage metabolism to counter *Salmonella enterica* serovar Typhimurium

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Question: The role of hypoxia-inducible factor-1α (HIF-1α) in regulating macrophage antimicrobial activity and host defense during *Salmonella enterica* serovar Typhimurium infection

Methods: The role of HIF-1α in host defense against *Salmonella* was investigated using genetic and pharmacological approaches to modulate HIF-1α activity in bone marrow-derived macrophages and mouse infection models. Intracellular bacterial growth, tissue pathogen burden, host survival, and macrophage metabolic responses were assessed. The contribution of itaconate was evaluated through deletion of immune responsive gene 1 (IRG1), the enzyme responsible for its synthesis.

Results: Increased HIF-1α activity under normoxic conditions promoted intracellular *Salmonella* replication, whereas HIF-1α deficiency reduced bacterial numbers in macrophages in vitro and decreased pathogen loads in the blood, spleen, and liver, resulting in prolonged survival of infected mice. Metabolic analyses revealed elevated production of the antimicrobial metabolite itaconate in HIF-1α-deficient macrophages. Deletion of IRG1 abolished the enhanced antibacterial effect associated with HIF-1α deficiency, demonstrating that increased itaconate production mediates this protective response.

Conclusions: HIF-1α limits macrophage antimicrobial activity during *Salmonella* infection by suppressing IRG1-dependent itaconate production. These findings identify HIF-1α–regulated metabolic pathways as potential targets for host-directed therapies aimed at enhancing antibacterial immunity against *Salmonella* infection.

WS19.04

Sex bias in host response to tuberculosis

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Tuberculosis remains the most prevalent bacterial infectious disease worldwide. The disease exhibits pronounced sex differences in both incidence and outcome, with men disproportionately affected and experiencing higher mortality. While these disparities have long been attributed to gender-related social and behavioral factors, a growing body of evidence suggests that biological sex may influence susceptibility to *Mycobacterium tuberculosis* (*Mtb*). Notably, our work using murine infection models—which allow the effects of sex to be examined independently of gender-associated confounders—consistently demonstrates increased susceptibility in males, as reflected by accelerated disease progression and premature death.

At the tissue level, we identified striking sex-specific differences in the organization of pulmonary TB lesions. Increased susceptibility in males was associated with a highly inflammatory lung environment dominated by monocytes and macrophages, indicative of a poorly organized and potentially pathogenic immune response. In addition, males developed significantly smaller pulmonary B cell follicles compared to females. As pulmonary B cell follicles are associated with protective immunity in both experimental models and human TB, their reduced size in males suggests impaired organization of adaptive immune responses.

Because tissue-resident B cells can regulate macrophage recruitment and activation, in part through the production of IL-10, we hypothesized that females are better able to counteract chronic inflammation via B cell-derived IL-10. To test this, we infected B cell-specific IL-10-deficient mice with M. tuberculosis HN878. Surprisingly, the absence of B cell-derived IL-10 resulted in only minimal transcriptional changes in females, whereas males displayed marked alterations in gene

expression. Notably, the loss of B cell-derived IL-10 abolished the sex-specific patterns of IFN-γ and IL-27 expression, reduced pulmonary bacterial burden in males, and conferred a significant survival advantage. Together, these findings identify B cell–derived IL-10 as a sex-dependent regulator of TB immunity, with a predominant and functionally consequential role in shaping immune responses in males.

Collectively, our findings demonstrate that biological sex is a key determinant of the pulmonary immune landscape during TB and underscore the need to incorporate sex as a critical variable in TB research and therapeutic development.

WS19.05

A cDC2–macrophage axis orchestrated by interferon regulatory factor 4 promotes chronic cutaneous leishmaniasis

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Introduction: In C57BL/6 mice, the outcome of cutaneous leishmaniasis (CL) depends on the infecting *Leishmania* species. Whereas an infection with *L. major* resolves spontaneously due to a T-helper cell (Th) 1 response inducing leishmanicidal nitric oxide, *L. mexicana* causes chronic skin lesions characterized by a mixed Th1/Th2 profile. We hypothesized that differences in the infection and/or functionality of myeloid cells at the infection site determine differential adaptive immunity and disease outcome.

Objectives: We aimed to define myeloid cell populations involved in self-healing versus chronic CL during the early phase of infection on a single cell level.

Materials and Methods: Fluorescently labeled *Leishmania* and flow cytometry were used to identify early host cells in infected skin of C57BL/6 mice. Single-cell RNA sequencing analyses (scRNA-seq) were performed with skin and draining lymph nodes of mice lacking the transcription factor interferon regulatory factor (Irf) 4 in integrin-alpha-X(CD11c)-positive cells (Irf4ΔItgax) and of wild-type (WT) littermates. RT-qPCR and immunofluorescence were used to further characterize the immune response.

Results: Flow cytometry identified granuloyctes, dendritic cells (DCs), monocytes and macrophages as early host cells for *Leishmania* parasites. Dermal cDC2s showed higher infection rates than cDC1s. To study the role of cDC2s, we analyzed Irf4ΔItgax mice, which have reduced levels of Irf4-dependent cDC2s in lymphoid organs. Whereas *L. major*-infected knockout (KO) mice and WT littermates showed similar self-healing disease, *L. mexicana*-infected Irf4ΔItgax mice developed only mild pathology and resolved the infection. RT-qPCR analysis revealed a stronger Th1 and reduced Th2 gene expression in infected Irf4ΔItgax mice. scRNA-seq of skin and dLN cells from Irf4ΔItgax versus WT mice at day 14 of infection revealed that cDC2 subclusters were present in both genotypes. However, Irf4-deficient cDC2s had lost their Th2-polarizing ligand profile (Icosl, Ox40l, Dll4, Aldh1a2) and showed impaired IL-2/STAT5 signaling. Both factors could contribute to the shift from a mixed Th1/Th2 gene profile seen in WT to the prominent Th1-related gene expression detected in Irf4ΔItgax mice. In addition, there was a near-complete loss of the Ccl24-producing M2-like macrophage subcluster and an accumulation of undifferentiated patrolling monocytes in the skin lesions of Irf4ΔItgax mice, indicating an Irf4-dependent macrophage differentiation checkpoint. Also, eosinophil infiltration was strongly reduced in Irf4ΔItgax skin. As we recently identified eosinophils as crucial drivers of chronicity in *L. mexicana* infection, this latter phenotype may account for the clinical healing of the KO mice.

Conclusion: Irf4 coordinates a multi-cellular amplification circuit, in which cDC2s and a M2-like macrophage subpopulation sustain eosinophil recruitment and differentiation of a Th2 response leading to the chronicity of *L. mexicana* infections.

WS19.06

Divergent patterns of autoantibodies against IFN-γ and systemic autoantigens in scrub typhus patients from Nepal (Young talent)

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Objective: Scrub typhus is a mite-borne, acute febrile illness caused by the obligate intracellular bacterium *Orientia tsutsugamushi* (OT) that is endemic in wide parts of the Asia-Pacific region. While the acute disease is governed by cytokines produced by innate and adaptive immune effectors, it has been associated in the post-acute phase with an increased risk for autoimmune diseases such as systemic lupus erythematodes (SLE) in epidemiological studies. We hypothesized that antibodies directed against cytokines and systemic autoantigens are prevalent in scrub typhus patients and could influence pathogenesis. These autoantibodies were measured in a cohort of acute Nepalese scrub typhus patients, in healthy Nepalese blood donors as well as German blood donors.

Methods: Sera from acute scrub typhus patients (n=112) from Nepal, Nepalese blood donors (n=49) and German blood donors (n=47) were tested for antibodies binding to 13 autoantigens related to SLE or other collagenoses and 6 cytokines (interferon (IFN)-α/-β/-γ, TNF, GM-CSF and interleukin (IL)-1-α), using a commercial multiplex bead array by Luminex® technology. Results were correlated with age and clinical parameters, e.g. OT bacteremia.

Results: In acute scrub typhus patients, SLE-related autoantibodies increased with age (10/13 specificities), whereas anti-cytokine autoantibodies did not; anti-IFN-γ antibodies showed a negative age correlation. Nepalese blood

donors exhibited higher baseline levels of both SLE-related and anti-cytokine autoantibodies than German donors. Acute scrub typhus was associated with further increases in anti-cytokine autoantibodies, whereas SLE-related autoantibody profiles largely resembled those of Nepalese controls. Unbiased clustering confirmed distinct distribution patterns for SLE-related and anti-cytokine autoantibodies. Anti-RNP/Sm antibodies correlated with higher bacterial DNAemia ($r = 0.24$, $p = 0.028$), whereas anti-IFN-γ antibodies were associated with lower bacterial loads (≈1-log reduction; $r = -0.20$, $p = 0.057$), suggesting opposing associations with pathogen control.

Conclusions: Autoantibodies in scrub typhus patients are heterogeneous and differentially linked to pathogen control. Antibodies against systemic antigens, but not those against cytokines, were highly prevalent in the healthy Nepalese population, suggesting that these antibodies are most likely not induced by OT infection, but rather pre-exist with the potential to get boosted. Surprisingly, binding antibodies to IFN-γ did not drive susceptibility but were associated with lower bacterial loads, indicating a potentially protective effect in OT infection. These data highlight previously unrecognized relationships between autoimmunity and host defense in acute scrub typhus.

WS22.01

Unify, align, and educate: Promoting photodynamic inactivation within the One Health framework by COST Action CA24127 PanEuCOPT

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Photodynamic Inactivation has gained attention as effective and applicable antimicrobial approach within the One Health framework. The procedure is based on the photosensitiser-mediated and light-induced overproduction of reactive oxygen species in target cells, by this killing all types of microorganisms by oxidative processes irrespective of resistance against conventional treatment. Despite the extensive body of scientific evidence demonstrating the high efficacy of Photodynamic Inactivation under laboratory conditions, its practical implementation is hindered by inconsistent terminology, heterogeneous experimental protocols, and insufficient training and awareness among practitioners in human and veterinary medicine, as well as in plant and environmental protection. The COST Action CA24127 Pan-European Commission on Photoantimicrobial Testing (PanEuCOPT) unites more than 150 experts from 28 countries under the shared vision *Unify, Align, and Educate* to advance the implementation of Photodynamic Inactivation in practical application. PanEuCOPT is open to researchers, clinicians, and industry professionals from COST and cooperating countries and actively invites new members to join the initiative. Within its first year, five dedicated Working Groups have been established: WG1 focuses on the standardisation of terminology, WG2 advances education and training initiatives, WG3 develops guidelines for testing methods, WG4 defines and harmonises illumination parameters, and WG5 coordinates dissemination and outreach activities. This presentation provides a brief introduction to Photodynamic Inactivation, presents the COST Action, summarises its progress to date, and outlines opportunities for active involvement in the network.

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WS22.02

***Coxiella burnetii* establishes a small cell variant (SCV)-like persistent form to survive adverse intracellular conditions**

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Introduction: Coxiella burnetii is an obligate intracellular zoonotic bacterium responsible for Q fever. Infections appear as either acute illness such as mild flu-like symptoms, pneumonia, or hepatitis or as chronic disease, most often endocarditis. Notably, chronic Q fever may develop months or years after the initial infection without clinical symptoms, indicating that C. burnetii can persist within the host.

Question: However, little is known about how *C. burnetii* persistence is induced or regulated?

Background: Our research demonstrated that during infection of hypoxic (0.5% O_2) primary macrophages, limiting citrate availability inhibits *C. burnetii* replication, but does not compromise bacterial viability, indicating bacterial persistence. Mechanistically, hypoxic conditions stabilize HIF1α, which in turn impairs STAT3 activity, leading to reduced levels of the TCA cycle intermediate citrate. Interestingly, *C. burnetii* requires hypoxic conditions for axenic growth, suggesting that oxygen limitation alone does not affect *C. burnetii* physiology, but hypoxia-induced changes in the host cell metabolism does.

Methods and Results: In this study, we aimed to characterize persistent *C. burnetii* in more detail. Therefore, we infected primary murine macrophages with *C. burnetii* under both normoxic (21% O_2) and hypoxic (0.5% O_2) conditions and analysed the gene expression profiles. Our findings indicate that under hypoxia, *C. burnetii* does not mount a stringent response, but instead transitions to an SCV-like form, representing a non-replicating persistent state. The observed phenotype of *C. burnetii* was reversable once the bacteria encountered favourable conditions (21% O_2) in host cells. Electron microscopy revealed that this SCV-like form was smaller, contained dense chromatin and had a thicker cell wall. Moreover, our data showed that the SCV-like persistent form of *C. burnetii* was more infectious, exhibited greater antibiotic tolerance, and was less susceptible to clearance by IFNγ activated macrophages.

Conclusion: In summary, hypoxic conditions lead to citrate limitation, creating a nutritional stress that triggers *C. burnetii* to enter an SCV-like persistent state. This adaptation enables the bacterium to survive under adverse conditions. Because the SCV-like persistent form is more resistant to antibiotics and less susceptible to clearance by activated macrophages, it impedes effective elimination of the bacteria. As a result, *C. burnetii* can persist till environmental conditions become favourable again.

WS22.03

Staphylococcus aureus CC8 – A One Health perspective

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Introduction: *Staphylococcus aureus* threatens human health, animal welfare, and food safety. Frequent host switches between humans and livestock -facilitated by domestication- enable this multi-host pathogen to cause infections across species, including chronic, persistent mastitis in cows that harms welfare and dairy production.

Objectives: Our aim is to identify the factors that enable adaptation to the bovine host, including the milk-rich environment of the bovine udder. We investigated closely related human- and bovine-associated strains of clonal complex 8 (CC8). In the United States, human-associated CC8 is a major cause of community-acquired (CA-MRSA) and healthcare-associated (HA-MRSA) methicillin-resistant infections. In contrast, bovine-associated CC8 is highly prevalent in the European Alps. The bovine-associated strains probably originate from a recent shift from humans, although the exact circumstances remain unknown.

Materials and Methods: We performed growth experiments in skimmed milk to assess metabolic adaptation to the udder environment. Using a transposon mutant library (NTML) derived from a human reference CC8 strain, we specifically examined transcriptional regulatory genes to evaluate their role in milk adaptation. We then examined the effect of SigB by targeted inactivation of the regulator in additional human-associated CC8 strains and compared growth in milk with their respective wild-type backgrounds. In addition, we assayed beta-haemolysin activity and phiSa3 phage integration in bovine-associated CC8 isolates.

Results: Screening of the mutant library revealed that the *sigB* mutant adapted fastest to milk. Human-associated strains lacking SigB adapted better to milk than strains with functional SigB. Reduced SigB activity is advantageous in the milk-rich environment of the bovine udder. This is shown by shorter adaptation times and faster growth. Phenotypic and genetic screening also showed that most bovine CC8 isolates did not secrete beta-haemolysin, whilst PhiSa3 phages were detected. This contradicts the previous assumption that the prophage is lost during *S. aureus* transition from humans to bovine, and that beta-haemolysin activity is typically detected in bovine strains.

Summary: *S. aureus* adapts to the udder through modulation of a central master regulator in the core genome and through mobile genetic elements. We discuss implications for zoonotic risk, animal health, and food safety from a One Health perspective, with emphasis on antibiotic resistance and the challenges of alpine dairying.

WS22.04

Genomic diversity and conserved plasmids in the 2025 EHEC O45:H2 outbreak strain

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Background: Germany experienced a major outbreak of enterohemorrhagic *Escherichia coli* (EHEC) serotype O45:H2 in 2025, mainly affecting Mecklenburg-Western Pomerania and North Rhine-Westphalia. More than 400 cases met the outbreak case definition, including 199 laboratory confirmed cases. The median age was 10 years (range 0–94 years). Boys were predominantly affected among children aged 0–5 years, whereas females accounted for most cases among adults older than 60 years. All 53 hemolytic–uremic syndrome (HUS) cases occurred in children, and three patients died. Although the outbreak source remains unresolved, molecular characterization of the isolates provide important genomic insights.

Methods: 58 EHEC outbreak isolates were collected between August and November 2025 and analysed by long-read whole genome sequencing (lrWGS) (PacBio® Sequel IIe system). *De novo* genome assemblies were generated and analyzed by core-genome multilocus sequence typing (cgMLST) (MBioSEQ Ridom Typer v.11.1, Ridom GmbH, Germany). In addition, plasmids were reconstructed, typed, and compared using a Mash distance-based clustering approach (MobSuite v3.1.8 in MBioSEQ RidomTyper).

Results: All isolates were confirmed as *E. coli* serotype O45:H2 carrying *stx2a* and *eaeA*. The outbreak strain belonged to ST301 and CT66411. cgMLST analysis showed pairwise allelic distances of 1 to 25, indicating a high core genome diversity of the strain compared to previous outbreaks. Despite this high variability, the plasmidome of the outbreak strain contained three highly conserved plasmids (mash distances < 0.0001): a 154.8 kb IncF plasmid, a 97.7 kb IncI-γ/K1 plasmid, and a 97.6 kb IncHI1B plasmid. The IncF plasmid encodes a MOBF relaxase as well as resistance genes against beta-lactams, chloramphenicol, kanamycin,

tetracycline, and heavy metals including silver, copper and mercury.

Conclusions: The 2025 EHEC O45:H2 outbreak strain showed substantial chromosomal heterogeneity but a highly conserved plasmidome, supporting a common origin. This observation demonstrates the crucial resolution provided by lrWGS for epidemiological outbreak investigations and revealed the added value of plasmid analysis in molecular epidemiology. In addition, the results support the adaptation of surveillance protocols by integrating plasmid analyses into monitoring strategies for critical pathogens.

WS22.05

Shiga toxin causes a coordinated upregulation of pro-inflammatory and stress-response pathways in a human 3D gut-on-a-chip model

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Introduction: Enterohemorrhagic *E. coli* (EHEC) are intestinal pathogens that can cause hemorrhagic colitis and the life-threatening hemolytic uremic syndrome (HUS). The cardinal virulence factor of EHEC is Shiga toxin (Stx), which is a potent AB5 toxin that irreversibly inhibits eukaryotic protein synthesis. We have recently shown that Stx breaks the epithelial barrier and causes inflammation in a human 3D gut-on-a-chip model after 24 hours (h) of treatment. Here, we further investigated the mechanisms underlying these phenotypes.

Methods: Caco-2 and HT29-MTX intestinal epithelial cells were cultured against an extracellular matrix in an OrganoPlate® 3-lane 40 plate (Mimetas) under continuous medium perfusion. Leak-tight intestinal tubules were treated with purified Stx1a or Stx2a for 6 h for RNA-sequencing (RNA-seq) analysis and 24 h or 48 h for immunofluorescence. RNA-seq was performed using total RNA enriched for mRNA followed by strand-specific library preparation and Illumina sequencing. Differentially expressed genes were subjected to KEGG pathway enrichment analysis using clusterProfiler. For immunofluorescence, fixed tubules were stained with antibodies against Stx and the tight junction protein Zonula occludens-1 (ZO-1) and visualized by confocal scanning microscopy.

Results: The Stx treatment induced a strong transcriptional response in the gut-on-a-chip model already after 6 h of treatment. Interestingly, we detected only enrichment of upregulated KEGG pathways in the treated versus untreated gut tubules, with the response to Stx1 and Stx2 being almost identical. The analysis revealed the coordinated upregulation of inflammatory, cytokine and stress-response pathways upon Stx treatment. In particular, we detected activation of the inflammatory signaling pathways TNFα, NF-κB, MAP kinase and IL-17 signaling, which are known to impair tight junction integrity thus resulting in epithelial barrier dysfunction. Indeed, using immunofluorescence we could show that the treatment with both Stx1 and Stx2 led to the downregulation and redistribution of the tight junction protein ZO-1. The concurrent enrichment of stress-response pathways like cellular senescence, p53, FoxO and Hippo signaling pathways further suggested for activation of epithelial remodeling programs.

Conclusion: Taken together, Stx treatment of a human gut-on-a-chip model results in a coordinated activation of pro-inflammatory and stress-related pathways, associated with epithelial barrier dysfunction and tissue remodeling.

WS22.06

A comparative study of the prevalence of multidrug-resistant Gram-negative pathogens in broiler and free-range chicken production systems in Indonesia and Zambia

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Question: Poultry production plays a key role in food security and source of houshold income in low- and middle-income countries. However, poultry can also serve as a reservoir of antimicrobial-resistant bacteria, including extended-spectrum β-lactamase (ESBL)-producing Enterobacterales. Evidence comparing antimicrobial resistance patterns between broiler and free-range production systems remains scarce. This study compared the prevalence of ESBL-producing Enterobacterales and multidrug-resistant isolates in broiler and free-range chickens to identify production systems associated with a lower antimicrobial resistance burden.

Methods: Using a harmonized protocol, a cross-sectional study was conducted in Indonesia and Zambia. Per country, 100 broiler and 100 free-range chickens were sampled from farms, markets, and abattoirs. Cloacal swabs were screened for ESBL-producing Enterobacterales and characterized using phenotypic methods and whole-genome sequencing (WGS). Structured questionnaires were administered to collect data on farm characteristics, management practices, and antimicrobial use. ESBL prevalence and multidrug resistance patterns were compared between production systems and countries.

Results: A total of 88 and 118 Enterobacterales isolates were recovered from samples collected in Indonesia and Zambia, respectively. The predominant species were *Escherichia coli* (74/88, 84.1%), *Klebsiella pneumoniae* (6/88, 6.8%), and *Enterobacter cloacae* (3/88, 3.4%) in Indonesia, and *E. coli* (98/118, 83.1%), *K. pneumoniae* (10/118, 8.5%), and *Shigella*spp. (8/118, 6.8%) in Zambia. Among *E. coli* isolates, resistance to third-generation cephalosporins was detected in 52/58 (89.7%) broiler and 5/30 (16.7%) free-range isolates from Indonesia, and in 73/103 (70.9%) broiler and 2/15 (13.3%) free-range isolates from Zambia. WGS identified *bla*CTX-M-55 (n=29), *bla*CTX-M-15 (n=16), and *bla*CMY-2 (n=1) among Indonesian isolates. In Zambia, *bla*CTX-M-55 (n=46), *bla*CTX-M-14 (n=8), *bla*CTX-M-

15 (n=5), *bla*CTX-M-27 (n=2), *bla*CTX-M-65 (n=1), and *bla*CMY-2 (n=1) were detected. In-depth analyses of the WGS data are still underway.

Conclusion: Third-generation cephalosporin resistance was substantially more prevalent among broiler than free-range chickens in both Indonesia and Zambia. These findings highlight intensive poultry farming as a potential driver of antimicrobial resistance and support targeted One Health interventions to reduce the dissemination of ESBL-producing bacteria.

WS23.01

National reference centers and consultant laboratories in Germany

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In the 30 years since their inception, the German National Reference Centers and Consultant Laboratories were faced with various threats posed by pathogens to public health in Germany and worldwide. Their crucial role within public health systems has been evident through various crises and their sustained funding is important for public health in both pandemic and non-pandemic times. This presentation is intended to provide an overview of the National Reference System, current challenges and an outlook for the future.

WS23.02

The animal kingdom – A large and diverse reservoir for diphtheria pathogens and zoonotic infections

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Background: Diphtheria is caused by toxigenic *Corynebacterium* spp. producing diphtheria toxin (DT), the major pathogenicity factor. The three potentially DT-producing species are the classical human diphtheria agent *C. diphtheriae* and the two zoonotic pathogens *C. ulcerans* (with increasing numbers of human infections in industrialized countries) and *C. pseudotuberculosis*. In recent years, the *C. diphtheriae* species complex (*Cd*SC) has been expanded based on genomic data, and in some instances, supported by biochemical and antibiotic resistance properties, now additionally including *C. belfantii, C. rouxii, C. silvaticum* and *C. ramonii*. The emergence of novel zoonotic species raises concerns regarding dissemination within the animal kingdom and zoonotic transmission to humans.

Methods: All *Cd*SC isolates originating from companion, farm, zoo and wildlife animals, as well as isolates from corresponding animal owners submitted to the German Consiliary Laboratory for Diphtheria between 2001 and 2025 were included in this study. Species identification was performed by biochemical differentiation and MALDI-TOF MS. Toxigenicity was verified by a real-time DT-PCR, optimized modified Elek-test, and a DT-detecting Lateral Flow Immunoassay. Antimicrobial susceptibility testing was performed according to EUCAST guidelines. Next-generation sequencing (NGS) analyses included average nucleotide identity (ANI) and core genome (cg)MLST.

Results: A total of 390 *Cd*SC strains isolated from pet animals (cats, dogs, rats), farm animals (horses, pigs, cattle, goats), wildlife animals (hedgehogs, foxes, wild boars, red deers, water rats, beavers) and captive wild animals (camelids, primates), representing all currently described *Cd*SC species, were included.

C. ulcerans was predominantly isolated. *tox*-positive strains accounted for 73% of all isolates and were most frequently isolated from skin lesions, throat/nose swabs, lower respiratory tract secretions/lung tissue, lymph nodes, and ear swabs. 77% of the *tox*-positive strains were also DT producing strains.

The two *C. ulcerans* sequence types ST-331 and ST-332 (also predominantly isolated in human zoonotic wound diphtheria cases) were found in various animals.

Conclusions: This study highlights the widespread distribution of all potentially toxigenic *Cd*SC species throughout the animal kingdom with a large potential for zoonotic transmission and diphtheria infections in humans. Asymptomatic carriers or symptomatic cats and dogs appear to represent the main zoonotic reservoir for human infections, while wildlife species living in close proximity to humans, such as hedgehogs, may play an increasingly significant role. This emphasizes the need for low-threshold interdisciplinary collaboration to monitor and control emerging zoonotic diphtheria transmission patterns.

WS23.03

Carbapenemases in Germany in 2025: Impact of changing the isolate inclusion criteria of the German National Reference Centre for Multidrug-resistant Gram-negative Bacteria

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Background: Multidrug-resistance in *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since hardly any innovative antimicrobial drug against gramnegative bacteria will be introduced within the next years. Among all resistance mechanisms the spread of carbapenemases is the most worrisome. However, the correct identification of

carbapenemases is still challenging and accurate molecular epidemiology of carbapenemases is urgently required.

Methods: The German National Reference Centre for Multidrug-Resistant Gramnegative Bacteria offers the free service of carbapenemase detection in bacterial isolates with elevated carbapenem MICs. All isolates are tested by a wide array of phenotypic and molecular methods. A bioassay based on cell-free extracts and WGS methods allow the detection of still unknown β-lactamases. In march 2025, the NRC had to revise the inclusion criteria for isolates to prevent capacity overload. Since then, a positive unspecific phenotypic test result (e.g. mCIM, zCIM, CarbaNP…) is required before sending strains to the NRC for all *Enterobacterales* and *P. aeruginosa*.

Results: A total of 8.307 isolates were investigated for carbapenemases in 2025. This represented a 18.6 % decrease compared to the same timepoint in 2024, reflecting the impact of the changed inclusion criteria. However, the number of carbapenemase producing isolates increased by 13 % with carbapenemases found in 4.973 *Enterobacterales* strains, 494 of *P. aeruginosa* and 387 of *A. baumannii*. The most frequent carbapenemases in *Enterobacterales* were OXA-48 (n = 1.153), OXA-244 (n = 812), NDM-5 (n = 775), NDM-1 (n = 705), KPC-2 (n = 589) and VIM-1 (n = 560), which were also found in various combinations, e.g. NDM-1/OXA-48 (n = 157). In *P. aeruginosa,* VIM-2 was the most frequent carbapenemase (n = 269), followed by NDM-1 (n = 86), IMP-1 (n = 28) and GIM-1 (n = 28). OXA-23 was again the most frequent carbapenemase in *A. baumannii* (n = 227), followed by OXA-72 (n = 128). Regarding the percentage of carbapenemase-positive isolates, the changed inclusion criteria had a massive impact with now 82.2 % of all *Enterobacterales* analyzed producing a carbapenemase (+ 20.1 percentage points), 80.6 % of *K. pneumoniae* (+18.4 percentage points), 77.2 % of *Enterobacter cloacae* complex (+24.1 percentage points) and 58.2 % of *P. aeruginosa* (+ 28.4 percentage points).

Conclusions: A large variety of different carbapenemases is constantly detected in Germany, especially in *Enterobacterales*. Compared to 2024, the total number of isolates analyzed was lower due to tightened inclusion criteria at the NRC, but nonetheless the number of carbapenemases detected increased, suggesting a further significant spread of carbapenemase producing strains in Germany.

WS23.04

Rethinking *Tropheryma whipplei* endocarditis: A systemic disease rather than a localized infection? Experience of the German Consiliary Laboratory

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Question: Whipple"s disease is a chronic systemic infection that may involve, in addition to intestinal affection, various organs such as joints, the central nervous system (CNS) and heart valves. Infective endocarditis (IE) caused by *Tropheryma whipplei* is usually considered as a localized infection, although it has also been reported as complication of classical Whipple"s disease. The condition is rare and difficult to diagnose due to the fastidious nature of the pathogen, resulting in limited data that relies primarily on small case series. The aim of this retrospective cohort study from the German Reference Laboratory was to evaluate cases of IE caused by *T. whipplei* with respect to additional organ involvement.

Methods: In a retrospective analysis covering the period from 2001 to 2026, cases of IE were identified from all specimens submitted to the German Reference Laboratory for *T. whipplei*. Detection of *T. whipplei* in explanted heart valves was based on specific PCR targeting the rpoB gene, 16S rRNA gene PCR with sequencing and fluorescence in situ hybridization (FISH). For the identified IE cases, patient characteristics were documented, and the results of additionally submitted specimens were evaluated.

Results: A total of 31 patients (24 male, 7 female) with IE caused by *T. whipplei* were recorded. The aortic valve was affected in most cases (n = 20). From 17 patients, additional samples were submitted, particularly urine, stool, cerebrospinal fluid, and duodenal biopsies. Of these, six patients were found to have positive results, some at the time of initial diagnosis and others during follow-up. CNS involvement was observed in two cases. For one patient, *T. whipplei* DNA was detected in synovial tissue after knee prosthesis removal, 8 months after initial IE diagnosis. Notably, one patient experienced relapse requiring two consecutive valve prosthesis replacements.

Conclusions: In approximately one-third of IE cases, additional organs were affected by *T. whipplei*, challenging the concept of a strictly localized infection. Although limited by the retrospective design and selection bias of samples submitted, our findings support the use of invasive sampling to investigate involvement of additional organ systems. Evidence on optimal antibiotic treatment is limited; in particular, classical *T. whipplei* therapy may require adaptation for IE and likely exceeds short-course treatments. Prospective studies are needed to establish standardized diagnostic and therapeutic approaches for this potentially fatal but treatable infection.

WS23.05

Antibiotic resistance in invasive and non-invasive H. influenzae isolates in Germany

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Introduction: *Haemophilus influenzae* (Hi) is a commensal pathogen of the respiratory epithelium. However, it can also cause a wide range of infections, from minor ear, nose and throat conditions to severe sepsis and meningitis. In this context, ampicillin, either alone or in combination with beta-lactamase inhibitors, and third-generation cephalosporins are the preferred treatment options. Antibiotic resistance surveillance is important in providing a basis for recommendations on the empirical use of antibiotic agents.

Objectives: To analyse the dynamics of the susceptibility rates of invasive and non-invasive Hi isolates to aminopenicillins and third-generation cephalosporins, and to compare the molecular

mechanisms of resistance. Furthermore, an analysis of the epidemiology of invasive and non-invasive infections in Germany was conducted.

Methods: Non-invasive isolates (n=208) were collected from a sentinel network of diagnostic laboratories in a prospective multicentre prevalence study conducted by the Paul-Ehrlich Society in the period 10/2022-03/2023. Invasive isolates (n=905) were collected in NRZMHi at the same time. Antimicrobial susceptibility testing was carried out using gradient agar diffusion (GAD) test. MICs were interpreted in accordance with EUCAST guidelines. β-lactamase production was tested using the Nitrocefin test. Sequencing of the ftsI gene, encoding penicillin binding protein 3 (PBP3) was performed to analyse the molecular mechanism of β-lactam resistance among all β-lactamase negative ampicillin resistant (BLNAR).

Results: The results showed no significant differences in the proportions of β-lactamase negative ampicillin-susceptible (BLNAS, 79.3% vs. 77.5%), β-lactamase positive ampicillin-resistant (BLPAR, 13% vs. 15%) and BLNAR (7.7% vs. 7.4%) in non-invasive and invasive isolates, respectively. Resistance to third-generation cephalosporins was rare in both non-invasive and invasive isolates (1.0% vs 1.3%). Most BLNAR exhibited PBP3 amino-acid substitutions of group II, most frequently IIb and IIa. Furthermore, 90% of isolates (n=9/10) carrying group III PBP3 mutation exhibited increased MICs against cefotaxime (≥0.125 mg/l). The present study revealed that, compared to encapsulated isolates (n = 104), NTHi (n = 801) exhibited a significantly higher propensity towards ampicillin resistance ($p < 0.0001$; Fisher's exact test). The vast majority of non-invasive isolates were also NTHi (204/208). All non-invasive ampicillin-resistant strains were NTHi (n = 42).

Conclusions: The findings generally indicated that resistance rates of invasive and non-invasive isolates were comparable. The molecular analysis revealed the presence of characteristic PBP3 mutations in BLNAR and cefotaxime-resistant isolates. Overall, the resistance rates to β-lactam antibiotics do not seem to have changed in Germany.

WS23.06

Characterization of a *Salmonella* Typhimurium ST19 clade associated with bloodstream infections and enhanced invasiveness

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Introduction: In Germany, *Salmonella enterica* is mainly associated with foodborne infections and largely causes self-limiting gastroenteritis. In contrast, invasive non-typhoidal *Salmonella* (iNTS) infections are particularly prevalent in Sub-Saharan Africa, with *Salmonella* Typhimurium strains of specific MLST sequence types (ST), primarily ST313 and ST19, being the main culprits behind iNTS cases in the region. At the German National Reference Centre for *Salmonella* (NRC), a rise in *Salmonella,* including *S.* Typhimurium, bloodstream infections have been detected during the last years. However, the specific *Salmonella* subtypes, mechanisms and genes of the pathogens driving invasive infections are largely unknown.

Goals: We aimed to identify genomic signatures and *in vitro* infection phenotypes of *S.* Typhimurium strains associated with invasive disease.

Materials and Methods: Over 4,500 clinical *S.* Typhimurium isolates (2020 to 2023) from Germany and from Sub-Saharan Africa were analyzed using bioinformatic tools to estimate their invasive potential. Based on phylogenetic information and prediction of invasiveness, isolates were classified into several clades and subsequently 96 isolates were analyzed in *in vitro* infection experiments to determine bacterial invasion and intracellular replication. Comparative genomic analyses, including gene presence/absence profiling, were used to identify candidate genes or genomic regions potentially associated with increased invasion capacity.

Results: Within *S.* Typhimurium phylogeny, a distinct *S.* Typhimurium ST19 clade showed a higher invasiveness index and higher cell invasion and/or intracellular replication capacity compared to other ST19 strains. Interestingly, this clade contained both German and African isolates. Gene presence/absence analyses identified several genomic loci potentially associated with this phenotype, providing a basis for further investigation through gene knockout experiments.

Summary: Within the phylogeny of *S.* Typhimurium ST19, we identified a clade containing strains from Germany and Africa that show higher invasiveness and intracellular replication capacity. Ongoing functional validation aims to pinpoint specific genetic signatures providing new insights into the evolution and virulence of non-typhoidal *Salmonella*.

WS24.01

Self-assessment of infection prevention and control preparedness among senior medical students: Implications for student-centered medical education

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Introduction: Infection prevention and control (IPC) represents a fundamental competency for all physicians as an essential component of patient safety. The revised German National Competence-Based Learning Objectives Catalogue for Medicine (NKLM) increasingly emphasis not only on theoretical knowledge, but also on practical skills, professional accountability, and interprofessional collaboration. Consequently, IPC teaching concepts should be tailored to aligned to students" educational needs, self- perceived competencies, and preferred learning formats to support sustainable competency development.

Aim: We assessed self-perceived preparedness in IPC among senior medical students and identified unmet education needs to develop a targeted educational intervention.

Material and Methods: At our university, IPC education comprises lectures in the third year of medical school and mandatory practical training in hand hygiene, dressing changes, operating room behavior, and catheter insertion and care in a dedicated simulated-based training environment

("Skills Lab"). We developed an eight-item questionnaire using multiple-choice and five-point Likert scale items to assess the perceived importance of IPC, sense of personal responsibility, self-rated competencies, need for additional training, and preferred learning formats. Medical students in their final practical year were surveyed during a mandatory IPC-workshop event on their first day in our hospital. Data were analyzed descriptively, and the findings were subsequently used to develop a novel student-centered teaching format.

Results: Over 18 months, 71 students from nine workshop groups were surveyed. More than 80% of respondents considered personal responsibility for IPC as high. However, substantial uncertainty was reported regarding dressing changes, isolation precautions, and the management of multidrug-resistant organisms. These topics were also identified as the highest priorities for further training. Online training modules, interactive seminars, and supervised ward-based teaching were rated equally favorable as educational formats. Based on these findings, we developed a blended-learning course combining an on-demand online multimedia course with structured bedside supervision and feedback, focusing on the dressing change procedures as part of the prevention of surgical site infections. The course is currently being evaluated in a pilot phase.

Conclusion: Senior medical students demonstrate high motivation and strong sense of professional responsibility for IPC while simultaneously reporting relevant deficits in practical competencies required for routine clinical practice. The survey findings directly informed the development of a targeted blended-learning intervention, addressing the competencies most frequently perceived as insufficient.

WS24.02

Desire vs. Reality: Implementing and evaluating a flipped classroom concept in medical microbiology and virology

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Background: Traditional lecture-heavy formats in medical education often struggle to foster flexibility and deep understanding. The flipped classroom (FC) model addresses these limitations by shifting passive listening to active pre-class preparation while reducing face-to-face time. We implemented this model in the Microbiology, Virology, and Hygiene course for third-year medical students (n≈120). Previously, the curriculum consisted of two weekly non-mandatory lectures (90 min each), a mandatory practical course, and a final oral examination. We hypothesized the FC model would increase flexibility and student satisfaction while improving learning outcomes.

Methods: The new format reduced voluntary face-to-face lecture time by 50% to one weekly session. Foundational knowledge was delivered via weekly online packages with 3–4 short videos (15–30 min each). In-person sessions focused on clinical cases, current outbreaks, Q&A sessions, and exam preparation. Evaluation included analysis of video access rates, qualitative feedback, a standardized end-of semester survey (grades 1–6, 1=best), and final exam performance.

Results: Initial participation and qualitative feedback were promising, with students praising the flexibility and quality of the videos. However, video access rates declined sharply over time, falling from 100% to 35% by the end of the semester, alongside a noticeable decline in in-person attendance. The resulting heterogeneous student preparation led to conflicting expectations during face-to-face sessions. Faculty reported that insufficient preparation significantly hindered the planned interactive teaching concept. Standardized evaluations were poorer than the five-semester historical average (overall grade 2.07 vs. 1.64). Both perceived learning gain (1.91 vs. 1.46) and perceived value of in-person sessions (2.71 vs. 2.03) decreased, whereas final oral exam grades remained stable (mean 1.8 vs. 1.9).

Discussion and Conclusion: The discrepancy between students" stated desire for flexibility and their actual engagement highlights a core challenge of self-regulated learning. Asynchronous formats without intermediate milestones may facilitate procrastination and reduce preparation motivation, suggesting that FC concepts require additional incentives such as mandatory e-quizzes or participation-based rewards. Although academic performance remained stable, the findings indicate that FC concepts require not only high-quality digital content but also clear external structures to support participation. Limitations include low survey response rates (30–55%) and potential bias of an unusually short winter term with increased student workload and time pressure. Beyond the challenges of self-study, the role of physical presence in medical education deserves broader consideration, since communication and collaborative decision-making are essential competencies for future patient care and should remain integral to the curriculum.

WS24.03

Improving hand hygiene performance using asynchronous personalized digital feedback and microlearning: Prospective longitudinal single-center-study

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Correct hand hygiene is essential for preventing healthcare-associated infections, yet maintaining high-quality hand disinfection in routine clinical practice remains challenging. Digital self-monitoring systems with personalized feedback and adaptive learning content may support sustainable behavioral improvement. The objective of this study was to investigate if repeated self-directed digital hand hygiene assessments combined with individualized feedback, adaptive microlearning clips, and motivational performance scoring improve hand hygiene performance over time.

A prospective longitudinal single-center study with repeated measurements was conducted at Jena University Hospital. Healthcare workers and medical students with patient-facing activities completed three self-directed hand hygiene

assessments over 8-12 weeks using a browser-based assessment platform and the DesiCoach 2Go (Heyfair GmbH, Jena, Germany). Each assessment consisted of two sequential procedures: first, participants performed hand coverage using a temporarily visible whitening dye ("coloring"), followed by a second step requiring complete removal of the dye using alcohol-based hand disinfection ("discoloring"). Insufficiently covered or incompletely disinfected areas ("gaps") were digitally documented via smartphone. Up to 40 predefined zones per hand were assessed for both coloring and discoloring procedures. Following the baseline assessment, two follow-up interventions were performed asynchronously and individually, with the first intervention scheduled after 2-3 weeks and the second after 8-12 weeks. Based on individual deficits, participants received personalized digital feedback, targeted microlearning clips, and an individualized motivational performance score integrating hand coverage deficits and disinfectant dosing quality (Fig. 1).

A total of 137 participants completed baseline assessment, with 97 and 75 participants completing follow-up assessments. Mean coverage errors decreased from 7.92 at baseline to 3.37 after 2 weeks and 2.67 after 6 weeks. Median errors decreased from 6.0 to 1.0. Correct disinfectant dosing improved from 75.2% at baseline to 92.0% after 6 weeks (just involving blind staining). Completely error-free assessments increased from 6.6% to 28.0%. Participants achieving ≥80% hand coverage increased from 64.2% to 90.7%, while clinically acceptable hand disinfection (areas of skin that come into frequent contact with the environment) increased from 10.9% to 41.3%. The largest improvements between baseline and second intervention were observed in fingertips, thumbs, and wrist regions (Fig.2, dark green).

Personalized digital feedback, adaptive microlearning, and motivational performance scoring may substantially improve hand hygiene performance without instructor-led training. Digital self-monitoring systems could represent a scalable strategy for promoting sustainable infection prevention competencies and workplace-based learning in healthcare settings.

Fig. 1

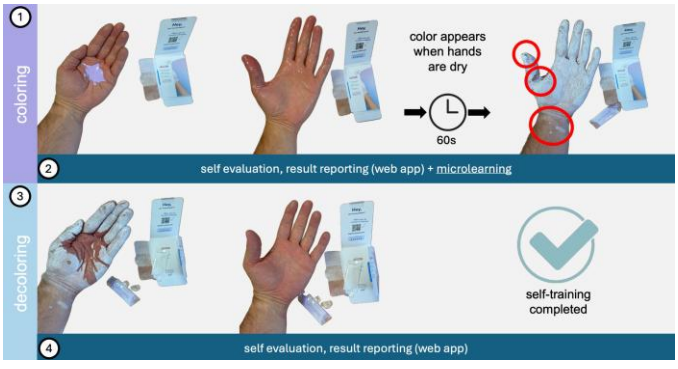

Fig. 2

WS24.04

Learning objectives for risk assessment and action-oriented risk communication in environmental medicine teaching in QB 06 at Witten/Herdecke University

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Background/Objectives: Climate vulnerability is often addressed in teaching and public health as heat, extreme weather events, and shifts in infectious disease patterns. In clinical practice, however, these risks intersect with a broad spectrum of environmental exposures, including air, water, chemicals, indoor environments, and physical factors, all of which help determine vulnerability in terms of exposure, sensitivity, and resilience. Thus, planetary health risks require environmental medicine competence among current and future physicians. At Witten/Herdecke University, a new approach to teaching and assessment in Cross-Sectional Area 06 "Clinical Environmental Medicine" has been successfully implemented, in which peer teaching through group presentations on environmental problems during Environmental Medicine Week plays a key role. This contribution develops a structured thematic and competency map that positions climate-related risks as a cross-cutting dimension and makes them usable for teaching.

Materials and Methods: A deductive content analysis was conducted along the dimensions of risk perception and risk communication, resilience, and prevention. The analysis was based on 68 teaching/presentation topics and responses from a total of 218 medical students who completed 80 statements from the Progress Test Environmental Medicine (PTU) in true/false format — true, false, don"t know — during the winter semester 2023/24 (N=92) and the summer semester 2024 (N=126).

Results: Risk assessment dominated the presentation topics, with a risk or hazard assessment already formulated in the title in 45 cases (66%). Action-oriented risk communication (13 topics, 19%), prevention (12 topics, 18%), and resilience in the sense of safeguarding system functioning (5 topics, 7%) were explicitly named much less frequently. Risk-related knowledge aspects also predominated in the PTU. Most items (72 items, 90%) primarily addressed risk assessment, whereas risk communication (10 items, 13%), prevention (9 items, 11%), and resilience (6 items, 8%) were represented only marginally by comparison.

Discussion/Conclusion: The structured thematic map of environmental medicine presentation topics prevents a narrowing of climate vulnerability to isolated risks and makes interfaces between environmental medicine, prevention, healthcare provision, and risk communication visible. Overall, the teaching and assessment topics in Witten/Herdecke University"s Environmental Medicine Week and in the PTU are strongly oriented toward risk assessment, whereas the dimensions of risk communication, resilience, and prevention are considerably less strongly represented in the curriculum.

Take-Home Message: Climate vulnerability becomes practically applicable in teaching and research when climate-related exposures are systematically integrated into the broader spectrum of clinically relevant environmental exposures and operationalized along the dimensions of exposure, sensitivity, and resilience.

WS24.05

Climate vulnerability as a teaching opportunity: What exposures, concerns, and resources do medical students report?

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Background/Research Question: Climate vulnerability can be described as interplay of exposure, sensitivity, and resilience, with individual resilience potentially buffering effects of adverse environmental stressors. In the context of climate change, healthcare professionals occupy a dual role: they are themselves exposed to climate-related stressors while also being required to recognize and treat climate-sensitive diseases and support preventive measures. Students usually have an understanding of climate change, but often show gaps regarding its specific health implications and action-oriented climate resilience. Our aim was to explore subjective climate vulnerability and educational needs among medical students.

Methods: In winter semester 2025/26, a voluntary, anonymous cross-sectional survey was conducted among medical students in their sixth semester during Environmental Medicine Week. A self-developed questionnaire comprising 20 items assessed expected climate-related exposures, physical and psychological symptoms, emotions/cognitions, and expectations regarding teaching (7 categorical items; 11 Likert-scale items rated from 1 to 5). Sensitivity and resilience were each assessed on a scale from 1 to 10. Climate vulnerability is calculated based on the students" self-assessments. For exploratory group comparisons, the sample was dichotomized at the median.

Results: A total of 61 students participated (38 female, 23 male; age M=24.83, SD=2.57; range 20–33). More than 90% expected exposure to heat waves, severe weather events, flooding, and dust/air pollution within the next five years. Concerns about consequences of climate change (M=4.13, SD=0.83) and perceived personal affectedness (M=3.70, SD=1.10) were high. The perceived climate-related changes caused negative emotions such as sadness (52.46%) and fear/anxiety (50.82%). A weak statistically significant association was found between perceived sensitivity and resilience (Spearman: r=−.258, p=.049). Students in the higher-vulnerability group showed, by construction, higher sensitivity (Z=−6.16, p<.001) and lower resilience (Z=−2.59, p=.010). Higher vulnerability tended to be associated with female gender, although narrowly not statistically significant (χ^2=3.80, p=.051). From the teaching sessions during Environmental Medicine Week, students most strongly expected knowledge about diseases caused by environmental influences (M=4.49, SD=0.77).

Discussion/Conclusion: Pilot data indicate a high level of anticipated climate-related burden and relevant climate emotions even during studies. The targeted development of climate competence can therefore be addressed both as a knowledge-related topic and as an issue of burden and resources, with learning objectives relating to risk assessment, care pathways, self-efficacy, and prevention.

Take-Home Message: Environmental medicine teaching should consistently combine evidence-based knowledge with the promotion of resilience and action-oriented risk communication.

SO04.01

Weiterentwicklung des Zweiten Abschnitts der ärztlichen Prüfung: Wo stehen wir aktuell und wo wollen wir hin?

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SO04.02

Medical microbiology and infection control throughout the medicine curriculum at the University of Groningen

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At the University of Groningen, the Medicine program annually admits 400 first-year students. The six-year curriculum comprises a three-year preclinical phase focusing on theoretical knowledge and core competencies, such as professionalism, communication, and scientific skills. The preclinical phase is followed by a three-year clinical phase emphasizing practical training across medical specialties. Students are educated through problem-based learning, preparing them as future physicians to meet the challenges of

increasingly complex and evolving healthcare. To monitor progress throughout the entire curriculum, a progress test is administered annually containing multiple-choice questions that cover all medical specialties, including medical microbiology.

Medical microbiology is integral to the curriculum, offering future physicians comprehensive education in infectious diseases, microbial pathogenesis, diagnostics, therapeutics, and infection prevention. In the first preclinical year, students study microbial diversity, host-pathogen interactions, immunology, and the fundamentals of diagnosis and treatment, supported by practical laboratory sessions featuring both culture-based and molecular techniques. The second and third preclinical years include lectures on clinical conditions and therapeutic strategies related to medical microbiology, including systemic bacterial infections and viral infections. Assessment in the preclinical phase includes multiple-choice exams.

The clinical phase begins with practical training in infection prevention and the application of diagnostic and therapeutic knowledge, including diagnostic and therapeutic stewardship and antimicrobial resistance. Practical examinations require students to manage clinical cases, including evaluation focusing on adherence to current guidelines for infection prevention, diagnostics, and treatment.

During clinical rotations, students encounter a range of infectious diseases across different specialties. The final clinical year includes an elective internship in microbiology and infection control, in which performance is assessed. This specialization allows students to advance their clinical microbiological reasoning and antimicrobial stewardship. Additionally, research projects in microbiology or infection control are available for students to further deepen their interest.

A revised preclinical curriculum is currently under development, designed to be future-oriented and to better prepare graduates for evolving roles in healthcare, with particular attention to appropriate care, sustainability, AI, prevention and the need for physicians in the first line of the healthcare system. The detailed implications for medical microbiology and infection control remain to be determined. The clinical phase will remain the same.

SO04.03
Structural differences between undergraduate medical education in Germany and Switzerland with a focus on medical microbiology and infectious diseases
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Introduction: Medical students in Germany (DE) and Switzerland (CH) are trained for professional roles as physicians within differently structured education systems. While DE applies a nationally regulated state-examination model with defined subjects, CH follows a Bologna-compatible Bachelor"s (BSc)/Master"s (MSc) structure with national competencies and greater curricular autonomy.

Objectives: The aim was to provide a structured comparison of undergraduate medical education in both countries, addressing admission procedures, including numerus clausus; study structure (e.g., classical va. Bologna model); clinical practica; MC/OSCE examinations; research elements; and curricular visibility.

Materials and Methods: A document-based analysis of publicly available regulatory and curricular sources was performed, including the German Licensing Regulations for Physicians, Swiss regulations on undergraduate medical education, the Swiss federal licensing examination and the competency-based Swiss Catalogue of Learning Objectives, PROFILES.

Results: Admission differs between both countries, with DE using a quota-based national allocation while in CH central preregistration with numerus clausus procedures and aptitude tests apply, based on cantonal specificities. DE implements a six-year state-examination programme whereas in CH a three-year BSc and three-year MSc programme is followed. At a practical level, DE requires nursing service, clinical electives and a 48-week final clinical year (PJ); CH includes longitudinal clinical teaching and an elective year/sub-internships. Three state examinations across the curriculum need to be passed in DE, whereas CH culminates in a federal licensing examination with MC and clinical-practical OSCE components. Research is more formally embedded in CH through the MSc thesis, while in DE it is less uniformly integrated and often linked to optional doctoral or faculty-specific programmes. Curricular visibility differs markedly. In DE, hygiene, microbiology, virology and infectious diseases are explicitly named in the licensing regulations, while in CH, these fields are mainly integrated into proficiency- and situation-based objectives, including diagnostic reasoning, nosocomial infections, immunocompromised patients and rational antibiotic prescribing.

Conclusions: Both systems lead, after six years of study, to a nationally regulated medical qualification and include substantial clinical practice components. Germany has a more discipline- and examination-regulation-based structure, whereas Switzerland follows a Bologna-compatible, competency-based model with greater scope for university-specific curricular implementation. Consequently, medical microbiology and infectious diseases exhibit different curricular visibility, characterized by explicit disciplinary anchoring in Germany versus a clinical, situation-based integration in Switzerland.

PS01.01
Potentially human-pathogenic *Vibrio* species and climate change: 13 years of monitoring coastal bathing waters in Schleswig-Holstein
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Introduction: The potentially pathogenic *Vibrio* species *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae* can cause wound infections, among other types of infection, in individuals exposed to seawater during swimming or wading. Although these infections are relatively rare, they are associated with high mortality rates of up to 50% and attract considerable media attention. Rising water temperatures in the North Sea, and particularly in the Baltic Sea, as a result of climate change

are expected to promote the proliferation of *Vibrio* species and, consequently, increase the incidence of human infections.

Aims: In the context of climate change, the aim of this study is to monitor the occurrence of potentially pathogenic *Vibrio* species in the coastal bathing waters of Schleswig-Holstein over extended time periods.

Materials and Methods: Following an initial comprehensive monitoring programme, current investigations of *Vibrio* bacteria are focused on bathing waters characterized by extensive shallow zones, where elevated water temperatures are most likely to occur, as well as by varying salinity conditions ranging from brackish to marine waters. The sampling sites are evenly distributed along the Baltic Sea coast of Schleswig-Holstein. Sampling is carried out in accordance with the Bathing Water Directive at a water depth of 1 m and a sampling depth of 30 cm. Microbiological analyses are performed using an accredited culture-based method employing CHROMagar™ Vibrio, followed by species identification via MALDI-TOF mass spectrometry, including the dedicated VibrioBase database.

Results: Based on the studies conducted, a four-tiered assessment scale was developed, ranging from non-detection to elevated concentrations. The occurrence of the three potentially pathogenic *Vibrio* species is strongly associated with water conductivity. The highest concentrations were detected in the Schlei estuary, where *V. cholerae* and *V. vulnificus* were particularly prevalent, whereas *V. parahaemolyticus* predominated along the Baltic Sea coast. Overall, bacterial concentrations were generally low or within the normal range. Fourteen bathing waters were directly comparable between 2014 and 2025; notably, higher concentrations were observed in 2025 despite lower water temperatures.

Conclusions: *Vibrio* concentrations measured in bathing waters of Schleswig-Holstein along the Baltic Sea coast and in the Schlei are generally within the expected, unremarkable range. In the long term, a slight increase in concentrations compared with 2014 appears likely; however, water conductivity and current water temperature exert a substantially greater influence on bacterial concentrations than any presumed long-term increase in average water temperature. Nevertheless, from the perspective of the public health authorities, regular annual monitoring of selected bathing waters remains necessary in light of ongoing climate change and the presence of extensive shallow-water zones at bathing sites.

PS01.02
Thermal inactivation of *Legionella* spp. and evaluation of *Brevundimonas aurantiaca* as a surrogate organism for hygienic assessment of decentralized domestic hot water systems

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Background: Rising energy costs and the need to reduce CO_2 emissions require new strategies for domestic hot water preparation, particularly in existing buildings. Heat pumps enable energy-efficient operation but often at reduced temperatures, which may promote the growth of *Legionella spp.* (30–45 °C). Decentralized instantaneous heaters offer a potential solution by enabling localized thermal disinfection at the point of use. However, reliable methods for assessing bacterial viability after thermal treatment are required.

Method: The effect of defined temperatures (37 – 80 °C) and exposure times (0-10 min) on the survival of different *Legionella* strains (*L. pneumophila* sg1 Philadelphia, sg1 OLDA, sg3, *L. anisa*) was investigated. Additionally, *Brevundimonas aurantiaca,* isolated from a plumbing emulator, was evaluated as a non-pathogenic surrogate organism. All strains were typed via the ELISA or MALDI-TOF MS. A cell suspension of ~1 × 10^8 CFU/mL were heat-treated before further testing. Viability and cellular integrity were assessed using three complementary approaches: culture-based methods (BCYE agar), qPCR/vPCR (microproof® *Legionella* Quantification LyoKit and the GoTaq® *Legionella* qPCR/vPCR Kit) and Flow cytometry. Flow cytometry employed SYBR Green I and propidium iodide staining to differentiate total, intact, and membrane-compromised cells.

Results and Conclusion: While culture-based methods indicated strong reduction or absence of growth after thermal treatment, qPCR/vPCR and flow cytometry still detected intact or potentially viable cells. This suggests the induction of a viable but non-culturable (VBNC) state rather than complete inactivation. Temperatures below 50 °C showed limited effects on both *Legionella spp.* and *B. aurantiaca*. Flow cytometry and molecular methods proved more sensitive than culture alone in assessing bacterial viability. These findings highlight the importance of temperature, exposure time, and physiological cell state for the hygienic evaluation of decentralized thermal treatment strategies and support the use of surrogate organisms in plumbing emulator studies.

PS01.03
The impact of sampling procedures on the microbiological assessment of dental unit waterlines

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Question: Microbiological monitoring of dental unit waterlines (DUWL) is essential for infection prevention and quality assurance in dental practices. However, the influence of different sampling procedures on microbiological results has not been sufficiently investigated.

The objective of our study was to assess the impact of various sampling procedures on the detection of microbiological contamination in DUWL.

Methods: Water samples from dental units (DU) were aseptically collected prior to and following flushing for a duration of 20 seconds for all transfer instruments (handpieces), the air/water syringe, and the ultrasonic scaler. To identify the influence of a potential contamination at the connection points, samples were also collected separately after disinfection and subsequent rinsing for all handpieces. Subsequently, each sample was subjected to independent analysis. All samples were tested for colony forming unit (CFU) per milliliter (mL) at 36°C in accordance with DIN EN ISO

6222:1999 and for the presence of *Legionella* sp. according to DIN EN ISO 11731:2019-03. CFU values above 300/mL were replaced by 300. The statistical significance of the differences between the results obtained from the various sampling methods was determined by employing the Wilcoxon test. A p-value less than 0.05 was considered to be statistically significant.

Results: A total of 537 samples were collected from 45 DU, with 90 samples obtained before and after flushing the air-water syringes and scalers. 357 samples were collected from transfer instruments/handpieces. Eleven DU were equipped with one or two micro-motors for the connection of handpieces and 34 DU were equipped with three micro-motors. 23 DU revealed CFU counts above the limit of 100 CFU/mL in at least one sample after disinfection/rinsing of handpieces with 19 samples from handpieces with CFU counts above 300/mL. After rinsing, eleven samples from air-water-syringes and 20 samples from scalers exhibited CFU counts that exceeded 100/mL.

A significant decrease in the mean number of CFUs was observed between samples collected after rinsing and after an additional disinfection procedure of the handpieces (119 versus 77 CFU/mL, p=0.013; 171 vs. 69 CFU/mL, p<0.001 and 176 vs. 68 CFU/ml, p< 0.001) and before and after rinsing the air-water syringes (131 vs. 83 CFU/mL, p = 0.021), but not after rinsing the scaler (140 vs. 135 CFU/mL, p = 0.469). The presence of *Legionella* sp. was detected in a single sample.

Conclusion: In order to assess the microbial load of procedural water from dental units, the sampling technique is of crucial importance. Our results reveal that it is necessary to take samples from the transfer instruments after disinfection of the connection point and subsequent rinsing, as well as to take a separate sample from the air-water syringe. Sampling the scaler may be non-compulsory.

PS01.04
Applied drinking water hygiene in technical systems: Automated on-site monitoring of viruses and bacteria

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Introduction & Objectives: The revised EU Drinking Water Directive (2020) mandates comprehensive risk management, including the monitoring of faecal indicators such as somatic coliphages, and pathogenic bacteria such as *Legionella* spp. and *Pseudomonas aeruginosa*. Conventional culture-based methods are too labor- and time-intensive to support a proactive and real-time risk management. Furthermore, current market solutions lack fully automated on-site sampling and detection from large water volumes at critical control points of drinking water distribution systems.

Methods: An automated monitoring system is being developed to detect the bacterial and viral parameter somatic coliphages, *P. aeruginosa*, and *Legionella* spp. The core innovation is a novel automated concentration module capable of capturing both viral (nanometer-scale) and bacterial (micrometer-scale) particles from large volumes while preventing clogging via filtration. The entire process including sampling, concentration, and species-specific molecular detection using qPCR operates within a sealed device. This eliminates manual handling, prevents operator errors and cross-contamination.

The proof-of-principle has been demonstrated that a concentration of bacteria is possible from water samples with recovery rates of >70 % for the depicted parameters. A multiplex qPCR was developed which detects *Legionella pneumophila*, *P. aeruginosa* and somatic coliphages T4. An online monitoring strategy is implemented allowing the automated sampling, the monitoring of the reservoir tanks and transfers the data obtained from the qPCR to an application on a smartphone/tablet device. An automated alert-system is implemented to inform users in case of threshold violations.

Discussion & Impact Integration with existing water conditioning systems (e.g., careBox®) allows for targeted, automated disinfection triggered only by acute contamination. For high-risk environments like hospitals, this technology offers the potential to replace costly point-of-use sterile filters with process-controlled hygiene surveillance, significantly reducing operating costs while enhancing supply security.

The presentation will provide insights into the system architecture, recent research results, and the resulting health and economic benefits for water suppliers and operators.

PS01.05
***Aspergillus niger* complex – Hygiene monitoring, source identification and remediation: Practical applications**

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Introduction: In a newly constructed room for water microbiological testing, which has been in use since June 2024, culture plates contaminated with mould have been observed since early November 2024 during the microbiological processing of drinking water samples. Of the 2,985 "Legionella culture plates" processed between 5 November 2025 and 27 February 2025, a total of 566 culture plates (19%) were contaminated with mould (*Aspergillus niger* complex), and of these, 330 culture plates (11%) were no longer usable. As part of quality assurance, a massive contamination of the indoor air with *Aspergillus niger* complex was detected, leading to the establishment of hygiene monitoring.

Objective: The objective of the intensive monitoring was to identify the source of the *Aspergillus niger* complex, determine the cause of the mould contamination and ensure its permanent elimination, as well as to prevent the spread of airborne contamination to adjacent laboratory areas.

Method: As part of routine hygiene monitoring, environmental hygiene testing methods—such as the measurement of mould in the air using impaction and sedimentation techniques twice a week over a 10-month period, as well as ad hoc random sampling of cultivable mould in materials—were carried out and analysed.

Results: The data collected between January and October 2025 from a total of 50 sampling campaigns involving over 500 individual samples indicated contamination with a pure culture of the *Aspergillus niger* complex. The total colony count showed highly fluctuating concentrations ranging from 0 CFU to 675 CFU per m³ of indoor air (active air sampling using the impaction method) and from 0 CFU to 100 CFU per 4-hour incubation period (sedimentation plates). In the course of the monitoring carried out, unsealed joints in a special exhaust air duct and recurring contamination from a recirculating air cooling unit were identified as likely sources of the *Aspergillus niger* complex.

Conclusion: The necessary remediation measures to eliminate the causes of mould growth and the maintenance of laboratory routines must be assessed on a case-by-case basis with regard to their implementation and closely monitored. As with many other hygiene issues, mould contamination can have multiple causes and cannot usually be attributed to a single source. The room"s design, the properties of the contaminant and, where applicable, air-handling units that exacerbate the problem can lead to the further spread and persistence of airborne spores within the room. Following several special cleanings of the room, microbiological activities were resumed in October 2025 after the room had stood empty for eight months. Since then, there have been no further instances of *Aspergillus niger* complex contaminating culture plates.

PS01.06
Resistance trends of vancomycin, teicoplanin and linezolid in *Enterococcus faecium* in Bavaria, Germany, 2020–2024

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Introduction: *Enterococcus faecium* (*ENCFAC*) is an important nosocomial pathogen with limited treatment options if resistance to glycopeptides or oxazolidinones occurs [1]. The NRZ for Staphylococci and Enterococci reported an increase in linezolid resistance among *ENCFAC* from 2020 to 2023, followed by a decline in 2024. Among vancomycin resistant enterococci (VRE) linezolid resistance increased in 2024 compared with 2020 and a shift to VanA was observed. [2]. The trend in resistance to vancomycin, teicoplanin and linezolid in *ENCFAC* in Bavaria will be outlined using the Datapool of the Bayerische Antibiotikaresistenz-Datenbank (BARDa) [3].

Material and Method: From 2020 to 2024, 24 to 30 Bavarian laboratories submitted their anonymized resistance data as SIR assessment to BARDa, evaluated according to the EUCAST standard. For each patient, only the first isolate for a period of 90 days is included. Screening samples are excluded. Trends are statistically analysed using a two-sided Cochran-Armitage trend test with Bonferroni-Holm correction for multiple testing.

Results: In the inpatient setting, BARDa showed a statistically significant decrease in vancomycin resistance among *ENCFAC* isolates from 31.0% in 2020 to 13.1% in 2024. Teicoplanin resistance also decreased statistically significantly over the same period from 15.5% to 8.2%. Meanwhile, linezolid resistance remained at a low level of around 0.7%. Teicoplanin resistance increased among VRE isolates, supposing a VanA shift from 2023 onwards. By 2024, 61.4% of VRE isolates were resistant to teicoplanin. Among VRE isolates, linezolid resistance peaked in 2023 at 2.9% (up from 1.0% in 2020).

Discussion: In the German surveillance tool ARS a sharp decline in both teicoplanin and vancomycin resistance has been observed in the inpatient sector for *ENCFAC* between 2020 and 2024, too [4]. In both BARDa and ARS, VRE has finally fallen below the European average of 16.9% since 2023 [1]. Unlike BARDa, which includes all materials, ARS only considers invasive isolates. BARDa data indicate a shift towards VanA for VRE from 2023 onwards as the NRZ postulates. Please note that samples submitted to the NRZ provide targeted insight into resistant isolates, as they are often selectively submitted and therefore do not represent the general population prevalence. VRE with linezolid resistance (LVRE) peak in both BARDa and NRZ in 2023. Low levels of linezolid resistance are reassuring, but warrant continued surveillance to rapidly detect any rise, including among LVRE.

[1] https://www.ecdc.europa.eu/en/publications-data/health-burden-infections-antibiotic resistant-bacteria-2016-2020
[2] Bender JK et al. Eigenschaften, Häufigkeit und Verbreitung von VRE in Deutschland – Update 2023/2024. Epid Bull 2026;11:3-18 | DOI 10.25646/13847
[3] https://www.lgl.bayern.de/gesundheit/infektionsschutz/barda/index.htm
[4] https://amr.rki.de/Content/Datenbank/ARS/ResistanceOverview.aspx

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PS01.07
Rapid identification of high-risk clone ST111 by *chrA*-PCR: Implications for infection control and outbreak management

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Introduction: The high-risk clone *Pseudomonas aeruginosa* ST111 is an increasing cause of hospital outbreaks frequently associated with environmental reservoir, particularly in intensive care units (ICUs). Detection of high-risk clones currently relies on resource- and time-intensive whole-genome sequencing (WGS). Here, we describe a novel PCR assay targeting the chromate transport protein *chrA* for rapid identification of the high-risk clone *P. aeruginosa* ST111. The assay was applied as a rapid screening tool during an external ICU outbreak caused by *P. aeruginosa*.

Methods: Between January 2019 and December 2024, core genome multilocus sequence typing (cgMLST) was performed on clinical and screening isolates of 4MRGN *P. aeruginosa* from non-paediatric inpatients at a tertiary care hospital. ST111 isolates were screened for potential specific marker genes.

PCR primers for the identified *chrA* gene were designed, and validated using *chrA*-positive ST111 and -negative non-ST111 isolates, as determined by WGS. During an outbreak in an external hospital, clinical and environmental isolates were sent to our laboratory and initially analysed using the *chrA*-PCR, followed by confirmatory WGS.

Results: A total of 20 internal clinical *P. aeruginosa* ST111 isolates were identified. Of these, 85.0% (17/20) harboured the *bla*VIM-2 carbapenemase gene. All ST111 isolates carried the *chrA* protein, which was consistently detected by the *chrA*-PCR. No false positive results were obtained for non-ST111 isolates. During investigation of an external outbreak (four clinical and three environmental isolates collected between June 2024 and June 2025), *chrA*-PCR was applied initially to all samples and confirmed the presence of *chrA* protein in all isolates, indicating ST111. Subsequent WGS confirmed ST111 in all PCR-positive isolates. The outbreak was successfully terminated following removal of contaminated wash basins.

Discussion: Effective infection prevention and control (IPC) measures are essential to contain outbreaks, particularly those involving high-risk clones such as *P. aeruginosa* ST111. While WGS remains the gold standard, it is time-consuming, whereas PCR-based methods offer nearly real-time detection with high concordance to WGS, facilitating earlier intervention and potentially reducing transmission. Despite the limited number of isolates, the consistent performance across both clinical and environmental samples highlights its robustness and practical applicability as a frontline screening tool.

Conclusion: The novel *chrA*-PCR represents an accurate and efficient diagnostic tool for the rapid identification of *P. aeruginosa* ST111 in outbreak settings.

PS01.08
Saving costs safely: Cost effectiveness and break-even-point analyses of single-use versus reusable bronchoscopes in the ICU

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Introduction: Continuous availability of bronchoscopy in the intensive care unit (ICU) is essential for adequate diagnostic and therapeutic management. The use of single-use flexible bronchoscopes (SFB) is often considered to reduces costs for repair, reprocessing and concerns for microbiological safety associated with reusable flexible bronchoscopes (RFB). Previous international studies have demonstrated the cost-effectiveness of RFB vs SVB in medium- to high- volume ICUs. However, to date, no study has evaluated the cos-t effectiveness of SFB vs RFB within the German ICU setting.

Aim: The aim of this study was to evaluate the cost-effectiveness of SFB vs. RFB in a German ICU setting.

Material and Methods: The study was conducted using data from the four ICUs of our tertiary care hospital over a two-year period. In-house reprocessing was available 24/7 and followed national guidelines. Data on the number of available RFB and performed procedures as well as data obtained from institutional records, manufacturer information, and empirically measured workflow parameters were collected. We compared cost for SFB, including acquisition costs per procedure and annual deprecation costs for light sources, and RFB, including annual fixed cost (repair, maintenance, microbiological surveillance, deprecation for RFB/ washer-disinfectors/ bronchoscopy towers/ light sources) and variable cost per procedure for reprocessing. The break-even-point for cost-effectiveness of RFB was calculated for the base- case scenario and for two scenarios considering higher maintenance and repair costs as well as price reduction for SFB.

Results: During the study period from 1 January 2023 to 31 December 2024, a mean of 1253 bronchoscopic procedures were performed annually in ICU I-IV. In total 20 RFB, three bronchoscopy towers, two washer-disinfectors and three light sources for SFB were available for bronchoscopic procedures in the ICU department. Annual fixed costs and variable cost per procedure were €108 258.02 and € 10.88 for RFB compared to € 2 558.50 and € 344.15 for SFB, respectively. The calculated break-even-point in the base-case scenario was 317 procedures, 50% increase in maintenance cost and 20% price reduction for SFB lead to break-eve-points of 358 and 399 procedures, respectively.

Conclusion: In the German ICU setting with a high bronchoscopy frequency, RFB are cost effective compared to SFB. Primary cost drivers were investment costs whereas personal and reprocessing cost were negligible.

PS01.09
Which leadership skills of infection and prevention control (IPC) teams may best empower clinicians regarding their hand hygiene compliance? Results of the IP-POWER trial's baseline survey on associations of capabilities, opportunities, and motivation with clinicians' assessments of "their" IPC team

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Leadership in hospitals can improve infection prevention and control (IPC).[1] In Germany, the LEAD-IC study has shown that management training of chief medical officers can initiate favorable changes in clinicians' IPC-related cooperation.[2] The IP-POWER trial (DRKS00031879)[3] applies the concept of leadership to IPC teams. The present analysis uses data from its baseline survey to examine which IPC teams" leadership skills are perceived by clinical staff as being most prominent, and which of these perceptions are associated with their capabilities, opportunities, and motivation regarding hand hygiene.

In 10 study hospitals, an online survey of clinical staff was conducted (2024-25). Complete cases are available for N=790 physicians and nurses who assessed their IPC team"s skills regarding employee development, personal influence, and environmental design. Scales from the LEaD Model©[4-5] were used, adapted to IPC in collaboration with the authors and

publisher. Hand hygiene opportunities, capabilities, motivation, and planning skills as perceived by the clinicians were assessed with scales developed and validated for surgical site infection prevention following the COM-B model[6] in the WACH trial (DRKS00015502).[7] They were adapted to hand hygiene as a single measure in IP-POWER. Statistically, general linear modelling (GLM) was employed.

Table 1 shows that among IPC teams" leadership skills, clinicians rated employee development lowest, followed by environmental design and personal influence. Among COM-B outcomes, they rated their planning skills lowest, exceeded by both motivation and capabilities, and opportunities. As Table 2 shows in regard to the associations between the two sets of variables, estimates associated with a shared variance of at least 5% (i.e., $\eta^2 \geq 0.05$) pertained to employee development and personal influence with planning, and to environmental design with opportunities (all $p<0.001$). Patterns were comparable across physicians and nurses.

Results indicate that IPC team leadership skills are differentially associated with clinicians" hand hygiene determinants. Skills in coaching, perspective taking, and giving feedback as well as instilling confidence and managing ambiguities correlate with planning skills, i.e., organizing for one"s own hand hygiene. This points to hand hygiene compliance as an implementation rather than a motivational issue. Managing conflicts and implementing changes seem to foster opportunities, i.e., resources and cooperation. These findings suggest that in order to effectively fulfil their leading roles, IPC team training should prioritize both interpersonal and organizational skills, including conflict management and work environments enabling hand hygiene compliance.

[1] doi:10.1186/s13756-016-0149-9

[2] doi:10.1186/s12909-023-04709-z

[3] doi:10.1136/bmjopen-2023-083806

[4] doi:10.1007/s11612-021-00582-w

[5] https://d-nb.info/1167955528

[6] doi:10.1186/1748-5908-6-42

[7] doi:10.2147/PRBM.S464335

Fig. 1

Table 1

Means with 95% confidence intervals (95%-CI) of the LEaD© and the COM-B scales

Scale	Mean (M)	95%-CI
Personal Influence (5 items)[a]	2.8	[2.82; 2.94]
Employee Development (10 items)[a]	2.2	[2.18; 2.31]
Environmental Design (6 items)[a]	2.7	[2.60; 2.72]
Capabilities (6 items)[b]	2.6	[2.61; 2.67]
Opportunities (6items)[b]	2.1	[2.02; 2.11]
Motivation (4 items)[b]	2.7	[2.67; 2.73]
Planning skills (2 items)[b]	1.6	[1.49; 1.63]

[a]LEaD© model (Likert-scales from 0 "never" to 4 "(almost) always")
[b]COM-B model (Likert-scales from 0 "not true at all" to 3 "definitely true")

Fig. 2

Table 2

Results of multiple multivariate general linear model with LEaD© scales as predictors and COM-B scales as outcomes

LEaD© predictors	COM-B outcomes	$F_{(1,786)}$	p-value	Partial Eta Squared (η^2)
Personal Influence[a,b]	Capabilities[c]	0.1	0.811	<0.01
	Opportunities[c]	15.9	<0.001	0.02
	Motivation[c]	2.9	0.091	<0.01
	Planning skills[c]	49.9	<0.001	0.06
Employee Development[a,b]	Capabilities[c]	3.3	0.070	<0.01
	Opportunities[c]	25.2	<0.001	0.03
	Motivation[c]	4.8	0.029	0.01
	Planning skills[c]	65.6	<0.001	0.08
Environmental Design[a,b]	Capabilities[c]	6.0	0.015	0.01
	Opportunities[c]	44.0	<0.001	0.05
	Motivation[c]	1.1	0.291	<0.01
	Planning skills[c]	6.6	0.010	0.01

[a]LEaD© model
[b]p<0.001 for all multivariate tests
[c]COM-B model

PS01.10
Synergistic mode of a ceftazidime-hexa-arginine-conjugate with resistance-overcoming properties

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Objectives: A non-toxic ceftazidime-hexa-arginine conjugate (CTZ-R6) was previously shown to surpass the antibacterial potency of the third-generation cephalosporine ceftazidime (CTZ) against multidrug-resistant bacteria by up to a 1000-fold [1], but mechanistic understanding remained elusive. Here, we investigated the mode of action of the conjugate in the Gram-positive and -negative model species *B. subtilis* and *E. coli*. Understanding of such conjugation approaches is essential to extend the lifespan of important antibiotics.

Methods: Determination of minimal inhibitory concentrations against multi-resistant clinical isolates, reference strains, and isogenic mutants by standardised broth microdilution. Investigation of target structures and lysis kinetics via specific bioreporters. Assessment of bacterial membrane potential and membrane integrity as well as cell morphology by microscopy. Determination of the penicillin-binding protein (PBP) target spectrum of CTZ-R6 in susceptibility tests with mutants devoid of individual PBPs.

Results: CTZ-R6 outperformed CTZ against multi-resistant Gram-negative isolates and synergised with avibactam. It passed the outer membrane of *E. coli* by self-promoted uptake and permeabilised this barrier to otherwise ineffective

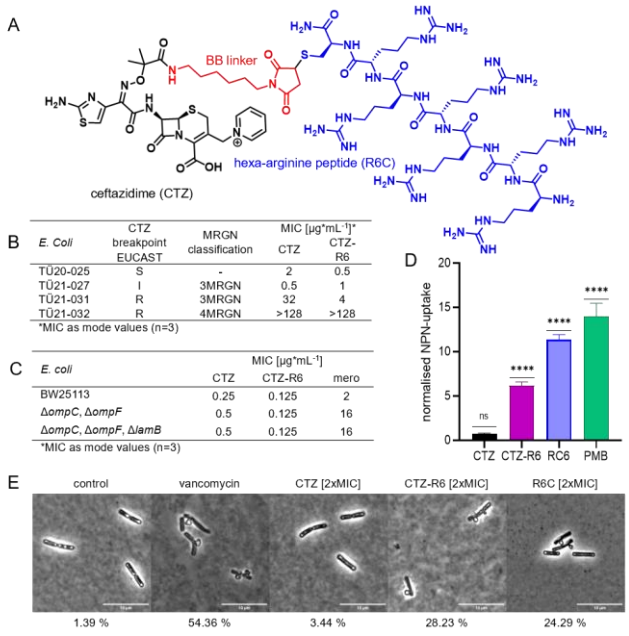
antibiotics, e.g. vancomycin. The antibacterial activity was not affected by the colistin resistance determinant MCR-1, and the cytoplasmic membrane potential remained intact, but membrane topology was perturbed. The peptidoglycan sacculus was damaged, resulting in β-lactam conform cell lysis kinetics. CTZ-R6 showed higher affinity for PBP2a of *B. subtilis*, lower affinity for PBP1a/b, and an overall more balanced PBP binding profile.

Conclusion: Both moieties contribute jointly to the increased antibacterial potency of CTZ-R6 with retained β-lactam-like characteristics. R6C destabilises the outer membrane, enabling porin-independent uptake. The peptide also interacts with the cytoplasmic membrane, facilitating access of CTZ to the PBPs in *B. subtilis* in a broader, more balanced manner which is beneficial for overcoming established resistance.

1. Werner, J., et al., *Conjugation of Polycationic Peptides Extends the Efficacy Spectrum of beta-Lactam Antibiotics.* Adv Sci (Weinh), 2024. 11(48): p. e2411406.

Figure 1. A) Structure of the conjugate CTZ-R6 (1783.12 g*mol^{-1}). B) Antimicrobial activity of CTZ and CTZ-R6 against clinical isolates. C) MICs for *E. coli* BW25113 and isogenic, porin-deficient mutants. Meropenem as positive control permeates through porins. D) Passage of the fluorophore NPN through the permeabilised outer membrane of *E. coli* (20 µM; n=3, normalised to the untreated control, ± SD). Negative control DMSO (1%), positive control PMB. ****: p<0.0001 (one-way ANOVA). E) Membrane protruding through areas of weakened peptidoglycan in *B. subtilis* after 15 min of compound exposure. Positive control vancomycin (n=3). Representative images of cells, >100 cells analysed per condition.

Fig. 1



B

E. Coli	CTZ breakpoint EUCAST	MRGN classification	MIC [µg*mL^{-1}]* CTZ	CTZ-R6
TÜ20-025	S	-	2	0.5
TÜ21-027	I	3MRGN	0.5	1
TÜ21-031	R	3MRGN	32	4
TÜ21-032	R	4MRGN	>128	>128

*MIC as mode values (n=3)

C

E. coli	MIC [µg*mL^{-1}] CTZ	CTZ-R6	mero
BW25113	0.25	0.125	2
ΔompC, ΔompF	0.5	0.125	16
ΔompC, ΔompF, ΔlamB	0.5	0.125	16

*MIC as mode values (n=3)

PS01.11
Establishing a structured cleaning compliance observation program at a university hospital

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Background: Ensuring adequate hospital cleaning requires both professional expertise and consistent compliance among cleaning staff. To systematically demonstrate and maintain cleaning quality, standardized compliance observations were introduced at our university hospital.

Objective: The aim of this project was to implement a structured and objective cleaning observation system to make process quality measurable and enable targeted improvements.

Methods: A 32-item observation checklist was developed to assess cleaning compliance. Using this tool, 50 audits were conducted over the course of one month during routine operations and subsequently analyzed statistically.

Results: The new procedure successfully enabled objective evaluation of cleaning performance. On average, two deviations from the defined standards were identified per observation. Error rates were lower in high-risk areas (1 deviation on average) compared to peripheral wards (3 deviations). The checklist has since been firmly integrated into both the hospital hygiene department and the external cleaning service to continuously monitor compliance and to derive targeted training measures based on the collected data.

Conclusion: Structured cleaning observations represent an effective tool for quality assurance. Objectifying the results provides the necessary data foundation for targeted retraining efforts, helping to sustainably close compliance gaps in everyday clinical practice.

PS01.12
No consistent differences in the maternal gut microbiome between Cesarean section with perioperative antibiotic prophylaxis and vaginal delivery: Results from the *MAMA* cohort study

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Introduction: Perioperative antibiotic prophylaxis (PAP) during Cesarean section (CS) is recommended by international gynecological guidelines to reduce maternal infectious complications. As microbiome-related consequences of antibiotic exposure are increasingly discussed in infection prevention and antimicrobial stewardship, prospective longitudinal clinical data remain limited.

Objectives: The Microbiome changes due to Antibiotic prophylaxis in Mothers At birth (*MAMA*) study aimed to scientifically address this knowledge gap. We investigated longitudinal changes in the maternal gut microbiome following CS with perioperative cefuroxime prophylaxis or vaginal delivery (VD) without antibiotic administration.

Materials and Methods: In this prospective longitudinal cohort study, pregnant women were recruited between May 2022 and October 2024. Participants provided self-collected stool samples during late pregnancy (T0; ≥32 gestational weeks), early postpartum (T1; 2-3 days postpartum), and three months postpartum (T2). Microbiome profiling was performed using 16S rRNA gene sequencing and QIIME2-based downstream analysis. Alpha diversity metrics, beta diversity analyses (Bray-Curtis, weighted and unweighted UniFrac), and longitudinal within-subject analyses were conducted. The study was approved by the Ethics Committee of Witten/Herdecke University and registered in the German Clinical Trials Register (DRKS00027305).

Results: A total of 31 women (CS: n=22; VD: n=9) contributed 71 eligible stool samples. No significant differences in alpha diversity were observed between delivery modes or across sampling time points. Beta diversity analysis using unweighted UniFrac demonstrated a statistically significant difference between delivery modes (Figure 1); however, the observed effect size was small (PERMANOVA: pseudo-F=1.67, R^2=0.024, p=0.005). The result was therefore likely influenced by differences in group dispersion rather than reflecting a robust biological separation. In line with this, no significant differences were observed using Bray-Curtis or weighted UniFrac distances. Longitudinal within-subject analyses revealed no evidence for differential microbiome shifts following CS with PAP compared with VD. Exploratory taxonomic analyses showed substantial inter-individual variability without consistent group-specific patterns.

Summary: In this prospective longitudinal cohort, routine perioperative prophylaxis with cefuroxime during CS was not associated with robust or persistent alterations of the maternal gut microbiome compared with vaginal delivery. Although subtle phylogenetic presence-absence effects cannot be excluded in this small cohort, no robust or persistent microbiome alterations were observed. Taken together, these findings support the microbiome-related tolerability of guideline-recommended PAP in Cesarean section and its continued role in surgical infection prevention.

Fig. 1

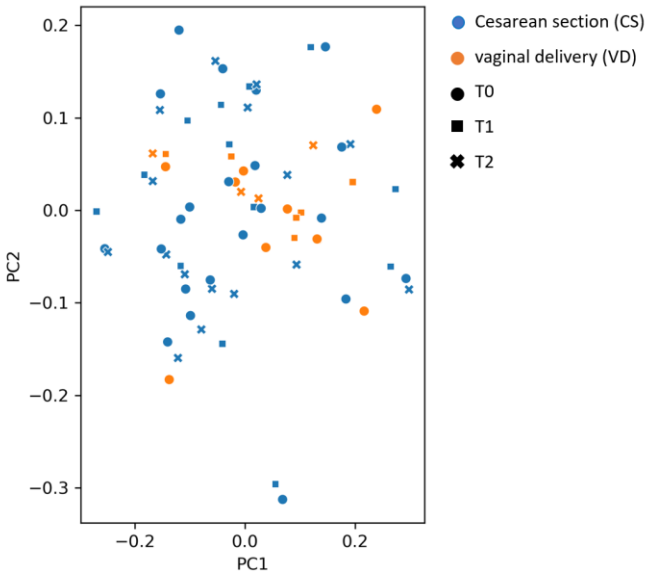

Figure 1. Principal coordinates analysis (PCoA) based on unweighted UniFrac distances showing beta diversity across delivery modes and sampling time points. Colors indicate delivery mode; symbols indicate sampling time points.

PS01.13

Surgeons' hand hygiene compliance during bedside visits: A matter of patient placement?

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Question: As a component of standard precautions (SP), hand hygiene (HH) according to the five moments defined by the World Health Organization has been determined to be the most effective measure for limiting spread of microorganisms and preventing healthcare-associated infections. Isolation precautions (IP) are implemented for patients infected or colonized with pathogens which require additional control measures. IP encompasses two key components: placing patients in single rooms and providing personal protective equipment. However, it has been demonstrated that outside of infection prevention contexts, IP can adversely affect patient safety and quality of care.

The aim of our study was to compare hand hygiene compliance (HHC) of surgeons during bedside visits under different precaution categories (SP and IP) and surgical wards.

Methods: A cross-sectional observational study was conducted to examine HHC during all bedside visits from Monday to Friday over a four-week period on two different wards. Observations were conducted by a single trained observer. HHC rates for the five WHO moments were analyzed overall and after stratification by precaution category (SP vs IP) and ward type (general surgery ward (GSW) vs surgery ward specialized on infectious diseases (IDSW)). For N=245 visits with at least one HH opportunity, multiple regression analyses were performed for HH indications for which HHC differences were found between SP and IP.

Results: HHC was assessed on the basis of N=712 opportunities during N=357 visits, resulting in an overall compliance rate of 59.7% for all indications. A total of 21.6% of all visits on the GSW and 74.5% of all visits on the IDSW took place under IP. HHC results exhibited a statistically significant discrepancy (p<0.001) when comparing the precaution categories across both wards for "all indications" (70.0.% for SP vs 43.0% for IP), "before patient contact" (78.7% vs 31.5%) and "after patient contact" (87.6% vs 69.4%). However, analyses stratified by wards revealed no significant HHC differences by precaution category whatsoever. Instead, the observed differences were exclusively attributable to different compliance rates between the two wards (p<0.001). HHC rates were 77.9% on GSW vs. 33.6% on IDSW for "all indications", 30.6% vs. 4.3% "before patient contact" and 90.0% vs. 62.8% "after patient contact". Regression analyses revealed a negative association between the number of HH opportunities and the HHC rate for all indications (β = -0.332; p<0.001).

Conclusions: The study demonstrates that the differences in HHC during bedside visits found under IP (vs SP) were entirely

due to ward type, i.e., specialization on infectious diseases (vs general surgery). HHC observed during visits on the specialized ward was substantially lower than in the GSW – despite the fact that the same surgical team was observed. Additionally, the study suggests that HHC should be addressed especially for bedside visits with five or more HH opportunities.

PS01.14

Characterization of phage host range and phage-antibiotic synergy in antibiotic-resistant *Klebsiella pneumoniae*

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Antimicrobial resistance is a growing global health concern, and *Klebsiella pneumoniae* is a clinically important pathogen due to its ability to rapidly develop resistance to antibiotics. Bac-teriophages (phages) have been studied as a potential alternative to antibiotic treatment. However, bacteria can also adapt to phage infection, which may reduce the effectiveness of phage therapy. Therefore, phage-antibiotic synergy (PAS) has been proposed as a strategy to improve antibacterial activity by combining different mechanisms of action.

The aim of this study was to characterize phage-antibiotic synergy against *Klebsiella pneumoniae*. This was achieved using a structured experimental workflow from large-scale screening through *in vitro* analysis to *in vivo* investigation in *Galleria mellonella*.

A total of 96 wastewater-derived phages were screened against 112 clinical and environmental *Klebsiella pneumoniae* isolates to identify suitable host-phage pairs. Host range screening revealed predominantly host-driven infection patterns, with susceptibility clustering by capsule type, particularly for K24 isolates. Based on these results, two *K. pneumoniae* isolates were selected for further analysis and tested with four phages and four antibiotics from different classes.

In vitro, PAS effects were variable and strongly dependent on the bacterial host, phage, antibiotic, and antibiotic concentration. Synergistic effects were primarily observed at antibiotic concentrations with residual antibacterial activity. The strongest and most reproducible *in vitro* effects were observed for the combination of *K. pneumoniae* isolate 24-KL00004 with phage Muc404B and ciprofloxacin, with amikacin showing less pronounced and cefotaxime only marginal effects.

These combinations were subsequently evaluated in the *Galleria mellonella* infection model. *In vivo*, phage treatment significantly improved larval survival compared to untreated controls. However, phage-antibiotic combinations did not outperform phage monotherapy.

Overall, this study demonstrated that PAS outcomes are highly dependent on bacterial isolate, phage and antibiotic individually, as well as in combination, and concentrations of the therapeutic agents. Furthermore, successful *in vitro* synergy does not always translate to *in vivo* efficacy. This highlights the need for careful experimental design and cautious interpretation when assessing PAS as a therapeutic strategy and underlines the need for validated personalized therapy.

PS01.15

The *Escherichia coli* population in hospital wastewater displays relative enrichment of carbapenemase but not ESBL genes

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Introduction: Hospital wastewater (WW) represents an important reservoir for multidrug-resistant (MDR) bacteria and may contribute to the dissemination of relevant resistance genes into municipal WW systems. This study investigated the population structure of susceptible a resistant *Escherichia coli* in the WW of a tertiary care hospital in northern Germany using a combined phenotypic and genomic approach.

Methods: Twenty-four-hour composite WW samples were collected on three separate sampling days in 2023. Isolates were recovered using both UTI agar (ThermoFisher Scientific) and ESBL agar (bioMérieux) to characterize the overall *Escherichia coli* population as well as 3rd gen. cephalosporin resistant subpopulations (3GCREC). A total of 170 *E. coli* isolates were analyzed by antimicrobial susceptibility testing and MLST and cgMLST typing after whole-genome sequencing (WGS).

Results: Phenotypic resistance as 3GCREC was confirmed for 10 (12.3 %) of 81 isolates from UTI agar, while carbapenem resistance (CR) was confirmed for 3 isolates (3.7 %). For 55 of 89 isolates (61.8 %) recovered from ESBL agar phenotypic CR was observed. Genomic analysis revealed a broad resistome with frequent occurrence of *bla*CTX-M-9, *bla*SHV-12, and *bla*OXA-48 and additional carbapenemase genes *bla*NDM-1, *bla*VIM-1, and *bla*OXA-244. cgMLST analysis displayed a highly diverse population with 32 different MLST sequence types (STs) for isolates from UTI agar (n=81). These isolates were dominated by ST10 (n=25), including a large cluster persisting across multiple sampling days. In contrast, isolates recovered from ESBL agar (n=89) showed lower diversity with 14 STs. These isolates were dominated by high-risk MDR clones including ST410 (n=41), ST131 (n=16) and ST409 (n=11). ST410 carrying *bla*OXA-48 represented the largest clonal cluster and was repeatedly detected in later samplings, indicating persistence or continuous reintroduction into the wastewater system.

Conclusion: These data demonstrate that hospital WW harbors a highly diverse *E. coli* population including 3GCREC high-risk clones and carbapenemase-positive lineages. Individual MDR clones can persist up to years in the WW system and might be overrepresented in studies using only selective media. The observation of 12.3 % 3GCREC isolates in the entire population is within the expectation range for clinical isolates. In contrast to the still low prevalence of carbapenemase-positive *E. coli* (CPE) in patients isolates *E. coli* recovered from WW using both, selective and non-selective, media displayed an unexpected relative enrichment of CR isolates. In particular, the predominance of *bla*OXA-48-positive lineages and the occurrence of high-risk clones were consistently reflected in both populations. Consequently, culturing approaches represent a suitable strategy for wastewater monitoring within a One Health framework targeting the environmental dissemination and persistence of antimicrobial resistance.

PS01.16

Evaluation of Fourier transform infrared spectroscopy (FTIR) for typing of *Klebsiella pneumoniae* complex isolates involved in hospital outbreaks, with clinical and environmental isolates from Tanzania

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Question: Multidrug-resistant *Klebsiella pneumoniae* (*Kp*) are among the major global threats in terms of Gram-negative bacteria. Rapid and reliable typing methods are crucial for outbreak detection/tracking and transmission surveillance. Fourier Transform Infrared Spectroscopy (FTIR) proved to be a reliable method for *Kp* typing, nevertheless, the cellular structures which drive FTIR-based differentiation are not yet completely clarified.

Objective: To investigate the correlation between FTIR spectral relatedness and locus K (KL), sequence type (ST) and SNPs/allelic mismatches.

Methods: 97 fully characterized *Kp* isolates (55 STs and 44 KLs), collected in Tanzania from children and livestock, were included. The dataset comprised 52 clonal isolates (18 different clones, defined by <15 SNPs) and 45 clonally unrelated isolates, either sharing ST/KL with the clonal isolates or being totally different. FTIR analysis was performed with the IR Biotyper® system (IRBT - Bruker Daltonics, Germany), using isolates cultivated at 35±2 °C for 20-24 h on Columbia blood agar (Becton Dickinson, Germany). Hierarchical cluster analysis (HCA) and a novel kNN-based approach (IR Tracker™) were applied for the FTIR/WGS (whole genome sequencing) comparison. Correlation between FTIR and WGS was assessed using the Adjuster Rand Index (ARI) and Pearson"s correlation (r).

Results: HCA showed high correlation of FTIR and the combination ST+KL (ARI=0.914). Correlation with KL and ST were 0.883 and 0.705, respectively. IR Tracker™ accuracy was 97.9% (95/97). The two errors included one isolate showing the same KL but a different ST, classified as highly related, and one isolate which was clonally related but classified as different. Moreover, recorded IR Tracker™ best match spectral distance values indicated robust correlation with genetic relatedness (r=0.86).

Conclusions: The findings of this study show that FTIR differentiation of *K. pneumoniae* is strongly associated with the combination of ST and KL, while the correlation with either ST or KL alone is lower. Isolates sharing the same ST+KL, but with genetic distance >15 SNPs, were recognized as not clonally related.as Also, isolates with the same KL but a different ST were correctly classified as different. IR Biotyper analysis showed a discriminative power close to WGS for the typing of *K. pneumoniae* below the species level.

PS01.17

Evaluation of a novel kNN-based approach for automated strain typing by Fourier-transform infrared (FTIR) spectroscopy

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Question: Fourier-transform infrared spectroscopy (FTIR) is an innovative method for reliable, quick, user-friendly and cost-effective microbial typing. However, the definition of conserved, unambiguous and operator-independent thresholds to assess similarities between strains and isolates is required to allow an implementation of FTIR into routine diagnostic workflows. In this study, the k-NN-based approach (IR TrackerTM) implemented in the IR Biotyper® software (Bruker Daltonics, Germany) was investigated using n=2434 genomically/epidemiologically well characterized isolates belonging to 13 different bacterial species, commonly involved in nosocomial infections (ESKAPEE group included).

Methods: Altogether, 1123 clonally related and n=1311 unrelated isolates from 41 independent outbreaks/surveillance contexts were included (Table 1). IR spectra were acquired from cultures on Columbia blood agar or Mueller-Hinton Agar after overnight incubation at 35 °C using the IR Biotyper® system (Bruker Daltonics, Bremen, Germany). The IR TrackerTM, based on the k-NN approach, provides a "best match" (indicating the "nearest" neighbors) as well as a distance score value as results, depicted in a color-coded heat-map related to the probability of clonal relationship (orange: high; grey: uncertain; blue: low). The IR TrackerTM was applied to the different datasets separately. Results were compared with traditional reference methods.

Results: The IR TrackerTM provided correct "best match results", with matching color-coding, in 96.1% (2338/2434) of

the assessed cases (ranging from 90.3% for *S. aureus* to 98.7% for *P. aeruginosa*). Correct "best match" results with uncertain color-coding were observed in 3.5% (80/2434) cases. Assignment of seven isolates (0.3%) was characterized as false negative, while the results for 5 (0.2%) isolates was considered as false positive (Table 1).

Conclusions: In this study, the k-NN-based automated FTIR approach showed very promising results. An extensive prospective validation in diagnostic routine settings will be necessary to assess its real-life performance, and could allow, if needed, to further fine-tune the thresholds as well as to optimize the method"s diagnostic accuracy in terms of sensitivity and specificity.

Fig. 1

ESKAPEE species (tot)	Result IR Tracker correct (n,%)	Result IR Tracker false positive (n,%)	Result IR Tracker false negative (n,%)	Result IR Tracker uncertain (clonal isolates) (n,%)	Result IR Tracker uncertain (unrelated isolates) (n,%)
K. pneumoniae (658)	636 (96.7)	0	2 (0.4)	7 (0.9)	13 (2.0)
P. aeruginosa (301)	297 (98.7)	0	1 (0.3)	0	3 (1.0)
E. cloacae complex (281)	270 (96.1)	0	0	4 (1.4)	7 (2.5)
E. coli (438)	428 (97.7)	5 (1.1)	0	5 (1.2)	0
S. aureus (155)	140 (90.3)	0	0	5 (3.2)	10 (6.5)
E. faecium (146)	135 (92.5)	0	0	6 (4.1)	5 (3.4)
A. baumannii (81)	77 (95.1)	0	0	0	4 (4.9)
K. oxytoca (58)	53 (91.4)	0	2 (3.4)	0	3 (5.2)
S. marcescens (46)	44 (95.7)	0	0	0	2 (4.3)
S. maltophilia (46)	45 (97.8)	0	1 (2.2)	0	0
Citrobacter spp. (72)	66 (91.7)	0	1 (1.4)	3 (4.2)	2 (2.7)
P. mirabilis (81)	78 (96.3)	0	0	1 (1.2)	2 (2.5)
A. xylosoxidans (41)	40 (97.6)	0	0	0	1 (2.4)
E. faecalis (30)	29 (96.1)	0	0	1 (3.3)	0
Total (2434)	2338 (96.1)	5 (0.2)	7 (0.3)	32 (1.4)	48 (2.0)

PS01.18
Evaluation of WGS and FTIR spectroscopy for *Candida auris* clinical isolates typing

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Question: *Candida auris* is an emerging nosocomial pathogen, which represents a global threat. Infections caused by *C. auris* are burdened by significant mortality rate, high resistance to antimycotic agents and disinfectants and high potential of person-to-person transmission. Global guidelines recommend an active surveillance for the rapid identification of colonization in high-risk patients. Nevertheless, clear and universal typing schemes and thresholds to assess relatedness between strains are still to be defined, especially regarding cross-transmission.

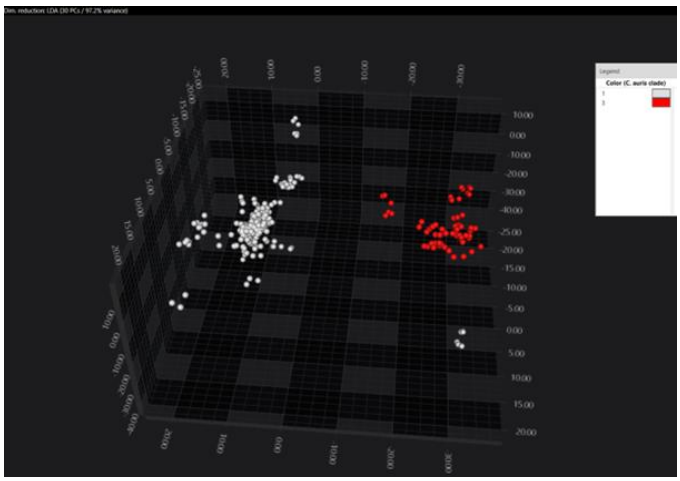
In this study, we compared Fourier-transform infrared (FTIR) spectroscopy with Whole Genome Sequencing for the typing of *C. auris*, investigating a dataset which comprised clonally related, likely unrelated strains and unrelated strains.

Methods: A total of N=73 *C. auris* strains (belonging to clade I and clade III), collected from 31 patients of Johns Hopkins healthcare system (Baltimore, Maryland, USA) between 2021 and 2024, were included in this study. The isolates, previously sequenced by WGS (Illumina, San Diego, USA), were analyzed with the FTIR-bases IR Biotyper® system (Bruker Daltonics, Germany). FTIR spectra were acquired from three independent cultures on Sabouraud Dextrose Agar (Becton and Dickinson GmbH, Germany) grown at 36 °C for 24±1 h. Principal component analysis (PCA) and linear discriminant analysis (LDA) were used to determine the centroid distances between the isolates, reported in a distance matrix. Comparison between genetic and infrared spectral distances was performed using a Mantel test [1] implemented in the PAST software v 5.2.2 [2]. The estimation of significance (one-tailed p-value) was conducted using ~10E6 permutations, and the lowest attainable p value in this instance is 1/(N+1).

Results: IRBT HCA results show a clear discrimination of the two clades, with further subclustering in each one the two clades (Figure 1). The clades exhibited an intra-class genetic distance between 0 and 35 (clade III) or 97 (clade I) alleles. IRBT spectral distance was in very good correlation with genetic distance (R=0.722, p<0.000001).

Conclusions. IR Biotyper showed a very good correlation with genomic data in terms of strains similarity. More data are needed with both methodologies to define clear and universal thresholds to assess the relatedness among different strains, especially in the grey area between the genomic thresholds reported in the literature between the one identified for samples of the same patient and the one identified for surely unrelated strains.

Fig. 1



PS01.19
Possible impact of the revised case definition of notifiable carbapenem-resistant Klebsiella pneumoniae: Evidence from the Bavarian Antibiotic Resistance Database (BARDa)

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Background: On 1 March 2025 Germany introduced a new, more restrictive phenotypic case definition for notifiable Carbapenem-resistant Enterobacterales (CRE) (1). The requirement to notify a detected carbapenemase remained unchanged. Because the new definition is more restrictive, a decline in reported cases is expected, reflecting the change in criteria rather than a true epidemiological decrease. The Bavarian Antibiotic Resistance Database (BARDa) is a surveillance system (2), continuously collecting phenotypical antimicrobial-susceptibility results from 30 laboratories (3) and

provides an excellent opportunity to examine how changes in case definitions could impact the number of notified cases. Due to the reports of increasing prevalence of Carbapenem resistant *Klebsiella pneumoniae* (4), this bacterium was selected to assess a possible impact of the change in reporting requirements.

Objective: To quantify the effect of the revised case definition on the number of *K. pneumoniae* isolates using retrospective data from the BARDa sentinel surveillance system.

Methods: Data for 2024-2025 were extracted from BARDa (participation unchanged during the study period). For each patient the isolate with the highest level of carbapenem resistance (clinical isolates + screening isolates) was selected. Two counting schemes were applied reflecting the change in case definitions:

Old case definition: any *i*solate that was carbapenem-resistant (R) or susceptible with increased exposure (I) to meropenem, imipenem or ertapenem had to be notified every 90 days per patient. New case definition: only meropenem-resistant (R) isolates are notifiable, and reporting is required once per 12 months per patient.

Results: Applying the new case definition on retrospective data from the BARDa sentinel would reduce the number of notifiable *K. pneumoniae* isolates from 648 to 406 isolates (reduction of 37.3%) for 2024 and from 586 to 356 isolates (reduction of 39.2%) for 2025. However, BARDa does not contain molecular carbapenemase test results, so isolates might still be notifiable if further molecular diagnostics are applied.

Conclusions: Based on the BARDa Data, the 2025 revision of the German CRE case definition could lower the number of notified carbapenem-resistant *K. pneumoniae* isolates in Bavaria by more than one-third, solely due to stricter phenotypic criteria and a longer reporting interval. Surveillance systems must account for this methodological effect when interpreting temporal trends in notified CRE incidence.

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(3)
https://www.lgl.bayern.de/gesundheit/infektionsschutz/barda/index.htm#teilnehmer

(4)
https://www.lgl.bayern.de/gesundheit/infektionsschutz/barda/trend.htm#pneumoniae

Fig. 1

Year	Isolates meeting old criteria (R or I to meropenem, imipenem, ertapenem, one isolate every 90 days)	Isolates meeting new criteria (Meropenem R, one isolate per calendar year)	Relative reduction
2024	648	406	37.3 %
2025	586	356	39.2 %

PS01.20

A comprehensive, multimodal, data-driven infectious disease and infection prevention and control program implemented in an adult intensive care unit

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Introduction: Critically ill patients often present with infectious diseases and have an increased risk of acquiring nosocomial (often device-associated) infections during their stay, such as pneumonia, urinary tract infections, or bloodstream infections. High adherence and competence regarding infection prevention and control and infectious diseases among the clinical team and supporting specialties are therefore crucial.

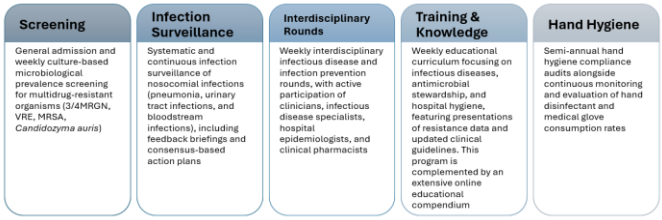
Methods: We describe and analyze the conception and results of a comprehensive, multimodal infection prevention and infectious disease program implemented in the adult intensive care unit (ICU) at the Diakonieklinikum Rotenburg (Germany). This interdisciplinary ICU comprises 22 plan beds in single and double rooms and managed 2,030 cases accounting for 4,740 patient days in 2025. The ICU provides comprehensive critical care services, including extracorporeal membrane oxygenation therapy.

Results: The multimodal infection prevention and infectious disease program encompasses the components illustrated in the figure. Universal admission and prevalence screening for multidrug-resistant organisms (MDRO) was initiated and fully implemented in the fourth quarter of 2025 and is actively supported by a traffic light system in the hospital information system. From January to mid-May 2026, this screening detected 3 patients with MRSA, 3 patients with VRE, and 2 patients with 4MRGN, prompting the immediate implementation of additional hygiene measures. During the winter months of 2025/26, admission screening was expanded to include influenza, SARS-CoV-2, and RSV, accompanied by the mandatory use of masks during any close patient contact. Standardized nosocomial infection surveillance initiated in early 2025 yielded results within the German national reference range for bloodstream and urinary tract infections for 2025. However, a signal for optimization emerged for ventilator-associated pneumonia (VAP) during the first year of observation (4.6 VAP cases per 1,000 ventilator days); consequently, a bundle of measures focusing on oral care and subglottic suctioning was consensually implemented. Hand hygiene compliance monitoring revealed pooled compliance rates exceeding 80% across all indications, with average hand disinfectant consumption equivalent to 42 hand rubs per patient day, alongside an average use of 45 pairs of disposable medical gloves per patient day. This highlights an opportunity for glove use optimization. The interdisciplinary rounds and educational training were well-received.

Discussion: The comprehensive program follows a transparent and participatory approach that actively involves all stakeholders. Through systematic data collection and analysis combined with a close-knit feedback culture, evidence-based decisions and tailored interventions can be made. The implementation of both infection surveillance and screening

was achieved without major hurdles. It has now become an established standard of care in our ICU.

Fig. 1



PS01.21
Resilience of medical examination gloves after disinfection: Evidence from BLS and ACLS simulations

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Background: Disinfecting examination gloves is a controversial measure for reducing nosocomial infections. In emergency settings, in particular, it can be an effective way of improving poor hand hygiene before time-critical invasive procedures. However, the chemical resistance of nitrile protective gloves to alcohol-based hand disinfectants, such as propanol and ethanol, is sometimes questioned, and their protective effect is assumed to be limited. Consequently, training concepts sometimes convey a limited protective effect. Currently, reliable data on the mechanical integrity of disinfected gloves under realistic conditions is lacking.

This study aimed to investigate the mechanical resilience of disinfected examination gloves compared to native ones in a realistic stress test. Cardiopulmonary resuscitation (CPR), as performed in certified simulation training courses of the American Heart Association (e.g. Basic Life Support (BLS) and Advanced Cardiovascular Life Support (ACLS)), served as a prototypical scenario. In addition to chest compressions, these training courses include other procedures (e.g. vascular catheter insertion, defibrillation, intubation and ventilation) that put mechanical stress on examination gloves.

Methods: Thirteen participants were trained in two groups as part of a low-fidelity simulation in accordance with BLS and ACLS standards. For half of the exercises, the nitrile gloves (Peha-soft nitrile Fino®, PAUL HARTMANN AG) were disinfected using an ethanol-based hand disinfectant (Sterillium med®, PAUL HARTMANN AG). After the exercises were completed, the worn gloves were collected and tested for mechanical integrity in accordance with DIN EN 455-1 (2022). Possible confounders were taken into account in the statistical analysis (e.g. glove size, handedness, and the everyday wearing of rings and watches). The study is registered (DRKS00036508).

Results and Conclusions: A total of 1175 examination gloves were tested. Disinfection of nitrile examination gloves did not influence the mechanical integrity according to DIN EN 455-1.

PS01.22
Longitudinal monitoring of SARS-CoV-2 immune trajectories in healthcare workers during the transition from the COVID-19 pandemic to the post-pandemic era (2021-2025)

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Question: Healthcare workers (HCWs) constituted one of the most intensely exposed and indispensable groups during the COVID-19 pandemic. Owing to their typically robust baseline health HCWs represent a quasi-population-level cohort under conditions of heightened viral exposure. This study characterizes the trajectory and durability of SARS-CoV-2-specific immune responses in HCWs across the course of the pandemic into the post-pandemic phase.

Methods: The CoVacSer study is a prospective, longitudinal cohort study conducted from September 2021 to December 2023. HCWs provided continuously serum blood samples alongside completing a study questionnaire at predefined intervals: 14 days, 3, 6, and 12 months following their latest COVID-19 vaccination or infection. Each new immunizing event restarted the follow-up timeline. From April 2024 onwards the study was continued as ARIPro study with two participations per year per HCW - before and after the winter/summer season. Anti-SARS-CoV-2-Spike IgG (Anti-S) levels were quantified using the SERION ELISA *agile* SARS-CoV-2 IgG assay (Institut Virion\Serion GmbH, Würzburg, Germany), Anti-SARS-CoV-2-Nucleocapsid IgG (Anti-N) levels using Elecsys® Anti-SARS-CoV-2 (Roche Diagnostics International AG, Rotkreuz, Switzerland).

Results: From September 2021 to October 2025, a total of 282 HCWs who participated at least once in both the pandemic (CoVacSer) and post-pandemic (ARIPro) study phases were included, resulting in 2,743 protocol-compliant observations. Stable baseline Anti-S immunity was achieved following the third COVID-19 vaccination and maintained through subsequent infections and vaccinations. Initial Anti-N seroconversion was not observed until spring/summer 2022 reaching peak median IgG levels after 2024 and 2025 summer season. A total of 12 (4.3%) HCWs remained Anti-N seronegative (all Anti-N <5 COI) throughout all study visits from 2021 to 2025.

Conclusions: Administration of the third vaccine dose enabled to establish a robust baseline Anti-S immunity. However, as the pandemic transitioned into the endemic phase, a gradual decline in Anti-S IgG levels became evident, indicating waning immunity. In contrast, widespread infections within the cohort - reflected by increasing Anti-N IgG levels - became apparent only later in the pandemic likely reflecting both the progressive relaxation of public health measures, the increasing spread of the Omicron variant and the transition to endemicity. The high post-pandemic Anti-N IgG levels suggest recurrent infections during the endemic phase. Notably, a small proportion of the

cohort remained persistently Anti-N seronegative suggesting that they may not have experienced detectable infection.

Figure 1: Development of Anti-SARS-CoV-2-Spike IgG levels from the COVID-19 pandemic to the endemic phase

Figure 2: Development of Anti-SARS-CoV-2-Nucleocapsid IgG levels from the COVID-19 pandemic to the endemic phase

Fig. 1

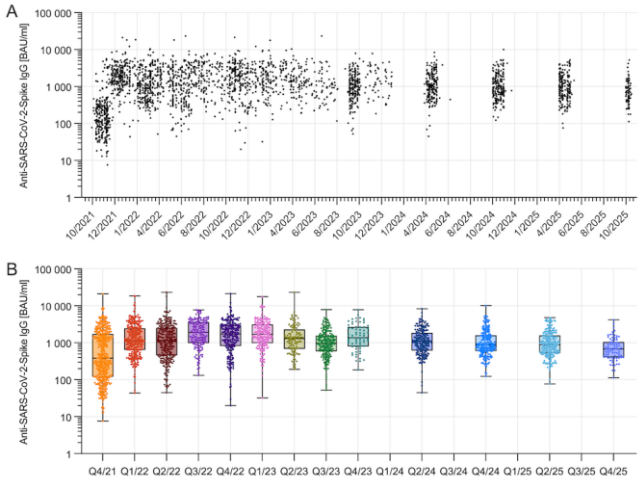
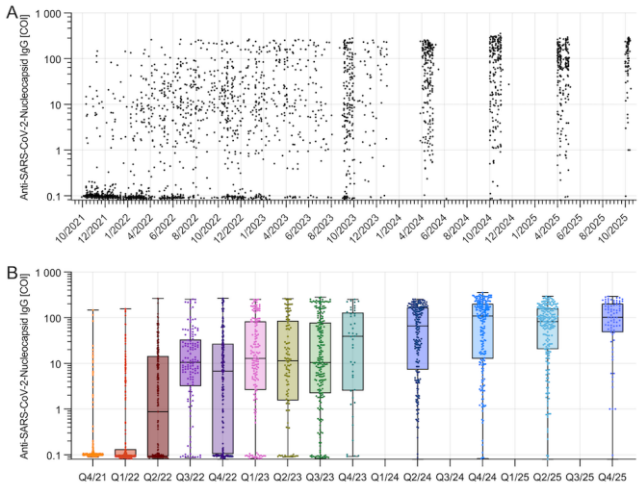


Fig. 2



PS01.23

Influence of acute respiratory infections and vaccination status on health-related quality of life and work ability in German healthcare workers

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Question: Healthcare workers (HCWs) remain highly exposed to acute respiratory infections (ARI) in the post-pandemic era, with potential consequences for psychological well-being, quality of life (QoL), and work ability. ARI as SARS-CoV-2 and seasonal Influenza may negatively affect individual health and the sustainability of healthcare workforce. Vaccination could help to maintain HCWs" well-being and professional functioning. This study investigates the impact of ARI and ARI vaccinations on psychological well-being and work ability among HCWs and explores whether vaccination is associated with improved QoL and work ability.

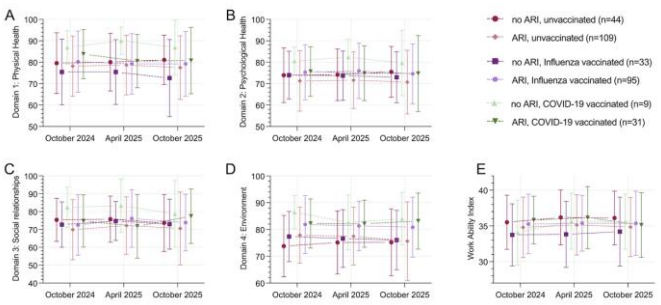
Methods: The ARIPro study is a prospective, longitudinal cohort study aiming to characterize the ARI immunity, epidemiology, QoL and work ability of HCWs. Participants complete a standardized questionnaire twice a year about recent ARI, ARI vaccinations and current work ability (work ability index (WAI)) and QoL (assessed via the four domains of WHOQoL-BREF). In total, 1,325 HCWs are enrolled, of which 360 have continuously participated over October 2024, April 2025, and October 2025. After checking for data completeness the remaining 321 HCWs were characterized into six subgroups according to their vaccination status (unvaccinated, seasonal Influenza or COVID-19 vaccination) and self-reported symptomatic ARI. Vaccinations were administered seasonally from September to December 2024.

Results: Both QoL and WAI remained relatively stable within a moderate to high range in all six groups across in the one-year follow-up from autumn 2024 to 2025. Individuals without any respiratory infection and COVID-19 vaccination showed some of the highest scores in all four domains of the WHOQoL-BREF. Scoring in Domain 4 suggested that uninfected HCWs with a COVID-19 vaccination have significantly higher QoL levels compared to unvaccinated. In general, unvaccinated HCWs with self-reported infection showed lower scores in the WHOQoL domains. Infection status in individuals with an Influenza vaccination showed little impact on QoL, with scores of vaccinated individuals reporting infection and not reporting infection remaining close together in both groups apart from Domain 1.

Conclusions: In this post-pandemic cohort of HCWs, QoL and workability remained overall stable at moderate to high levels over one year. However, respiratory infections were associated with lower QoL scores, particularly among unvaccinated individuals, whereas vaccinated HCWs generally showed more stable outcomes. COVID-19 vaccination was associated with higher QoL scores in certain domains among uninfected participants, suggesting a potential protective effect on well-being and professional functioning.

Figure 1: Longitudinal trajectories of health-related QoL and work ability stratified by ARI status and vaccination group between October 2024 and 2025.

Fig. 1



PS01.24

TDM for vancomycin training and communication strategies for dose optimization in clinical practice – A laboratory-led stewardship intervention

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Background: Therapeutic Drug Monitoring (TDM) is recommended for the parenteral use of vancomycin. Use of TDM can improve outcome of patient, decrease adverse event and toxicity based effects. However, we frequently encounter levels of vancomycin outside of the therapeutic range. The objective of this study was to improve parenteral treatment with vancomycin through immediated structured feedback of measured concentrations.

Methods: During a 20 month period all measured results for vancomycin TDM derived from samples sent by hospitals to our laboratory were included into this before and after study. During the intervenvention period (8 month) all results of vancomycin levels >25mg/l or <10mg/l were immediately phoned to healthcareworkers on the respective ward as a emergency result with the advice to improve dosing and consult clinical microbiology or infectious diseases irrespective of dosing intervalls. Our primary outcome was prevalence of samples within the target zone. Additional secondary outcomes were amount of patients with more than two vancomycin TDM samples taken.

Results: During the study period a total of 1528 sample from 272 patients were measured. During the intervention 540/797 (67.5%) of samples were within the target range compared to 490/731 (67.03%) during the historic control. Samples with a concetration of <10mg/l did not differ between control (152/731; 20.79%) and intervention (153/797; 19.20%). Respectively, samples with a concetration of >25mg/l slightly increased from 79/731 (10.81%) to 93/797 (11.67%).

Overall rates of patients with >2 samples taken did increase from 70/146 (47.9%) to 72/126 (57. 14%). During the intervention fewer patients were only sampled once 37/126 (29.37%) compared to the control 50/146 (34.25%). Especially in patients receiving with more than 2 trough level drawings the target concentration was hit more frequently in the intervention phase compared to the control phase.

Conclusion: Preliminary evaluation of the data showed no relevant impact regarding the primary outcome. However, higher rates of resampling and fewer patients with only one sample were detected during the intervention phase. Further in depth analysis an additional research could provide insight into barries to improved dosing of vancomycin in a hospital setting.

PS01.25

Perceptions of infection prevention and control measures across COVID-19 pandemic: A Google Maps review analysis in German and Spanish hospitals

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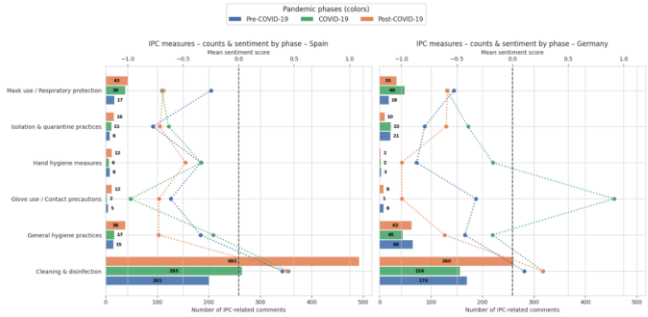
The COVID-19 pandemic and infodemic [1] significantly underscored the crucial role of information dissemination and public perception in navigating a global health crisis. Infection prevention and control (IPC) measures, such as mask and glove use, have become major topics in public discourse [2]. While traditional surveys are limited in scope, time, and cost, crowdsourced reviews offer large-scale, real-time, and cost-effective data [3]. This study aims to examine hospital reviews related to IPC measures in German and Spanish hospitals, and their sentiments before, during, and after the pandemic.

Google Maps reviews from 30 University Hospitals in Germany and 32 in Spain were collected and translated into English. The data were categorized into pre-pandemic, pandemic, and post-pandemic following WHO definitions. Keyword-based search was applied to identify IPC measures within the comments, whereas natural language processing-based sentiment analysis was performed by combining VADER and DistilBERT.

The analysis of 39,677 reviews (from 2011-2025) wherein 1991 (~5%) referred to six different IPC-measures (Figure 1; displays the volume (bars) and sentiment (scale −1 to +1) of IPC-related reviews per measure, stratified by pandemic phase and country). The overall amount of IPC-measures reviews did significantly differ ($p<0.001$) between the pre-pandemic (N=492), pandemic (N=574) and post-pandemic (N=925) period. While both countries showed significant temporal changes across the phases ($p<0.001$) for "cleaning and disinfection" and "mask use", Spain additionally showed a significant difference in "general hygiene practices" ($p=0.001$). Furthermore, overall sentiment regarding IPC measures declined from pre-pandemic to post-pandemic ($p = 0.01$); however, no significant change in sentiments was observed within either country.

Our findings confirm a transition of public health-related concerns to digital platforms facilitated by the pandemic. Patients translated public discourse, like mask use or disinfection, into their hospital reviews. However, no significant differences were observed in hand hygiene, isolation, or glove usage reviews. While the overall sentiment between pre- and post-pandemic declined, it was possibly driven by topic volume rather than a change in attitude towards IPC for both countries. Further analysis is needed to understand the differences across countries in behaviours related to hospital reviews and to use this information in improving patient involvement and safety.

Fig. 1



PS01.26

Mining perspectives on infection prevention and control in emergency care: A topic modeling analysis of online reviews from German hospitals

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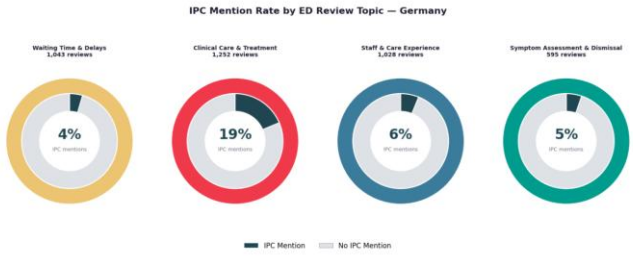
While digital platforms became increasingly important in day to day life, Google Maps reviews provide a scalable and low-cost source of patient feedback in healthcare perceptions (Dornaleteche et al., 2026). The emotional charged quality of Emergency Departments (EDs; Fenton et al., 2014) is associated with higher number of reviews across Germany and high patient dissatisfaction. Thus, the aim of this study was to identify key themes in ED patient reviews and examine whether hygiene/infection prevention and control (Hygiene/IPC) represents a salient concern within patient-reported experiences.

Google reviews from 36 German university hospitals were screened to identify ED reviews (2019-2025) using keyword matching and semantic similarity. ED reviews were clustered via BERTopic, an automated approach that encodes review as a numerical vector capturing its meaning (embeddings), identifies structurally similar reviews (UMAP and HDBSCAN), and extracts the most distinctive words per group (c-TF-IDF). Hyperparameters were optimised for topic coherence, yielding clinically interpretable themes. Hygiene/IPC mentions in these reviews were further identified through an expert-curated keyword set.

A total of 3,918 ED reviews were classified into four thematic categories. The most frequently identified topic was 'Clinical Care & Treatment' (n = 1,252) representing 32% of the total reviews and displaying a mean rating of 2.02. Followed by 'Waiting Time & Delays' (n = 1,043; 26.6%; rating:1.58), 'Staff & Care Experience' (n = 1,028; 26.2%; rating: 3.15) and Symptom Assessment & Dismissal (n = 595; 15.2%;1.63). Furthermore, Staff care & care experience had significantly higher review ratings compared to the rest of the topics ($p < .001$). Within the total reviews 374 (9.5%) mentioned the topic of hygiene/IPC. The proportion of reviews mentioning hygiene/IPC differed significantly across topics ($p < .001$; Figure 1). Reviews mentioning hygiene/IPC displayed a significantly lower sentiment than those that did not ($p = .004$). Within the topic "hygiene/IPC" the highest number of mentions were in reviews about clinical care and treatment (234; 18.7%) followed by staff and care experience (63; 6.1%), symptom assessment and dismissal (31; 5.0%), and waiting time and delays (46; 4.0%).

Using Google Maps reviews as a novel approach to unstructured hospital feedback showed a distinct, frequent spontaneous concern about waiting times and delays. Staff and care experience reviews were significantly more positive than all other topics, suggesting that staff-patient interactions remain a robust driver of positive sentiment despite the stressful ED environment. Hygiene/IPC-related reviews carried significantly more negative sentiment than non-hygiene/IPC topics, suggesting that self-perceived "hygiene shortcomings" elicit strong emotional responses. In addition the findings indicate that hygiene-related concerns arise primarily in the context of direct clinical care.

Fig. 1



PS01.27

Distribution and genomic diversity of carbapenem-resistant gram-negative bacteria in hospital and urban wastewater systems

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Background: Carbapenem-resistant Gram-negative bacteria, particularly carbapenemase-producing strains, represent an increasing challenge for the management of hospital-acquired infections. Wastewater from healthcare facilities may contribute to the dissemination of resistant bacteria into urban sewer systems, raising concerns about wastewater treatment plants (WWTPs) as possible reservoirs of antimicrobial resistance genes and about their effectiveness in reducing resistant pathogens. In addition, wastewater-based surveillance may serve as a valuable tool for monitoring carbapenem resistance in healthcare settings. This study examined the occurrence and distribution of carbapenem-resistant bacteria in hospital and municipal wastewater in a metropolitan area in Germany.

Methods: Wastewater samples were obtained at three sampling points between March 2024 and January 2025 from three hospitals, as well as from the influent and effluent of a WWTP. Samples were cultured on chromogenic screening agar supplemented with carbapenem, and up to 30 morphologically distinct colonies were selected from each sample. Bacterial species were identified using MALDI-TOF/MS. PCR assays were performed to screen for five major carbapenemase gene groups: *bla*$_{\text{NDM-like}}$, *bla*$_{\text{OXA-48-like}}$, *bla*$_{\text{KPC-like}}$, *bla*$_{\text{VIM-like}}$, and *bla*$_{\text{GES-like}}$. A subset of isolates underwent whole-genome sequencing (WGS). Multilocus sequence types and antimicrobial resistance gene profiles were determined. Comparative genomic analyses were conducted to explore potential relatedness between hospital-associated and environmental isolates.

Results and conclusions: Across all sampling sites and time points, 11 bacterial genera were detected. *Escherichia* and *Klebsiella/Raoultella* spp., both commonly associated with nosocomial infections, were the dominant genera. *Citrobacter* and *Aeromonas* spp. were also frequently recovered. The distribution of carbapenemase genes differed between

sampling locations, and the composition of bacterial genera changed over time. In wastewater from hospital 1, bla_{KPC} was the most frequently detected carbapenemase gene, whereas bla_{GES} was predominant in hospital 2. Notably, sequence types (ST) identified among the most common genera included strains previously associated with both clinical and environmental sources. *E. coli* ST399, which has been linked to hospital outbreaks, was detected in wastewater from hospitals 1 and 3. In contrast, *E. coli* ST5295, more commonly reported from environmental settings, was isolated from hospital 1 wastewater. Overall, these results demonstrate the dynamic and interconnected nature of clinical and environmental reservoirs of carbapenemase genes within hospital wastewater systems.

PS01.28
What a pity? Whole-genome sequencing reveals genotype-phenotype discrepancy in increased detection of 2MRGN *Acinetobacter pittii* in the neonatal intensive care unit

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Introduction: Neonates in the neonatal intensive care unit (NICU) are highly vulnerable to healthcare-associated colonization and infection due to prolonged hospitalization, close care and invasive interventions. While surveillance and infection prevention efforts among Gram-negative pathogens have predominantly focused on *Acinetobacter baumannii*, *Acinetobacter pittii* is an emerging member of the *Acinetobacter calcoaceticus-baumannii* complex associated with hospital-acquired infections, including bloodstream infections, and clinically relevant resistance determinants. Reports of carbapenem-resistant, virulence-associated *A. pittii* from septicemic neonates underline its relevance for neonatal surveillance and outbreak investigations.

Aim: We investigated increased detection of *A. pittii* in a neonatal and pediatric intensive care setting by epidemiological assessment and whole-genome sequencing, focusing on transmission and genomic resistance determinants.

Materials and Methods: Weekly NICU screening is performed using rectal and throat swabs according to national guidelines. In addition, we carried out WGS-based molecular surveillance of multidrug-resistant pathogens and event-driven sequencing if transmission was suspected. Molecular typing is performed by short-read sequencing-based core-genome multilocus sequence typing (cgMLST). Cases were defined as patients admitted to the NICU, pediatric intensive care unit or adjacent pediatric wards with detection of *A. pittii* in any microbiological specimen.

Results: Over two weeks, five 2MRGN *A. pittii* isolates were investigated. Increased detection was first recognized in the NICU (n = 3). One pediatric and one external neonatal comparison isolate were additionally included. All detections represented colonization. In cgMLST, all isolates differed by >100 alleles, ruling out clonal transmission. Phenotypic microbiology classified all isolates as 2MRGN, with meropenem susceptibility and ciprofloxacin susceptibility at increased exposure. However, WGS identified OXA-type β-lactamase alleles, including *blaOXA-421, blaOXA-417* and *blaOXA-500*, indicating a notable relevant genotype–phenotype discrepancy with carbapenemase-associated resistance determinants despite phenotypic carbapenem susceptibility.

Conclusion: Prospective WGS-based surveillance supported IPC decision-making by ruling out clonal transmission despite increased detection of 2MRGN *A. pittii*. The discrepancy between OXA-type β-lactamase detection and meropenem susceptibility underlines the need to interpret genomic resistance findings in relation to species background and phenotype. As an emerging non-*baumannii Acinetobacter* species, *A. pittii* should be considered in molecular surveillance of high-risk settings, as it has been reported to harbor clinically relevant OXA-carbapenemases and may serve as a reservoir for transferable resistance determinants.

PS01.29
Genomic characterization of third-generation cephalosporin-resistant *Escherichia coli* across urban wastewater sources

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Introduction: The spread of antimicrobial-resistant *E. coli*, particularly ESBL-producing strains, in environmental reservoirs represents a growing health concern. Urban wastewater (WW) systems serve as important interfaces between clinical and community settings, where resistant bacteria and resistance genes originating from human sources can be detected. The Molecular Monitoring of Biodiversity (MOMOBIO) project investigates the distribution of third-generation cephalosporin-resistant *E. coli* (3GCRE) in urban systems using culturomics and whole genome sequencing (WGS).

Methods: During a sampling campaign in 2025, WW samples were collected from seven locations representing different sources, including a hospital (2x effluent), a WW treatment plant (WWTP, 2x influent), two residential areas and an industrial area. The abundance of 3GCRE was quantified using chromID® ESBL agar (bioMérieux). 133 *E. coli* isolates were subjected to whole-genome sequencing and analyzed using the MBioSeq Ridom Typer (Bruker) for core-genome multilocus sequence typing (cgMLST), classical MLST, and resistance gene detection using the integrated AMRFinder.

Results: Variable concentrations of 3GCRE were detected across all WW sampling sites ($2.5x10^4$- $4.2x10^6$ CFU/L), with the highest and lowest concentrations observed in the two residential areas. cgMLST analysis revealed a diverse population structure with 35 different classical STs, with ST131 (n=20) and ST69 (n=15) being most prevalent. The largest cluster of clonally related ST616 isolates included eight isolates observed in the industrial site and one hospital effluent WW. Two smaller clusters displayed clonally related isolates

between the industrial site and hospital WW (ST131, n=3; ST46, n=2), whereas other clusters were limited to individual WW sampling sites. The β-lactamase gene *bla*CTX-M group predominated, along with combinations including *bla*CTX-M/*bla*SHV. In twelve isolates (9.0 %) grown on ESBL agar, *bla*OXA48 group carbapenemase genes were detected. For other resistance determinants (*sul*, *dfrA*, and *tet* genes), a high abundance was observed, with varying combinations even within genetic clusters.

Conclusion: Urban WW systems contain diverse 3GCRE, reflecting monitoring points for antimicrobial resistance originating from human activities. Despite overall genetic diversity, few clonally related isolates were identified across sampling sites, suggesting the distribution of similar strains in the human population or within the interconnected WW system. Notably, the prevalence of carbapenemase genes in 3GCRE of WW samples is higher than expected from clinical data. These findings underscore the importance of integrating environmental surveillance into One Health strategies to better understand and mitigate the spread of antimicrobial resistance. The ongoing longitudinal sampling MOMOBIO project, combined with metagenomics and ecological modeling, will further clarify resistance dynamics and environmental drivers.

PS01.30
The caveats and (im)possibilities of tracking outbreaks directly from swabs in neonates

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Metagenomics enables the study of microbial communities, and has advantages in monitoring transmission events. We aimed to track Enterobacterales in neonates admitted to the Neonatal intensive Care Unit in the Medical Center – University of Freiburg for a 12 month-period using long-read metagenomic sequencing.

We collected weekly anal and nasal swabs from 302 patients (nl=1,351), and performed metagenomic sequencing from enriched swabs using ONT, and simultaneous cultures were sequenced using Illumina. We predicted transmission events from the metagenomic data and compared them with those from culture. We focused on the 90% most abundant species: *E. coli*, *E. cloacae*, and *Klebsiella spp.*. Contaminants from the metagenomic samples were identified using the Stop-Check-Go system, and BIOM files were used for further diversity with Phyloseq and network analyses with igraph.

Detecting sequence types (STs) involved in transmission events required more samples using pure cultures compared to metagenomes, where lower number of samples still detected more patients per week (7 and 12, respectively). We found 61 STs potentially involved in transmission events, with 5 STs not detected by pure cultures. With metagenomics, we were able to detect putative transmissions on average 1 week earlier compared to cultures. In addition, 41% of the patients were overlooked in *E. coli* using cultures, 33% in *K. oxytoca*, 25% in *E. cloacae* complex, and 22% in *K. pneumoniae*.

Using metagenomics, we explored the roles of patient in the transmission networks: peripheral (with less connections), bridges (connecting independent transmissions), and core (staying longer on the ward). We found that core patients had a higher risk of Enterobacterales acquisition (Pearson, R2=0.79 p-value=3.81e-38). Those patients were relevant due to a higher level of involvement in different transmission networks. In this patient group, use of antibiotics was significantly extended (p=8e-04), and patients were born by c-section and premature.

Our findings highlight the added value of metagenomic surveillance for the detection and characterization of Enterobacterales transmission in clinical settings. Compared to conventional pure-culture approaches, metagenomics enabled identification of a greater number of patients involved in transmissions, revealed additional STs missed by culture, and facilitated earlier detection of transmissions. Culture-based methods substantially underestimated strain diversity and patient colonization. The frequent detection of multiple STs within individual patients further demonstrates the complexity of colonization dynamics and emphasizes the limitations of single-colony isolation strategies for outbreak investigations, highlighting the possibilities when integrating metagenomic surveillance into routine diagnostics.

PS01.31
Wastewater metagenomics for AMR surveillance: UniCARD improved detection of clinically relevant resistant pathogens

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Introduction: Antimicrobial resistance (AMR) is an escalating global health threat and increasingly limits treatment options, particularly for vulnerable patient populations [1]. Wastewater surveillance has emerged as a promising tool for monitoring of AMR at population level, capturing resistance signals from community, healthcare, and environmental sources [2]. However, reliable detection of antibiotic resistance genes (ARGs) in metagenomic data remains challenging, and existing approaches may fail to identify clinically relevant resistance determinants or provide limited biological context.

Methods: We developed UniCARD (https://github.com/IKIM-Essen/uniCARD), an enhanced ARG detection workflow integrating curated resistance knowledge with expanded protein sequence information while preserving established resistance classifications. Performance was assessed using 436 clinical isolates with matching VITEK 2 resistance phenotypes, as well as wastewater metagenomes from the influent of wastewater treatment plants, intra-city subsewersheds, and clinical wastewater. UniCARD was compared to the standard Comprehensive Antibiotic Resistance Database (CARD) workflows and the machine-learning approach DeepARG.

Results: In clinical isolates, UniCARD showed improved concordance between detected ARGs and observed resistance phenotypes compared with standard CARD-based analyses. In wastewater metagenomes, UniCARD consistently identified a broader resistome than CARD, increasing detection of ARG diversity across different wastewater sources. Importantly, recommended CARD filtering reduced ARG detection to limit uncertain assignments, whereas UniCARD mitigated this sensitivity-specificity trade-off, enabling broader ARG detection while preserving biologically meaningful resistance annotations. Comparison with DeepARG in wastewater samples highlighted clinically relevant differences: UniCARD identified carbapenem-resistant Pseudomonas aeruginosa, while these resistance determinants were not detected by DeepARG. These findings demonstrate that enhanced ARG detection can reveal resistance signatures that may otherwise remain undetected in wastewater surveillance datasets.

Conclusion: Wastewater metagenomics offers a scalable approach for AMR surveillance across communities and healthcare-associated settings, but its clinical value depends on reliable ARG detection. UniCARD improved resistome characterization and enabled detection of clinically important resistance determinants while maintaining biological interpretability. These findings support the use of improved metagenomic workflows to strengthen wastewater-based AMR surveillance and complement conventional clinical monitoring.

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PS01.32

Strong associations between carbapenem consumption and resistance across 26 German hospitals from 2023 and 2024: results from the ARVIA integrated surveillance approach

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Since 2008 and 2014, respectively, the surveillance systems for antimicrobial resistance (Antibiotika-Resistenz-Surveillance, ARS) and antimicrobial consumption (Antibiotika-Verbrauchs-Surveillance, AVS) have been established at the Robert Koch Institute (RKI) within the framework of the German Antibiotic Resistance Strategy (DART). ARVIA (Integrated Analysis of ARS and AVS Data) jointly analyses antimicrobial resistance and consumption data from both surveillance systems at the hospital level and provides timely feedback to participating institutions.

A recent extension of ARVIA combines data across multiple hospitals. Against the background of increasing carbapenem consumption in Germany, this study aimed to quantify associations between carbapenem use and resistance density across 26 hospitals.

Using whole-hospital data from January 2023 to December 2024, aggregating all isolates and total antimicrobial consumption per institution, associations between carbapenem consumption (DDD/100 patient days) and resistance density (R/1000 patient days) were analysed for *Acinetobacter baumannii* complex, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

Resistance counts were modelled as a function of antimicrobial consumption and time using mixed-effects Poisson regression, including patient days as an offset and hospital-specific random effects to account for between-hospital variability in baseline resistance, temporal trends, and consumption effects. Model estimates are reported as incidence rate ratios (IRRs). In addition to same-quarter analyses, lagged models incorporating antimicrobial consumption from one and two preceding quarters were fitted to account for potential delayed effects on resistance patterns.

A total of 1,177 isolates were included for *Acinetobacter baumannii* complex, 47,886 for *Escherichia coli*, 13,764 for *Klebsiella pneumoniae*, and 13,239 for *Pseudomonas aeruginosa*. The one-quarter lagged model for *Escherichia coli* met the predefined ARVIA significance threshold of $\alpha = 0.01$ with an IRR of 1.28 ($p = 0.009$), indicating that each 1 DDD/100 patient days higher or lower carbapenem consumption would correspond to a 28% higher or lower resistance density, respectively. By comparison, 1 DDD/100 patient days is close to the average monthly carbapenem consumption across participating hospitals during the study period (1.13 DDD/100 patient days). For *Klebsiella pneumoniae*, significant associations were observed in same-quarter (IRR 1.57, $p = 0.002$) and one-quarter lagged analyses (IRR 1.37, $p < 0.0001$). A significant same-quarter association was also observed for *Pseudomonas aeruginosa* (IRR 1.20, $p < 0.001$). No significant association was found for *Acinetobacter baumannii* complex.

To conclude, a significant association was found between carbapenem administration and carbapenem resistance for three major nosocomial pathogens. Prudent use of carbapenems is a key component in curbing carbapenem resistance.

PS01.33

Prevalence of MRSA and third-generation cephalosporin and/or carbapenem resistant gram-negative bacteria colonisation in international children and adults prior to radiation therapy – screening results from a German proton therapy centre from 2021 to 2025

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Introduction: Data on colonisation with MRSA and third-generation cephalosporin (3GC)- and/or carbapenem-resistant gram-negative bacteria (in Germany classified as 3MRGN/4MRGN) before start of radiation therapy are limited. As proton therapy is offered at only a few European centres and patients often travel internationally, pre-treatment screening is relevant. All patients are screened for MRSA

before treatment per local standard. International patients may have prior contact with healthcare systems in endemic regions, which additionally warrants 3GC/carbapenem-resistant gram-negative screening. Since the technique is particularly suited to paediatric radiotherapy, a high proportion of cases at the study centre were children (C). This study assessed colonisation prevalence in children and adults (A) prior to proton therapy and analysed possible geographic patterns.

Methodology: We retrospectively analysed pre-treatment screening data from patients treated at a German proton therapy centre between 2021 and mid-2025. Results were documented in MetaIPSS® (KMS GmbH). Analyses were performed separately for children (<18 years) and adults (≥18 years).

Results: A total of 2,442 MRSA screenings (C: 1,169; A: 1,273) were performed. Twelve were positive, including 6 children (0.51%) and 6 adults (0.47%). On an annual basis, MRSA prevalence rates in children were 0% (2021), 0.38% (2022), 0.54% (2023), 1.70% (2024) and 0.45% (2025). For adults, the figures were 0.56% (2021), 0% (2022), 0.41% (2023), 0.48% (2024) and 1.02% (2025). Positive MRSA results were obtained from patients in Germany, Finland, Romania, Ireland and the United Kingdom.

305 screenings for 3GC- and/or carbapenem-resistant gram-negative bacteria were performed (C: 270, A: 69). Five 3GC-resistant E. coli cases were detected: two children (0.74%) and three adults (4.35%). Detections occurred only in three years: two children (10.00%) in 2024, two adults (16.67%) in 2022 and one adult (7.14%) in 2025. No positive cases were found in other years. Affected patients came from Germany, Austria, Spain and Turkey.

Conclusion: MRSA prevalence was below European reference values of 1.3% [1] to 2.0% [2] for adults and 1.2% to 2.7% for children [2,3], with no MRSA detected in some years. We see no indication of increased MRSA risk in international radiation therapy patients. 3GC-resistant E. coli colonisation occurred sporadically. The seemingly high annual rates rest on very small case numbers and likely reflect imported cases rather than an elevated baseline. Overall prevalence was 0.81% in children and 3.57% in adults, above and below the published values of 0.75% and 9.5%, respectively [4,5]. Pre-treatment screening in line with accepted standards remains useful for early identification of individual cases. There is no evidence of increased admission prevalence in an international paediatric patient cohort referred r to proton therapy, though these results should not be overinterpreted.

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PS01.35

Glove use among missed hand hygiene opportunities: Observations from a university hospital

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Background: Hand hygiene is one of the most effective measures for preventing healthcare-associated infections. Recent recommendations by the German Commission for Infection Prevention in Healthcare and Care Facilities (KRINKO) and the World Health Organization (WHO) have highlighted that medical gloves are frequently used beyond their intended indications and may interfere with the performance of indicated hand disinfection.

Aim: This analysis aimed to investigate the association between glove use and hand hygiene compliance in a university hospital.

Methods: Standardized hand hygiene observations were conducted in 2025 within the framework of the German national hand hygiene campaign *Aktion Saubere Hände*, based on the WHO "5 Moments for Hand Hygiene". Observations were performed on 22 wards, including 15 intensive care units. A total of 5,866 hand hygiene opportunities were recorded. Hand hygiene compliance was assessed for each WHO indication. In addition, glove use was documented and analyzed in relation to missed hand hygiene opportunities.

Results: Overall hand hygiene compliance was 66.1%. Compliance was lowest before aseptic procedures (49%) and highest after patient contact (80%). Among omitted hand hygiene opportunities, glove use was documented in 49% of cases before patient contact, 61% before aseptic procedures, 40% after body fluid exposure risk, 25% after patient contact, and 21% after contact with patient surroundings. The highest proportion of glove use among omitted hand hygiene opportunities was observed before aseptic procedures (61%).

Conclusions: Glove use was frequently documented among missed hand hygiene opportunities, particularly before aseptic procedures and before patient contact. These findings support current concerns that gloves may be perceived as a substitute for indicated hand disinfection and may therefore represent a barrier to optimal hand hygiene practice. Educational interventions should emphasize the distinct roles of hand hygiene and glove use in infection prevention and promote indication-based glove use. Particular attention should be paid to aseptic procedures, where both the lowest hand hygiene compliance and the highest proportion of glove use among missed hand hygiene opportunities were observed.

PS01.36

5-year analysis: Do MRSA colonised healthcare workers contribute to nosocomial transmission?

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant nosocomial pathogen that is also transmitted between patients through the hands of healthcare workers (HCW). While this transmission route is well established, the impact of MRSA colonised HCW on the transmission to patients is not fully understood. Depending on the hospital regulations, admitted patients undergo routine MRSA screening on admission as part of the hospitals infection prevention strategy. However, HCW are diagnosed either in private settings or screening efforts. There are considerably different approaches among German hospitals regarding work restrictions and recommendations for MRSA colonised HCW. This study therefore aims to understand to which extend MRSA is transmitted from MRSA positive HCWs to patients during a five-year study period.

Methods: This retrospective, single center study analysed MRSA isolated from HCW and patients at a German University hospital between January 2021 and January 2026. All isolates originated from sporadic MRSA detections or during screening efforts of a medical intervention of the HCW. During the study period, the isolates of all MRSA positive HCW were compared with the MRSA isolates from patients. To identify potential transmission events and to investigate possible transmission routes, the genomic relatedness was assessed using whole genome sequencing (WGS) and subsequent core genome multilocus sequence typing (cgMLST) of all isolates. Epidemiological data was reviewed to support the findings and to evaluate the evidence within the hospital.

Results: During the five-year study period, in total 234 MRSA isolates from patients and ten MRSA isolates from HCW were compared using WGS and subsequent cgMLST. Of the ten isolates from HCW, only a single isolate matched to a patient isolate with an identical cgMLST profile. Epidemiological investigations revealed that this HCW was screened during an outbreak investigation in accordance to the national guidelines. The remaining nine isolates had no hit among any of the 234 MRSA isolates collected from patients.

Conclusion: In our study, we did not detect any MRSA transmission between HCW and patients outside of an outbreak situation. These findings suggest that standard hygiene measurements and infections prevention protocols might by sufficient to prevent MRSA transmission from sporadically colonized HCW to patient.

PS01.37

Regional genomic patterns define VRE epidemiology in Central Europe: Insights from the VRE DACH 10/2020 study

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Background/Aim: While data on the molecular epidemiology and virulence factors of vancomycin-resistant *Enterococcus faecium* (VRE) is increasing, it remains largely limited to outbreak reports, unsystematic sampling, and single-centre studies. Consequently, current knowledge is fragmented and potentially biased by isolate selection. To address this gap, we established the VRE DACH 10/2020 Study, comprising all first VRE isolates (n = 2,261) obtained in October 2020 from nearly all university medical centres in Germany (n = 34), Austria (n = 9), and Switzerland (n = 7), as well as three additional microbiology laboratories. This represents the first comprehensive overview of VRE epidemiology in the DACH region.

Methods: All confirmed VRE isolates (n = 2,216) underwent short-read sequencing (Illumina). After quality control using AQUAMIS (n = 2,187), isolates were included for further analysis using Bruker MBioSeq and Ridom Typing, covering glycopeptide resistance genotypes, MLST, core-genome MLST, and pairwise comparisons. Secondly, the isolates were long-read sequenced on an Oxford Nanopore platform for hybrid genome assembly. Circular genomes were obtained for 1,788 isolates, enabling accessory genome and bacteriocin analyses. Data analysis is ongoing in May 2026.

Results: Of 2,187 VRE isolates, 2,149 (98.3%) originated from Germany, and 63.2% (n = 1,383) were screening isolates. Additional isolates were obtained from urine (n = 382), blood cultures (n = 53), and other or unknown sources (n = 369). VRE/vanB predominated overall (65.1%, n = 1,423), with marked regional variation: vanB accounted for 86.6% and 87.5% of isolates in southwestern and northwestern Germany, respectively, while vanA reached 58.3% in western Germany.

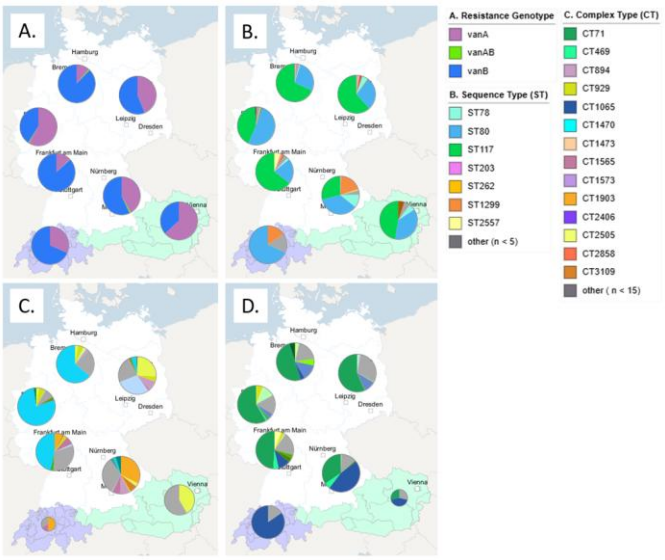
ST117 (n = 1,104) and ST80 (n = 724) were dominant, while only five additional STs included ≥15 isolates (ST1299, ST78, ST2557, ST262, ST203). Genotype distribution showed strong geographic clustering. Among vanA isolates, ST80/CT1470 predominated in western Germany, ST1299/CT1903 in southeastern Germany and Switzerland, and ST117/CT2505 and ST117/CT929 in northeastern Germany and Austria.

Plasmid analysis showed that the *vanA* operon was mainly located on highly conserved 40–45 kb plasmids enriched in multiple insertion sequences (IS1216E, IS1182, IS1380, ISL3, IS30) and additional resistance genes (*erm, aphA, aadK, satA, sasA*). High sequence homology was observed among plasmids within identical CTs across regions. Plasmid distribution was strongly linked to specific genotypes and frequently associated with bacteriocin genes (i.e., *bac43, bac51, entA*).

Conclusions: Our study reveals a geographically structured VRE population in the DACH region, highlighting the need for systematic molecular surveillance as a standard practice to enable comparable real-time genomic interpretation across regions and institutions and to support effective implementation of WGS in infection control.

Figure 1. Geographical distribution of VRE genotyp

Fig. 1



PS01.38

Hygiene/infection prevention and control (H/IPC) and sustainability: First results of a nationwide structured expert survey on personal protective equipment (PPE)

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Introduction: Hygiene/Infection prevention and control (H/IPC) is intrinsically linked to sustainability by preventing infections and avoiding future resource-intensive interventions. However, H/IPC measures contribute to the healthcare system"s ecological footprint and some practices are perceived as 'hygienic' by non-specialist staff, yet are not supported by H/IPC experts.

Aim: This abstract is part of the HOPE project (Hospital hygiene preventing emissions, G-BA Innovationsfonds 01VSF23019) and focuses on personal protective equipment (PPE) and potential modifications in defined clinical situations according to H/IPC safety and ecological footprint.

Material and Methods: A structured survey was conducted via REDCap, involving IPC experts (leading IPC physicians/ IPC nurses) from 152 German hospitals (>600 acute care beds). The survey assessed 34 scenarios involving PPE in a range of medical procedures. These scenarios were selected through a rapid review and problem-centred interviews and were designed to reflect both H/IPC recommendations and real-world clinical practice (Figure 1). A second survey targeted sustainability experts (academics, hospital sustainability managers) rating the same scenarios according to their ecological resource-saving potential. Scenarios were categorised according to defined cut-offs (see Figure 1) as A) low-risk/high-saving, B) low-risk/low-saving, C) high-risk/high-saving or D) high-risk/low saving.

Results: A total of 48/304 IPC experts (15,8%) and 42/50 sustainability experts (84,0%) fully participated. Overall, 64,7% of scenarios were rated as low hygienic risk (A+B), while 32,4% combined low risk with substantial resource-saving potential (A; Figure 1.I). In contrast, 14,7% were categorized in C showed high saving potential but relevant hygienic concerns. Notably, 71,4% of scenarios addressing single-use medical gloves were assigned to category A (B:21,4%, D: 7,1%, Figure 1.II), whereas all those addressing protective gowns fell under B (60,0%) or C (40,0%).

Conclusions: H/IPC and sustainability assessments reveal distinct patterns enabling categorisation of scenarios and identification of routines performed under the guise of H/IPC as hygiene standards, alongside potential for safe and sustainable modification. Single-use medical gloves appear, suitable for reduction, whilst those addressing protective gowns require careful evaluation due to conflicting risk and sustainability profiles. As sustainability assessments may be perception-based and influenced by H/IPC risk appraisal, future work within the HOPE project will integrate objective data, including life cycle analyses, into a decision matrix.

Fig. 1

I.

Exemplary scenario	Hygiene/IPC experts: hygienic risk	Hygiene/IPC experts: Mean	Sustainability experts: resource saving potential	Sustainability experts: Mean	Category
Single-use medical gloves					
Omission of single-use medical gloves during the administration of intradermal, subcutaneous, and intramuscular injections (e.g. vaccination)	low	85,7 SD=26,1	high	73,8 SD=33,9	A
Omission of single-use medical gloves during examinations without contact with mucous membranes, blood, or wounds (e.g. blood pressure measurement, temperature measurement, pulse measurement, auscultation, otoscopy)	low	89,1 SD=28,4	high	90,3 SD=16,9	A
Omission of single-use medical gloves during the preparation of infusions or syringe drivers (except when handling cytotoxic or hazardous substances)	low	83,1 SD=32,4	high	75,7 SD=33,4	A
Omission of single-use medical gloves during all manipulations of vascular access devices in the absence of blood flow / removal of vascular access devices	low	68,8 SD=35,1	moderate	46,5 SD=38,6	B
Omission of single-use medical gloves during the insertion of vascular access devices / peripheral venous cannulae (PVC) / administration of intravenous (i.v.) injections	moderate	51,3 SD=37,4	moderate	34,0 SD=32,3	D
Protective gowns					
Omission of protective gowns when present in an isolation room without direct patient contact	low	74,5 SD=32,3	moderate	60,9 32,3	B
Omission of protective gowns for visitors: in general	low	86,3 SD=27,4	moderate	65,2 SD=32,3	B
Omission of protective gowns for visitors: in the intensive care unit	low	82,8 SD=30,1	moderate	44,9 SD=38,6	B
Omission of protective gowns for visitors: when visiting isolated patients	moderate	49,8 SD=35,8	high	79,6 SD=23,9	C

II.

PPE product group / Category	Overall N=34 scenarios	Single-use medical gloves N=14 scenarios	Protective gowns N=5 scenarios
A: low-risk/high-saving potential	32,4%	71,4%	0,0%
B: low-risk/low-saving potential	32,4%	21,4%	60,0%
C: moderate-risk/high-saving potential	14,7%	0,0%	40,0%
D: moderate to high risk/moderate to low saving potential	20,6%	7,1%	0,0%

Figure 1: I. Comparison of mean values** with standard deviation (**SD**) for assessments by hygiene/infection prevention and control (**H/IPC**) experts (hygienic risk; 0 = extremely risky, 100 = no risk) and sustainability experts (resource-saving potential; 0 = low, 100 = high) regarding exemplary scenarios involving personal protective equipment (**PPE**) across various medical procedures. Total number of scenarios n = 34. Arbitrary Cut-offs: >67 = low hygienic risk/high resource saving potential, 33-66 = moderate hygienic risk/resource saving potential, <33 = high hygienic risk/low resource saving potential. **Categories:** A = low hygienic risk and high resource saving potential; B = low hygienic risk but low resource saving potential; C = moderate hygienic risk but high resource saving potential; D = moderate to high hygienic risk and moderate to low resource saving potential. **II. Proportions of categories of scenarios, overall and for exemplary PPE product groups.

PS01.39

The plasmid network of carbapenemase-producing Enterobacterales in a hospital in Germany: Identifying resistance genes and preventing hidden transmission

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Data from the National Reference Center for Gram-negative Hospital-Acquired Pathogens in Germany, derived from patients with infections and colonization, show that there has been an increase over the years in carbapenemase-producing Enterobacterales (CPE) (1). Among these multidrug-resistant bacteria, OXA-48 is the most commonly detected carbapenemase in Germany. OXA-48-type carbapenemases are predominantly located on plasmids, which can be exchanged via horizontal gene transfer between bacteria of the same species and between bacteria of different species within the gut or in biofilms. Plasmid transfer therefore plays a significant role, alongside the direct transmission of CPE between colonized patients and their environment, in the further spread of carbapenem resistance (2).

The major aim of this study was to distinguish between clonal and plasmid-mediated transmission of CPE in order to prevent hidden transmission within complex patient movement networks and their environment at the hospital. To this end, four patient isolates containing OXA-48-positive pathogens and three OXA-48-positive environmental isolates were subjected to whole-genome sequencing using both short-read and long-read technologies. The analysis of resistance plasmids using long-read sequencing (ONT sequencing, Oxford Nanopore Technology) was intended, in particular, to identify plasmid transfer between different species within the patient as well as between patients and the environment. Bioinformatic analysis identified two variants of the pOXA-48 plasmids, which differ due to the loss of a DNA segment approximately 30 kb in size that is relevant for conjugation. SNV (Single Nucleotide Variant) analyses showed that both plasmid variants are nearly identical in their shared backbone. A cgMLST analysis revealed a clonal relationship for two *Raoultella planticola* isolates, whereas no clonal relationship was found in other isolated OXA-48-positive species. Nearly identical pOXA-48 plasmids detected in those isolates suggest a plasmid-mediated transmission across species boundaries.

The combination of short-read and long-read sequencing data enables precise whole-genome sequencing as well as the reliable detection of pOXA-48 plasmids. Therefore the approach can help to elucidate clonal and plasmid-mediated transmission patterns of CPE within patients, between patients, and between patients and their environment. We are now shifting from a reactive to a proactive approach and can implement targeted hygiene measures before these multidrug-resistant pathogens spread further and cause outbreaks of infection.

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PS01.40

Colonization with ESBL-producing Enterobacterales among hospitalized patients in Sierra Leone

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Background: Antimicrobial resistance places a high burden on low- and middle-income countries. The extensive use of third-generation cephalosporins contributes substantially to the selective pressure driving the emergence of extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E). This study investigated the prevalence of ESBL-E colonisation among hospitalised patients in Sierra Leone and identified risk factors associated with colonisation.

Methods: During a pseudo-longitudinal study at Masanga Hospital, Sierra Leone (09/2024–03/2025), each patient was screened once for ESBL-E colonisation at a random time point during hospitalisation. Rectal swabs were cultured on chromogenic agar selective for ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*. Species identification was performed by MALDI-TOF and antimicrobial susceptibility testing using Vitek. The ESBL phenotype was confirmed by double-disk diffusion, and risk factors were statistically analysed. Isolates were stratified by day of hospitalization (1 to >7), and ten isolates per group and species (n = 160) were randomly selected for whole-genome sequencing (Oxford Nanopore Technologies).

Results: Of the 454 patients, 92 % were colonized with ESBL-E (ESBL-*E. coli*: 84 %, ESBL-*K. pneumoniae*: 56 %) any time after admission. While colonization with ESBL-*E. coli* fluctuated between 76-92% during hospitalization, colonization rates with ESBL-*K. pneumoniae* significantly increased during hospitalization from 42% (day 2) to 92% (day 6, $p<0.001$).

Multivariate analysis identified increasing age ($p=0.007$) and contact with cats ($p=0.049$) as significant risk factors for ESBL-E colonization. Notably, all patients presenting with chronic wounds (n=59) were colonized with ESBL-E ($p=0.007$).

A total of 638 ESBL-E isolates (382 ESBL-*E. coli*, 256 ESBL-K. *pneumoniae*) were recovered. In addition to resistance to third-generation cephalosporins, highest resistance rates were detected against trimethoprim/sulfamethoxazole (83%), ciprofloxacin (68 %), and gentamicin (46%). The predominant ESBL gene detected was blaCTX-M-15 (86%).

WGS of 80 isolates per species assigned ESBL-*E. coli* and ESBL-*K. pneumoniae* to 46 and 52 different sequence types (STs), respectively, indicating a highly diverse bacterial population structure (diversity index: 0.98). Selected clades of both species were exclusively identified in patients sampled more than two days after admission, suggesting potential hospital-associated acquisition.

Conclusion: ESBL-E colonization was highly prevalent already at admission. Together with high genetic diversity, these findings suggest extensive community transmission. The observed increase in ESBL-*K. pneumoniae* colonization during hospitalization points to possible nosocomial transmission. Targeted infection prevention and control strategies adapted to resource-limited settings may therefore be required to prevent ESBL-E acquisition in initially non-colonized patients.

PS01.42
Effect of swab type on brilliance VRE chromogenic agar screening for vancomycin-resistant Enterococcus faecium: A retrospective analysis of routine diagnostic data

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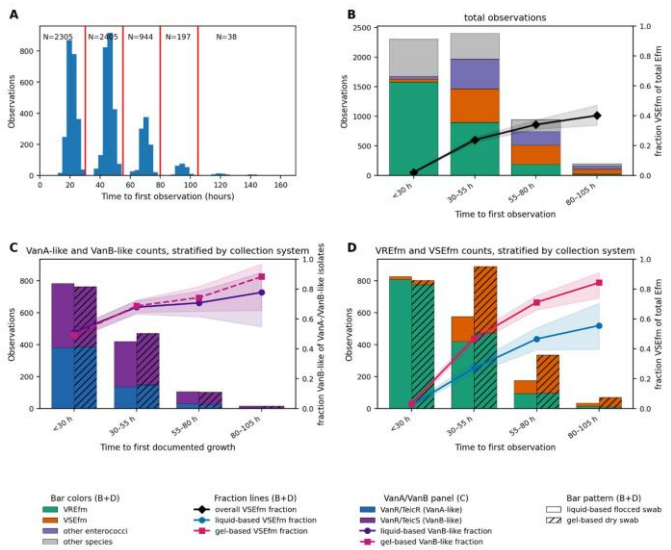
Background: Chromogenic agar-based screening for vancomycin-resistant enterococci (VRE) is widely used in routine diagnostics. Prolonged incubation increases the recovery of vancomycin-resistant Enterococcus faecium (VREfm) at the cost of reduced specificity including growth of vancomycin-susceptible E. faecium (VSEfm). In our routine diagnostic setting, two different swab systems are used, allowing assessment of whether the used swab systems modify this known temporal effect.

Methods: We retrospectively analyzed 5,834 routine VRE screening observations processed on Oxoid Brilliance VRE agar between 2020 and 2025. Outcomes were classified as VREfm, VSEfm, other enterococci, or other species. Swab systems comprised liquid-based amies-medium flocked swabs and gel-based amies-medium rayon swabs. A subset of isolates with vancomycin/teicoplanin susceptibility data was additionally stratified into VanA-like (VanR/TeicR) and VanB-like (VanR/TeicS) phenotypes.

Results: Prolonged incubation progressively enriched VSEfm and non-target organisms. Among E. faecium isolates, each additional 24 h was associated with an approximately four-fold increase in the odds of VSEfm relative to VREfm. Importantly, swab type significantly modified this effect: gel-based rayon swabs showed an approximately 1.5-fold steeper increase in VSEfm growth over time compared with liquid-based flocked swabs. VanA-like and VanB-like phenotypes were distributed similarly between swab systems, although later incubation intervals showed increased proportion of VanB-like isolates.

Conclusions: The specificity of chromogenic VRE screening is strongly influenced by incubation time and is additionally modified by specimen collection system. In our setting, incubation duration longer than 30 hours, particularly in combination with gel-based rayon swabs, substantially increased the recovery of presumptive VRE colonies, which require confirmatory testing and ultimately prove to be VSEfm.

Fig. 1



PS01.43
Azole Resistance in Germany remains low in clinical *Aspergillus fumigatus* isolates in 2020-2024

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Introduction: *Aspergillus fumigatus* can cause life-threatening disease. Azole-resistant *A. fumigatus* (ARAF) have become a major clinical concern. Environmental azole fungicide use is a possible cause for resistance development. Environmental resistance is mainly linked to L98H mutations in its *cyp*51A gene.

Objectives: To obtain representative data on resistance proportions and resistance mechanisms in A. fumigatus clinical isolates, we describe ARAF in systematically collected data in Germany from 2020-2024 from two datasets.

Methods: Resistance proportion was analysed using routine diagnostic data on *A. fumigatus* from 2020-2024 from a voluntary, laboratory-based surveillance system involving continuously participating facilities that cover approximately one-third of the German population (Antibiotika-Resistenz-Surveillance, ARS). The first clinical isolate per quarter per patient was included, reflecting infections. A. fumigatus isolates voluntarily sent to the National Reference Centre (NRZMyk) from 2020-2024 were sequenced to determine the *cyp*51A genotype. Isolates are sent for reference identification and resistance testing.

Results: In ARS, from 2020-2024, testing against azoles was available for 1,120 *A. fumigatus* infections (N=17,836, 6%) with 1.3% (95% CI: 0.7%-2.4%) being resistant against azoles. Resistance proportions varied by year from 0.0% (95% CI: 0.0%-2.5%) in 2024 to 3.8% (95% CI: 1.6%-8.6%) in 2023 and

by region from 0.7% (95%CI: 0.1%-3.6%) in West and 1.6% (95%CI: 0.6%-4.7%) in South-East of Germany. In 131 ARAF isolates sent to the NRZMyk, the mutation L98H accounted for 67% of isolates. Mutations considered to be of environmental origin were found in 87% of ARAF.

Conclusion: The overall proportion of ARAF in *A. fumigatus* infections in Germany from 2020-2024 remains low with <4%. L98H is the most common mutation found in Germany, which suggests predominant environmental resistance development. Single centres in Germany and specific patient groups might have higher resistance proportions. Local and national continuous surveillance is important to guide antimicrobial stewardship.

PS01.44

Supporting prescribing physicians in Germany — AntibioResDE presents current resistance data for drug-bug-combinations, Germany 2026

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Introduction: Current information on antibiotic resistance is essential for appropriate antimicrobial treatment. Under EU legislation, resistance information for antibiotics is included in Section 5.1 of the Summary of Product Characteristics (SmPC) to support treatment decisions in view of the resistance situation. However, these documents may not always reflect the latest data. For prescribing physicians, accessing current resistance data can therefore be challenging.

Goal: The AntibioResDE project aims to provide up-to-date, easily accessible information on antibiotic resistance in Germany through easy-to-use data visualisations on a website. By presenting resistance data for relevant antibiotic pathogen combinations (drug bug combinations) (DBC), it seeks to support empiric prescribing, promote appropriate antibiotic use, and help prevent resistance development and treatment failure.

Materials and Methods: Resistance data are derived from the Antibiotic Resistance Surveillance database at the Robert Koch Institute. As of 2024, it included data from 965 inpatient care facilities, including 762 general hospitals, and 29 400 outpatient practices. Resistance proportions and 95% confidence intervals are calculated in R/RStudio for each DBC. Combinations with fewer than 70 isolates are excluded. Intrinsic resistance is based on EUCAST information, and antibiotics are classified according to WHO Access, Watch, Reserve (AWaRe) classification and the German definition of reserve antibiotics under Section 35 SGB V. The Federal Institute for Drugs and Medical Devices verified licensure of DBC.

Results: The AntibioResDE data tool displays resistance proportions for DBC using categories aligned with product information: <10%, 10-50%, and >50%. Intrinsically resistant combinations are highlighted separately, while combinations with insufficient data are marked in grey. Antibiotics are labelled by AWaRe category and by § 35 SGB V. Tooltips provide the pathogen name, antibiotic, AWaRe category, resistance proportion, 95% confidence interval, and number of isolates. Pathogens and antibiotics can be selected by group or search function for rapid access in clinical practice.

Summary: AntibioResDE provides a practical platform for accessing and interpreting current antibiotic resistance data in Germany. It is intended to complement, not replace, individual clinical judgement, SmPC information, guidelines, and patient-specific factors. By improving access to current resistance data, AntibioResDE supports targeted antibiotic use and contribute to preventing antimicrobial resistance and treatment failure. www.amr.rki.de/Content/AntibioResDe/Datentool.aspx

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PS01.45

Fourier-transform infrared spectroscopy identifies dialysis panels as an environmental reservoir in a prolonged KPC-producing *Klebsiella pneumoniae* outbreak in an intensive care unit

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Introduction: Germany is considered a low-prevalence country for carbapenem-resistant *Enterobacterales* (CRE). In our hospital, intensified environmental cleaning followed by control sampling is performed after discharge of patients colonized or infected with CRE. During such surveillance, a KPC-producing *Klebsiella pneumoniae* isolate was recovered from a dialysis panel in an intensive care unit (ICU). As a prolonged outbreak of KPC-producing *K. pneumoniae* had occurred in a different ICU a few months earlier, environmental screening was extended to all dialysis panels in four ICUs.

Objectives: We aimed to assess the extent of CRE contamination of ICU dialysis panels and to investigate potential clonal relationships between environmental and outbreak isolates using Fourier-transform infrared spectroscopy (FTIR).

Materials and Methods: A total of 131 environmental swabs were collected from outlet nozzles and drains of 47 dialysis panels in four ICUs: ICU1: 9 panels/18 swabs; ICU2: 9 panels/27 swabs; ICU3: 8 panels/24 swabs; and ICU4: 21 panels/62 swabs. Carbapenemase genes were confirmed by PCR (Cepheid, Krefeld, Germany). Strain typing was performed using FTIR spectroscopy (IR Biotyper®, Bruker Daltonics, Germany). Hierarchical cluster analysis and principal component analysis were used for exploratory analysis and clustering.

Results: CRE were detected on 25/47 dialysis panels. The proportion of contaminated panels differed between ICU. ICU1: 4/9; ICU2: 9/9; ICU3: 4/8; and ICU4: 8/21. In total, 40 CRE isolates were recovered, comprising 22 *Klebsiella* spp. (including nine *K. pneumoniae* and 13 *K. oxytoca)*, four *Enterobacter* spp., nine *Citrobacter* spp., three *Escherichia coli*, one *Providentia rettgeri* and one *Morganella morganii*. Different carbapenemases were detected, including NDM, KPC, OXA-48 and VIM. FTIR analysis showed that all

Citrobacter spp. and *E. coli* isolates represented singletons, whereas several clusters were observed among *Klebsiella* spp. For *K. oxytoca*, one cluster comprising five isolates and three additional clusters comprising two isolates each were identified. Importantly, one KPC-producing *K. pneumoniae* isolate recovered from a dialysis panel in ICU4 clustered with five KPC-producing *K. pneumoniae* outbreak isolates obtained from the same ICU a few months earlier, indicating the dialysis panel as the probable environmental reservoir and transmission source.

Summary: CRE contamination of ICU dialysis panels was frequent and involved multiple species and carbapenemase types. Regular environmental surveillance of dialysis panels, including drains and outlet nozzles, should be considered as part of infection prevention in ICUs. FTIR spectroscopy proved useful for rapid outbreak investigation and revealed the probable transmission route of a prolonged KPC-producing *K. pneumoniae* outbreak.

PS01.46
From target modification to global regulation: adaptive evolution driving β-lactam resistance in *Listeria monocytogenes*

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β-lactam antibiotics remain the mainstay of therapy for invasive infections caused by *Listeria monocytogenes*, although recently emerging clinical isolates with reduced β-lactam susceptibility indicate that adaptive resistance evolution may compromise treatment efficacy. Previous work identified a W428R substitution in the essential penicillin-binding protein PBPB1 that reduces ampicillin susceptibility, but paradoxically increases ceftriaxone susceptibility, suggesting that target modification can disturb the intrinsic cephalosporin-resistance network (1).

Here, we investigated secondary adaptive events that compensate for this phenotype and expand β-lactam resistance. Suppressor mutants were selected from a *pbpB1* W428R background under increasing β-lactam pressure and characterized by quantitative minimal inhibitory concentrations (MIC) profiling against ampicillin, ceftriaxone, meropenem and penicillin G. Genetic and structural mapping was used to place candidate mutations into a mechanistic framework.

We identified a suppressor mutation in *rpoD*, encoding the housekeeping sigma factor σ^A. The F187L substitution lies within the highly conserved region 2.3, a promoter-melting hotspot enriched in aromatic residues that stabilize open promoter DNA during transcription initiation. In MIC assays, the *pbpB1* W428R mutant showed a pronounced reduction in ampicillin susceptibility and a marked loss of intrinsic ceftriaxone resistance. Introduction of the *rpoD* F187L mutation into this background further decreased susceptibility to ampicillin, meropenem and penicillin G, and restored cephalosporin resistance. Most prominently, meropenem MICs increased by approximately two orders of magnitude relative to the parental reference strain.

Together, these data support a two-step adaptation model in which an initial cell-wall target mutation alters β-lactam susceptibility and is subsequently compensated by global σ^A-dependent transcriptional reprogramming. This regulatory adaptation appears to stabilize the altered cell-wall state and expand the resistance phenotype beyond the initially affected antibiotic. Our findings highlight compensatory transcriptional adaptation as a previously underappreciated evolutionary mechanism shaping intrinsic β-lactam resistance in *L. monocytogenes*. A better understanding of such adaptive resistance pathways may help to anticipate the emergence of β-lactam resistance and identify regulatory vulnerabilities relevant for future antimicrobial strategies.

(1) Wamp, S., Wagner, R., Schuler, F., Krüttgen, A., Flieger, A., & Halbedel, S. (2026). A *pbpB1* mutation causing reduced β-lactam susceptibility in clinical *Listeria monocytogenes* isolates. Emerg Microbes Infect, 15(1), 2616949.

PS01.48
Differential effects of recombinant IL-17 and IL-22 on the response of A549 epithelial cells to *S. aureus* and *C. albicans* infection

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Introduction: The commensal fungus *C. albicans* and the Gram-positive bacterium *S. aureus* frequently coexist as part of the normal flora on human mucosal surfaces and skin. However, they can cause severe systemic infections, in immunocompromised patients. Unlike *S. aureus*, *C. albicans* rarely causes invasive pneumonia, but it often massively colonizes the lung during mechanical ventilation. Epithelial barrier integrity plays a central role in host defence against pathogen dissemination. Cytokines involved in mucosal immunity, including IL-22 and IL-17, modulate antimicrobial responses and barrier function.

Objectives: We aimed to investigate the effects of rIL-17 and rIL-22 on the adherence and invasion of *C. albicans* and *S. aureus* in A549 cells and to assess associated changes in epithelial barrier permeability upon infection.

Methods: An in vitro lung epithelial cell model was established using A549 cells. Pathogen adhesion and invasion were assessed by CFU plating and fluorescence microscopy. Changes in epithelial barrier integrity were evaluated by transepithelial electrical resistance (TEER) measurements using Transwell inserts. Cellular damage was quantified using an LDH cytotoxicity assay.

Results: Pretreatment with rIL-22 significantly reduced bacterial burdens following infection of A549 cells with *S. aureus* compared to the untreated control, whereas rIL-17 did not. In addition, rIL-22 showed a trend towards reducing cell damage in the LDH assay. Barrier integrity was enhanced by rIL-22 pre-treatment in the infected group, as reflected by higher TEER values compared to the other infected experimental groups. In contrast, rIL-17 or rIL-22 pre-treatment

did not affect fungal burdens, TEER values or LDH release upon *C. albicans* infection compared to the untreated control.

Conclusion: These findings indicate a pathogen-specific protective role for rIL-22 in *S. aureus* infection, characterized by reduced bacterial burden and preserved epithelial barrier integrity, whereas no protective effects were observed during *C. albicans* infection.

PS01.49
IL-22 induced by *C. albicans* reduces the severity of invasive *S. aureus* pneumonia in mice

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Introduction: The commensal fungus *C. albicans* and the bacterium *S. aureus* frequently coexist in humans and can cause severe systemic infections in immunocompromised patients. Clinically, *S. aureus* commonly induces pneumonia and disseminates from the lung, whereas *C. albicans* almost never causes invasive pulmonary disease, even in mechanically ventilated patients with high fungal burdens.

Objectives: We aim to investigate the different behavior of *C. albicans* and *S. aureus* in the lung, and to assess how pulmonary pre-colonization with *C. albicans* influences subsequent *S. aureus* infection.

Methods: We established a lung pre-colonization/infection model using Balb/c mice. Mice were administered *C. albicans* intranasally at day 0 and infected intranasally with *S. aureus* on day 1. At 24, 48 and 96 hours post *S. aureus* infection, lung, liver and kidney bacterial/fungal burdens as well as lung immune responses were analyzed.

Results: Following intranasal administration, pulmonary loads of both pathogens declined over time; however, only *S. aureus* disseminated to liver and kidney, closely mirroring the clinical situation. Pulmonary pre-colonization with *C. albicans* markedly altered the lung immune landscape and resulted in enhanced recruitment of neutrophils and CD11b dendritic cells upon *S. aureus* infection. Cytokine profiling revealed a pronounced induction of IL-1β, IL-17, and IL-22 during fungal colonization followed by bacterial challenge. Importantly, *C. albicans* pre-colonization protected mice from lethal *S. aureus* lung infection. Neutralization of IL-22 abrogated this protective effect, identifying IL-22 as a critical mediator to *S. aureus* induced pneumonia. Early administration of recombinant IL-22 was sufficient to significantly enhance early survival during a lethal *S. aureus* pneumonia.

Conclusion: We established a physiologically relevant dual pathogen lung model and identified IL-22 as a key immunological switch determining the outcome of *S. aureus* lung infection.

PS01.50
PlaD, a novel type IVB secreted effector protein of *Legionella pneumophila*

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Introduction: *Legionella pneumophila,* the causative agent of a life-threatening pneumonia, intracellularly replicates in a specialized compartment in lung macrophages, the *Legionella*-containing vacuole (LCV). During infection *L. pneumophila* secretes proteins, among others phospholipases, into the lumen of the LCV and the host cell cytoplasm via its type II (Lsp) and type IVB (Dot/Icm) secretion systems. At least 15 phospholipases A, which divide into the patatin-like proteins, the PlaB-like proteins and the GDSL hydrolases PlaA, PlaC and PlaD, are encoded in the genome.

Goals: We here focus on the characterization of the phospholipase PlaD and shows various differences to the other GDSL hydrolases PlaA and PlaC. We aim to understand the importance, secretion path, and mode of action in infection and activation mechanism of PlaD.

Materials and Methods: We investigated the mode of secretion of PlaD by means of Western blotting and protein translocation assay. Additionally, we determined its binding to various lipids and its interactions with eukaryotic proteins by means of lipid-protein-overlay assays, proximity ligation, and pull down assays. Further, we analyzed the localization of PlaD during infection via immunofluorescence microscopy.

Results: We showed that, during infection, PlaD is Dot/Icm-dependently injected into the host cell cytoplasm where it localizes to distinct organelles. Moreover, we demonstrated that PlaD binds to a subset of phosphoinositide species and interacts with a class of regulatory proteins of the host cell. Additionally, our data revealed that the C-terminal half of PlaD is essential for its secretion and phosphoinositide binding but dispensable for interaction with the regulatory proteins.

Summary: Based on its Dot/Icm dependent injection into the host cell cytoplasm, we classify PlaD as a novel type IVB secreted effector protein of *L. pneumophila*. We propose that PlaD is involved in the regulation of host cell signaling cascades during infection.

PS01.51
Histopathologically confirmed biliary invasive fungal infection impairs survival after liver transplantation

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Background & Aims: Invasive fungal infections (IFIs) are increasingly recognized in liver transplant (LT) recipients, yet graft-specific biliary IFI remains poorly defined and likely underdiagnosed. We evaluated the clinical impact and risk factors of histopathologically confirmed biliary IFI in chronic allograft failure following LT.

Methods: In this single-center retrospective cohort study, 144 transplant episodes (1990–2019) in 131 patients with explanted grafts (re-LT n=141; death n=3) were stratified by the presence (n=41) or absence (n=103) of histopathologically proven IFI. Overall survival was analyzed using a Cox model. A time-dependent Cox model evaluated the association between microbiological fungal detection, antifungal therapy, and the composite endpoint of graft loss or death. Fungal amplicon sequencing of bile was compared with routine culture and molecular diagnostics.

Results: IFI was detected in 28.5% of explanted grafts and independently associated with a twofold increase in 5-year mortality (HR 2.03; p=0.026), with the strongest effect in the first year (70.3% vs. 93.2% survival). Microbiological fungal detection during follow-up was strongly associated with graft loss or death (HR 6.23; p<0.001), while concurrent antifungal therapy markedly attenuated this risk (interaction HR 0.09; p<0.001). Non-anastomotic biliary strictures (OR 8.5) and hepatic artery thrombosis (OR 3.4) were independent predictors of IFI. Sequencing was largely concordant with culture and molecularpathological results but detected additional fungal signals in culture- or molecularpathological-negative samples.

Conclusions: Biliary IFI is a frequent and clinically relevant feature of chronic allograft failure and associated with impaired survival. Microbiological fungal detection identifies a high-risk phase potentially amenable to antifungal intervention. These findings support systematic fungal surveillance and risk-adapted antifungal strategies in LT recipients with biliary complications and vascular compromise.

PS01.52

Monocyte-mediated immune responses to co-Infections with *Candida albicans* and *Staphylococcus aureus*

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Candida albicans and *Staphylococcus aureus* are common human commensals but also opportunistic pathogens capable of causing life-threatening systemic infections. More than one in four cases of candidemia is estimated to be polymicrobial, with *S. aureus* among the most frequently co-isolated pathogens. Despite increasing clinical recognition, the immunological complexity of these polymicrobial infections remains poorly understood and is associated with high mortality rates.

Here, we investigated human monocyte responses to polymicrobial infection using *C. albicans* cell-wall mutants to dissect the contribution of outer-layer mannans to innate–adaptive immune coordination. Primary human monocytes and autologous T cells were challenged in vitro with *C. albicans* and/or *S. aureus*.

Flow cytometric analyses revealed that N-mannan deficiency significantly impaired monocyte recognition, uptake, activation, and MHC-II-mediated antigen presentation. In contrast, the O-mannan-deficient *mnt1Δ/mnt2Δ C. albicans* mutant induced a synergistic response in combination with *S. aureus*, resulting in altered T-cell survival, shifts in the CD4:CD8 ratio, and modulation of early activation markers.

Notably, these cellular phenotypes were not sufficiently reflected by multiplex or ELISA-based quantification of cytokines, chemokines, and effector molecules, despite detectable release of monocyte-derived T-cell-modulating cytokines including TNF-α, IL-23, and IL-1β.

Ongoing studies employ single-cell transcriptomics, extended 72-h co-culture systems, and characterization of cell-death modalities to further define mechanisms underlying adaptive immune priming during polymicrobial infection. In addition, comparative analyses using diverse *S. aureus* and *Staphylococcus epidermidis* isolates aim to determine strain-specific effects on monocyte–T-cell communication and inflammatory signatures.

PS01.53

Detection of *Candidozyma auris* subpopulations with low echinocandin susceptibility

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Introduction: *Candidozyma auris* is an emerging human fungal pathogen which is distinguished from other *Candida* species by its easy transmissibility between patients and a frequent resistance to the commonly used antifungal drug fluconazole. Treatment of *C. auris* is further complicated by a swift development of echinocandin resistance and low-level susceptibility to amphotericin B. During antifungal drug susceptibility testing of clinical *C. auris* isolates with gradient diffusion tests, we observed for some strains the presence of macrocolonies in the inhibition zone. This phenomenon was then made the subject of further experiments.

Objectives: The aim of the study is to find out how frequent these macrocolonies are and if the phenotype can also be found by other experimental approaches such as population analysis profil (PAP) assays. Finally, we wanted to know if these colonies display a lower echinocandin susceptibility over prolonged time periods.

Material and Methods: As echinocandins are primarily used for the treatment of systemic *C. auris* infections, we investigated 34 bloodstream isolates which were obtained from different patients. As ten of these strains derived from initial colonizers, we also included the primary isolates, totalling the number of investigated strains to 44. Gradient diffusion tests and population analysis profile (PAP) assays were used to

identify low-level-susceptible subpopulations. These cells were further tested for their susceptibility to micafungin in several growth tests and additional antifungal drug susceptibility assays.

Results: We performed micafungin gradient diffusion tests with the selected 44 strains. For 14 of them (32.6%), large colonies within the inhibition zone were observed. Consequently, PAP assays were performed with isolate NRZ-2020-0224 which is showing the presence of large colonies and NRZ-2024-0690 which did not. After plating onto RPMI1640 agar with 2 mg/L micafungin, we observed a survival rate of 28% for NRZ-2020-0224. No colonies were detected for NRZ-2024-0690 under the same conditions. However, when surviving NRZ-2024-0690 colonies were obtained for another micafungin gradient diffusion test, the assay revealed no more clean inhibition zones. Instead, no inhibition at all or only zones of delayed growth which resembled a phenotype sometimes seen for gradient diffusion test with azoles could be identified.

Conclusion: Our results indicate that subpopulations with a lower susceptibility to echinocandins do exist in *C. auris*. However, it remains the subject of future experiments to identify if this phenomenon can be either linked to heteroresistance or tolerance. Additionally, it might be interesting to examine if isolates showing this phenotype can be linked to echinocandin treatment failures.

PS01.54

Stress resilience of multidrug resistant *Nakaseomyces glabratus* isolates

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Background: *Nakaseomyces glabratus* (syn. *Candida glabrata*) is a leading cause of invasive candidiasis, second only to *Candida albicans*. Multidrug resistance (MDR) is an emerging problem in the treatment of systemic *N. glabratus* infections. Administration of echinocandins is considered first-line therapy as resistance is less frequent compared to azoles. However, the number of isolates showing reduced susceptibility to both drug classes is increasing, limiting treatment options to amphotericin B or combinatory therapy. MDR is often associated with fitness costs for the fungal cell although it is not fully understood how MDR affects fungal fitness and how comparable *in-vitro* stress susceptibility is to *in-vivo* stress resilience and virulence of clinical MDR isolates.

Objectives: The aim of the study is to perform large-scale stress screenings of clinical resistant and multiresistant *N. glabratus* isolates to comprehensively characterize their *in-vitro* stress resilience and overall fitness. Based on these data, we will start infection assays with *Galleria mellonella* larvae to examine the virulence of MDR isolates.

Material and Methods: Based on the large strain collection from the German National Reference Center for Invasive Infections, we collected two clinical isolates which were susceptible to all antifungals and 22 isolates which displayed resistance to either one or more antimycotics. The laboratory strain CBS138 was used as reference. For all strain growth assays in a 96-well plate format with different media and stress conditions were performed. Additionally, we examined all strain for mutations in genes known for their association with antifungal drug resistance such as *FKS1*, *FKS2* or *ERG3*. The established *Galleria mellonella* infection model was used to study fungal virulence.

Results: Compared to the reference strain CBS138, we found that the two susceptible clinical isolates NRZ-2015-067 and NRZ-2016-191 displayed better fitness in YPD and under cell wall, osmotic and oxidative stress conditions. Interestingly, most of the resistant and MDR isolates outperformed CBS138 under cell wall and oxidative stress, while they were comparable to the reference under osmotic stress. When glycerin is used as only carbon source, CBS138 performed better than all clinical isolates. However, this changed during the combination of glycerin and oxidative stress where nearly all resistant and MDR isolates grew better than CBS138. Although partially displaying growth arrests under *in-vitro* stress conditions, the isolate NRZ-2016-252 was fully virulent in the *Galleria mellonella* infection model.

Conclusion: Our results indicate that MDR isolates of *N. glabratus* can display a certain level of fitness and stress resilience which is not much different than that for susceptible strains. We also found first evidence that MDR isolates can still be fully virulent - at least in an insect infection model and even despite small growth defects under *in-vitro* conditions.

PS01.55

Vulvovaginal Candidiasis – *In vitro* characterization of isolates, combined with patient samples, reveals the multifaceted nature of this infection

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Vulvovaginal candidiasis (VVC) is the second most prevalent infection of the female reproductive system, affecting 75% of women at least once during their reproductive years. Up to 10% of women suffer from recurrent episodes, experiencing symptoms such as itching, swelling, pain, and fissures, yet it is also affecting the patients mental health. Even though *Candida albicans* is the predominant pathogen, other non-*albicans* species (NAC) are being isolated more frequently, especially in patients who have undergone frequent fluconazole treatment. Therefore, characterizing the virulent traits of different *Candida* species alongside assessing correlates of host inflammation may enable personalized therapies and circumvent the development of antifungal resistance. One project, the DeVEnIR (Defining VVC – Elements of Infection and Remedy), aims to acquire insights into VVC pathogenicity, including the roles of the microbiome, metabolome, immune system, and pathogen characteristics.

Our study focuses on the isolated *Candida* species from healthy women (n = 4), women with VVC (n = 17) and RVVC (n = 31), including samples from non-infectious periods (n = 9). In line with other studies, *C. albicans* and *C. africana* were the most prevalent species, but some non-*albicans Candida* (NAC) species were also isolated, including *Nakaseomyces glabratus* (*Candida glabrata*), *Pichia kudriavzevii* (*Candida krusei*), and *Candida parapsilosis* as well as *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*. The isolates were characterized and grouped according to their morphology, as well as their interaction with epithelial and immune cells. Live-cell imaging revealed highly diverse phenotypes in terms of (pseudo)hyphae formation and growth rate. The isolates also differ in their ability to grow and adhere to epithelial cells. Here, LDH and cytokine release from epithelial cells also depend heavily on the isolate. Vaginal swab samples from the patients also revealed altered albumin and IL-1β levels. Therefore, the potential of some isolates to activate the inflammasome in macrophages was tested. While some triggered IL-1β release in primed immune cells, only a few were able to initiate the cascade. Overall, the presence of albumin modified these signals.

These findings shed light on the variable pathogenicity of (R)VVC isolates and, together with the patient samples, strongly suggest that (R)VVC is a very heterogenous disease. This unique dataset allows exploring the different facets of (R)VVC as well as patient stratification, which will be essential in the identification and development of individualized therapies.

PS01.56
Interkingdom biofilms in chronic wounds: the collaboration of *Candida albicans* and *Staphylococcus aureus* against conventional wound antiseptics

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Aim: Biofilms are a major problem in infected wounds. This does not necessarily have to be bacterial burden; fungal and yeast infections can increase the damage to the wounds and reduce the chances of healing. According to previous studies, about 20% of chronic wounds are colonized by fungi. It is well known that biofilms are more tolerant to antimicrobial treatments, but the composition of these communities and the resulting interactions can also have an influence. For example, Candida can exacerbate the (wound-) infection of *S. aureus* even though under normal conditions these are typical microbiome microorganisms.

Method: *C. albicans* and *S. aureus* were observed in single- and mixed species human biofilm models, which consisted of bloodplasma, immune cells and the specific microorganisms in single or co-cultivation. Hereby, the tolerance to the common clinical antiseptics octenidin-dihydrochloride/phenoxyethanol and polyhexamethylene biguanide was examined using quantitative suspension method (QSM), and their spatial distribution in the biofilm was analyzed using confocal laser scanning microscopy.

Results/ Discussion: In our study, the presence of *S. aureus* triggered an increase formation of (pseudo-) hyphae. These hyphae can produce better penetration and invasion of the host tissue. This could also enable *S. aureus* to penetrate tissue more effectively, as it appears to adhere to the hyphae in the process. Furthermore, this study observed that S. aureus exhibited a grwoth advantage when cultured togehther with C. albicans.

Conclusion: Wound biofilms do not necessarily consist solely of bacteria. Fungi are also active in chronic wounds. However, due to their eukaryotic physiology, they react differently to antimicrobial substances and can form unfavorable alliances with bacteria. In conclusion, this translational study once more highlights the importance of non-specific antimicrobial treatment.

PS01.57
Proteomic profiling of antifungal drug responses of *Aspergillus fumigatus* to elucidate drug mechanisms of action

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Introduction: The human fungal pathogen *Aspergillus fumigatus* has become increasingly resistant to clinically used antifungals agents, in particular to triazole-based drugs. This has led to high mortality rates in patients with azole-resistant invasive aspergillosis. The discovery of new antifungal agents, especially those with novel modes of action (MoA), is therefore urgently needed. However, due to the closer evolutionary relationship between eukaryotic fungi and humans in comparison to bacteria, the number of fungal-specific targets for drug development is rather limited. This often leads to the rediscovery of drugs with known MoA.

Objectives: To address this challenge and to learn more about how antifungal agents affect fungal cells, we set out to establish an expression-based proteomics workflow with the intention to construct a drug-specific proteomic response library based on the profiling of anti-*Aspergillus fumigatus* compounds. Our goal was also to test whether this library can correctly classify new antifungal agents in terms of their mode of action.

Material and Methods: We selected eleven antifungal compounds including all current drug classes in clinical use for building up the antifungal drug response library. Their effect on conidial germination and hyphal growth were tested in bioassays to determine the optimal drug concentrations. Liquid cultures of *A. fumigatus* were exposed for four hours to the

individual compounds. Proteome profiles were determined by LC-MS/MS on an Ultimate 3000 nano RSCL system connected to an Orbitrap Exploris 480 instrument.

Results: Our analysis revealed that the strongest proteomic responses were linked to processes involved in "detoxification" and "defence" against environmental insults. This applied in particular to biosynthetic drugs, which suggests an evolved response programme to natural products. Based on our response library, we successfully identified MoA-specific signature proteins, enabling the prediction of similar MoAs. Our strategy allowed us to distinguish between biosynthetic and synthetic drugs, and uncovered drug-specific induction patterns of biosynthetic gene clusters (BGCs). We also validated our approach by using three additional drugs, including azalomycin F, a natural product with a predicted new MoA. Notably, we identified the drug-specific increase of poorly characterised transcription factors, which suggests complex regulatory circuits of antifungal drug resistance, which are now investigated further.

Conclusions: Selected *A. fumigatus* signature proteins (98) based on an antifungal drug-specific proteomics response library allow to predict whether an antifungal compound has a new mode of action.

PS01.58

Mechanisms of tissue invasion and pathogenicity of *Fusarium keratoplasticum* and *Fusarium petroliphilum* in structural corneal models

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Background: Within the *Fusarium solani* species complex (FSSC), *Fusarium keratoplasticum* and *Fusarium petroliphilum* are major causative agents of severe fungal keratitis. However, the precise pathophysiological differences in their invasiveness and early host-pathogen interactions remain poorly understood. This study aims to comparatively characterize the pathogenicity, tissue-specific infection patterns, and induced inflammatory responses of these species using 3D-Hemi-Cornea models and ex vivo porcine cornea models.

Methods: Controlled infection studies were performed using defined strains of *F. keratoplasticum* and *F. petroliphilum*. Human 3D-Hemi-corneal models and an ex vivo infection model utilizing enucleated porcine eyes served as experimental systems. Vertical tissue invasion and hyphal penetration depth were analyzed via standard histology (PAS/H&E) and complemented by immunohistochemistry (IHC) to evaluate epithelial barrier integrity and in situ expression of inflammatory markers. To quantify host cell damage, lactate dehydrogenase (LDH) release was measured in the culture supernatants. Additionally, supernatants were collected to evaluate the secretion of key pro-inflammatory cytokines and chemokines (e.g., IL-6, IL-8, and IL-1β) via immunoassay.

Results: Both *F. keratoplasticum* and *F. petroliphilum* successfully adhered to the ocular surface and disrupted the epithelial barrier in both model systems. Initial evaluations and ongoing quantitative analyses indicate distinct species-specific differences. While both strains induced a significant increase in LDH release, correlating with incubation time and infection progression, preliminary data suggest variations in the kinetics of stromal invasion. Furthermore, IHC and cytokine profiling revealed distinct expression patterns and secretion profiles of tissue-derived factors, indicating an isolate-dependent modulation of the early innate immune response of the cornea.

Conclusion: The establishment of 3D corneal models and ex vivo porcine eyes allows for a differentiated assessment of species-specific virulence factors within the FSSC. Combining histological and immunohistochemical imaging with cytotoxicity assays and cytokine profiling contribute to a better understanding of clinical disease progression and highlight the necessity of precise species differentiation for future therapeutic strategies.

PS01.59

Therapeutic efficacy of inhaled N-chlorotaurine in a mouse model of fungal pneumonia

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Question: The therapeutic efficacy of the endogenous antiseptic N-chlorotaurine (NCT) applied via inhalation was investigated in *Lichtheimia corymbifera* and *Aspergillus fumigatus* pneumonia in the mouse.

Methods: Specific pathogen-free female C57BL/6JRj seven-week old mice were immune-suppressed with cyclophosphamide or cortisone acetate. After 7 days, they were inoculated intranasally with 1.5x10E7 spores of *Lichtheimia corymbifera* or 6.5 x 10E6 spores of *A. fumigatus*. Inhalations with 0.1%, 0.5%, 1.0% (55 mM) or 2.0% NCT solution or 0.9% sodium chloride three times daily for 10 min started one hour after inoculation and ended after 15 days.

Results: In mice euthanized on day 2 in all groups (n = 5), fungi in the lung and other signs of pneumonia were found. Challenge with *L. corymbifera* was survived by 7 of 9 mice in the 1% NCT group, but only by 1 of 9 in the control group ($p = 0.0049$). Accordingly, the count of colony-forming units per pro ml homogenised lung in the test group came to 1.60 log10 (1.30; 1.99; median, quartiles), while it was 4.26 log10 (2.17; 4.53; median, quartiles) in the control group. Challenge with *A. fumigatus* was survived by 8 of 9 mice in the 0.5%, 1.0% and 2.0% NCT groups, but only by 1 of 9 in the control group ($p < 0.01$ for each concentration versus saline). There was no difference between the two ways of immune-suppression. With 0.1% NCT, 4/9 mice died ($p = 0.03$ versus the higher NCT-concentrations; $p = 0.0035$ versus control). The fungal load came to 5.28 (4.46; 5.70; median, quartiles) colony-forming units per ml lung homogenate in the control group and to 1.3 (median; maximum 2.45) in the 1% NCT group in mice immune-suppressed with cyclophosphamide ($p = 0.0004$). Values were similar in cortisone groups ($p = 0.0023$). Secondary parameters showed respective significant differences between test and control groups.

Conclusions: Early treatment with inhaled NCT demonstrated highly significant efficacy in fungal pneumonia in the mouse model. A concentration of 1% NCT appears to be optimal, which fits to a clinical phase I study and to case experiences with inhalations in humans.

PS01.60
SET2 sets the sex: Epigenetic control of gametocyte fate in Plasmodium falciparum

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Transmission of *Plasmodium falciparum* depends on the successful differentiation of male and female gametocytes, a process driven by epigenetic reprogramming. Histone H3 acetylation and methylation are central to this regulation, yet how chromatin states shape sexual commitment and sex-specific development remains poorly understood. The histone methyltransferase SET2 catalyzes H3K36 di- and trimethylation and has sofar been implicated in transcriptional repression during blood-stage development.

Here, we identify SET2 as a critical regulator at the onset of gametocytogenesis. Using two independent *set2* knockout lines, we show that SET2 is dispensable for asexual replication but essential for productive gametocyte formation. SET2-deficient parasites undergo aborted gametocytogenesis, with rare gametocytes displaying severe morphological defects, including elongated cell tips, disrupted microtubule organization, and loss of membrane integrity. Male sexual development is particularly affected, as indicated by defective exflagellation and aberrant flagellar structures.

Comparative RNA-Seq analyses reveal extensive transcriptional deregulation in SET2-deficient ring stages including downregulation of genes associated with sexual commitment and male and female differentiation, like ones encoding AP-2G and key sex-specific regulators, alongside pathways controlling cytoskeletal organization, mitochondrial function, and lipid metabolism. In contrast, mature gametocytes exhibit comparatively limited transcriptional changes, suggesting that SET2 acts early to license developmental trajectories that later become fixed.

Together, our findings position SET2 as a pivotal epigenetic regulator that couples chromatin state to the decision-making processes underlying gametocyte induction and sex determination. By controlling transcriptional thresholds required for male and female development, SET2 emerges as a key molecular switch governing malaria parasite transmission, highlighting histone methylation as an attractive target for transmission-blocking interventions.

PS01.61
Molecular epidemiology of *Clostridioides difficile* – High diversity in a northern tertiary care hospital

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Introduction: *Clostridioides difficile* is able to persist in hospitals due to its ability to form disinfection-tolerant spores. Nosocomial outbreaks of this pathogen are described. Using various methods, e.g., ribo-typing, a number of hypervirulent clones (e.g. RT027) were identified. The aim of our study was the detailed molecular characterization of the *C. difficile* population in a northern German tertiary care hospital and the analysis of potential transmission events.

Material and Methods: GDH-positive stool samples (n=196) from 01-12/2023 were used for bacterial culture independent of toxin detection. Positive culture was observed in 71.9 % (n=141). Up to date 107 isolates were characterized by WGS and subsequent analyzed via MBioSEQ Ridom Typer (Bruker) for MLST, core genome MLST (cgMLST) and toxin/resistance gene identification. For epidemiological assessment of potential transmission events, a high probability of transmission was assumed, if patients were admitted to the same ward with overlapping time periods or in quick succession (<4 weeks) whereas a medium probability was assumed for patients being treated simultaneously on different wards within the same department. The patient clinical severity assessment was based on the CDI-KISS criteria.

Results: Of the 141 cultured isolates 116 were toxigenic. Using classical MLST, a high diversity was observed among the sequenced 107 isolates. In total 41 different sequence types were identified, ST2 (n=15), ST8 (n=8), and ST3 (n=7) being the most common. There were only a few *C. difficile* isolates with sequence types associated with hypervirulent clones. No ST1 associated with RT027 was observed. Analysis of cgMLST distance identified three clusters of three isolates and seven clusters of two isolates within a six-loci distance. Nine of the ten clusters contain toxigenic *C. difficile* strains. By reviewing epidemiological data, a high probability of transmission was assumed for three clusters and a medium probability of transmission for one cluster. For the remaining clusters, the probability of transmission appears to be low. Regarding the clusters with high to medium probability, all isolates were toxigenic. One according patient of each of two high-probability-clusters and the medium-probability-cluster died within 30 days of diagnosis. No further severe cases were observed within the high to medium probability clusters.

Conclusions: A high diversity of *C. difficile* isolates was observed. No longer transmission chain was identified and possible transmission events were sporadic. Transmission was observed for toxigenic isolates. There was no MLST sequence type found in the data set, associated with the known hypervirulent strain RT027/ST1, indicating that this clone is still rare in northern Germany. The combination of molecular and classical epidemiology showed, that the transmission of *C. difficile* does not necessarily occur only in hospital settings.

PS01.62

Genomic landscape of *C. freundii* as an emerging pathogen in bloodstream infections across four university hospitals in Germany

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Objectives: *Citrobacter freundii* is increasingly recognized as a significant opportunistic pathogen responsible for a range of infections, including bloodstream infections (BSI). Simultaneously, an increasing occurrence of multidrug-resistant *C. freundii* strains, especially those that produce carbapenemase and are responsible for nosocomial as well as community-acquired infections, is observed. The objective of our research was to investigate the genomic landscape of BSI *C. freundii* gathered from four university hospitals in Germany between 2016 and 2023.

Methods: *Citrobacter* isolates (n = 257) from different species were collected from human blood cultures. Out of these, 102 isolates were selected for whole-genome long-read sequencing and taxonomically reclassified, with a focus on the species *C. freundii* and *C. braakii*. The phylogenetic relationships of 52 *C. freundii* genomes were evaluated following their detailed analysis. For comparative genomics, an additional publicly available set of 169 complete genomes was used.

Results: A total of 94 complete genomes that passed quality control were generated. 52 of these were genomically assigned to *C. freundii*. The genomes of *C. freundii* showed significant genetic diversity and were classified into 27 different MLST types, with ST396, ST18, ST19, and ST98 being especially common. Phylogenetic analysis revealed the existence of four clades in the *C. freundii* population. The genomes were discovered to be dispersed across the global population. Virulence gene analysis revealed 36% of BSI *C. freundii* containing T6SS^{i1} and approximately 15% T6SS^{i2}. Using a *Galleria mellonella* infection model, the T6SS^{i1} positive isolates demonstrated enhanced pathogenicity compared to those lacking the T6SS operon.

Conclusions: The study found that *C. freundii* isolates from bloodstream infections display significant genetic diversity, with ST396, ST18, ST19, and ST98 being the most prevalent. Different genetic clades exhibited varying levels of pathogenicity, with T6SS^{i1} carrying ST396 isolates demarking as potential high-risk pathogens.

The members of the CITROBSI-Study group are as follows (in addition to those listed above): Silke Peter and Kristina Schmauder from Tübingen, David Tobys and Harald Seifert from Köln, Dennis Nurjadi and Jan Rupp from Lübeck and Moritz Fritzenwanker, Parisa Golbargtaklimi, Jan-Paul Herrmann, Markus Weigel, Max Pfister, and Jane C. Falgenhauer from Giessen.

PS01.63

Adaptive plasmid evolution in clinical *E. coli*: Amplification of blaCTX-M genes

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Introduction: Antimicrobial resistance is a major global healthcare challenge. In particular, extended-spectrum betalactamase (ESBL)-producing *Escherichia coli* are of major concern because treatment options are limited. ESBL genes like blaCTX-M are commonly encoded on plasmids in clinical isolates, facilitating their transmission and spread.

Goals: The aim of this study was to investigate the microevolution and characteristics of *bla*CTX-M-encoding plasmids carried by two clinical *E. coli* isolates. A further goal was to assess IS*26*-mediated gene amplification as a successful evolutionary strategy and its clinical relevance.

Materials and Methods: Two clinical multidrug resistant *E. coli* isolates harbouring IncF plasmids with insertion sequence (IS)*26*-mediated *bla*CTX-M gene amplifications were used as model strains. Both isolates underwent an *in vitro* evolution experiment over 30 passages with or without subinhibitory ceftazidime exposure. Antimicrobial susceptibility testing, long-read whole genome sequencing (PacBio) and bioinformatic plasmid analysis were performed. Plasmid curing generated plasmid-free strains to determine the contribution of plasmids to resistance and bacterial fitness. Growth and conjugation experiments were used to evaluate fitness costs and transferability.

Results: Under ceftazidime exposure, both plasmids showed an increased *bla* gene amplification, which correlated with stronger phenotypic resistance, including an up to 10-fold increase in ceftazidime resistance in one strain. Co-selection effects resulted in increased resistance to ciprofloxacin, meropenem and piperacillin/tazobactam despite the absence of direct exposure. Chromosomal variation remained limited, indicating that adaptation was predominantly plasmid-driven. Plasmid curing restored susceptibility phenotypes, confirming the central role of plasmid-encoded resistance genes. Fitness effects were host- and plasmid-dependent, ranging from minimal burden to significant fitness gain. Both plasmids retained conjugative activity, enabling horizontal dissemination alongside the ESBL genes.

Summary: IS*26*-mediated amplification of plasmid-encoded ESBL genes represents a dynamic and clinically relevant evolutionary mechanism enabling bacteria to rapidly adapt to antibiotic exposure. Our findings highlight how subinhibitory antibiotic exposure may accelerate resistance evolution and co-selection of multidrug resistance. They further underline the importance of genomic surveillance to detect high-risk plasmids and guide infection control and antimicrobial stewardship in clinical practice.

PS01.64

Sequencing-based resolution of fitness dynamics in mixed gonococcal populations under antibiotic exposure

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Introduction: The increasing use of doxycycline post-exposure prophylaxis (doxyPEP) and doxycycline pre-exposure prophylaxis (doxyPrEP) has raised concerns regarding the potential selection and persistence of resistant *Neisseria gonorrhoeae* (NG) lineages. Antibiotic-associated selective pressure may alter bacterial fitness and influence competitive population dynamics, favoring strains carrying resistance-associated genetic determinants. Understanding these dynamics is essential for evaluating the long-term epidemiological implications of doxycycline-based prevention strategies.

Objectives: This study aimed to establish a sequencing-based approach for quantitative monitoring of mixed gonococcal populations and to assess the impact of doxycycline-mediated selection on NG population dynamics and resistance-associated genetic determinants.

Materials and Methods: NG isolates with 10 different sequence types (ST) were co-cultivated with or without doxycycline. Relative isolate abundances were assessed using a sequencing-based analytical framework independent of selective markers. Short-read sequencing data were processed through a customized computational workflow integrating MLST-associated profiling and multilayer normalization procedures to account for technical variability. Relative ST abundance reconstructed from normalized allele-specific signal patterns, and deviations from the initial population structure (t0) were used to estimate comparative fitness dynamics under different experimental conditions.

Results: The initial t0 populations demonstrated highly reproducible ST distributions with only minor variability regarding frequency between replicates. Comparative analyses revealed condition-dependent shifts in population structure under doxycycline exposure compared to control conditions. Distinct ST-specific dynamics were observed, with certain ST showing relative enrichment under antibiotic pressure, whereas others declined in abundance. These opposing trends indicate differential competitive behavior within mixed populations and suggest an association between doxycycline-associated selective pressure and adaptation-linked genetic backgrounds. Overall, the observed population shifts support the presence of heterogeneous fitness responses among individual NG lineages under antibiotic exposure.

Conclusion: Sequencing-based population profiling enabled robust monitoring of competitive dynamics within mixed NG populations without the use of selective markers. Doxycycline exposure was associated with pronounced alterations in population composition consistent with resistance-associated adaptation and fitness trade-offs. These findings illustrate the utility of sequencing-based population profiling for investigation of NG population dynamics und antibiotic pressure and highlight that genotypic surveillance is an important tool to assess developments regarding NG in the context of doxycycline-based prevention strategies.

PS01.65

Investigating putative correlations of clinical case presentation and outcome with *Klebsiella pneumoniae* resistance- and hypervirulence marker gene carriage in hospitalized patients

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Introduction: Hypervirulent *Klebsiella pneumoniae* strains (hvKp), in contrast to classical multidrug-resistant *K. pneumoniae* (MR-non-hvKp) strains, can cause invasive community-acquired infections in healthy patients of all ages. In this study, *K. pneumoniae* isolates from hospitalized patients were analyzed by whole-genome sequencing (WGS). Clinical presentation and outcome, including hospital length of stay, in-hospital mortality, need for invasive ventilation, pneumonia, bloodstream infection, and urinary tract infection were assessed for potential correlations with carriage of resistance and/or hypervirulence traits.

Goals: This retrospective cohort study aimed to characterize in univariate and multivariate analyses the association between genetic resistance and virulence markers and clinical outcomes in patients infected with *K. pneumoniae*.

Materials and Methods: For the study, 95 *K. pneumoniae* isolates (November 2017–March 2024) were preselected and characterized by WGS. Further, the strains were analyzed in four subgroups (MR-hvKp, MR-non-hvKp, S-hvKp, S-non-hvKp) and additionally in a simplified two-group analysis (hvKp vs. non-hvKp). Clinical outcomes were analyzed using univariate methods (Chi-square, ANOVA/Kruskal-Wallis tests), and evaluated through multivariate regression models for hospital stay and mortality. Explorative statistical methods (hierarchical cluster analysis, PCA) supported the differentiation of genetic subpopulations.

Results: Multidrug-resistant hypervirulent isolates (MR-hvKp) were significantly associated with prolonged hospital stays ($p<0.05$). Multivariate analyses confirmed patient age and MR-hvKp status as independent predictors for extended hospital stays. Further, in the two-group analysis, multidrug resistance was associated with increased virulence gene carriage and prolonged hospitalization. Temporal trend analyses indicated a decline of several antibiotic resistances and a simultaneous increase in selected virulence factors over time. Hypervirulence markers alone did not independently predict in-hospital mortality or clinical manifestations; patient age and comorbidities were more influential.

Summary: The study enabled comprehensive statistical analyses of different *K. pneumoniae* pathotypes. The combination of multidrug-resistance and hypervirulence gene carriage, often called convergent hvKp, significantly correlates with extended hospital stays. However, the marker genes alone seem to be insufficient predictors for clinical manifestations or outcome. These findings suggest that genetic characteristics may supplement clinical decision-

making but currently do not offer a standalone basis for prognosis.

PS01.66

Prevalence of extended-spectrum β-lactamase and multidrug-resistant producing *Enterobacterales* in dogs in Hesse, Germany

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Introduction: Antibiotic resistance is a persistent problem in human and veterinary medicine. Within a One Health framework, pet animals are increasingly recognized as potential reservoirs of resistance genes, yet systematic surveillance in companion animals remains limited in Germany. Furthermore, systematic data on multidrug-resistant organisms (MDROs) in pets outside clinical settings are scarce. This study therefore investigated the prevalence and molecular characteristics of MDROs in fecal samples of dogs in non-clinical settings in Hesse.

Methods: A total of 209 fecal samples from eight animal shelters and 44 private households from 13 cities were collected on six consecutive days from March 2 to 7, 2025 and screened for MDROs using a broad panel of selective agar media. Isolates were identified by MALDI-TOF mass spectrometry and the resistance phenotype characterized by disc diffusion, antibiotic gradient strips and VITEK®2 analysis. Whole-genome sequencing was performed for molecular characterization, and transfer of ESBL plasmids from *Enterobacterales* isolates was investigated by liquid mating assays. Epidemiologically relevant sequence types (ST) were clustered in EnteroBase v1.2.0 to show their genetic relationship.

Results: A prevalence of (8/209) 3.8 % for ESBL/AmpC-producing *E. coli* was identified, whereas no MRSA, VRE, and CRE were detected. All isolates examined demonstrated resistance to the tested penicillins and first-generation cephalosporins. The 13 identified *Enterobacterales* isolates belonged to 10 different STs. Three predominant STs, ST95, ST405 and ST69 were identified. These isolates were closely related to strains (≤20 allelic differences) originating from environmental, animal and human sources. Eleven of the 13 isolates harbored an ESBL/AmpC-carrying plasmid, whereas one isolate possessed a chromosomal ESBL, and one isolate carried a mutation in penicillin-binding protein 3. Replicon types of the F (n=9), H (n=1), X (n=4) and Col (n=1) families were detected. Among ESBLs, CTX-M-14 was most frequent (n=4), followed by CTX-M-27/-1 (n=2 each) and SHV-12 (n=1). Two AmpC types, ACT-12 and DHA-1, were also identified (n=1 each). Nine of 11 plasmids were successfully transferred to a human *E. coli* recipient strain. The *bla*$_{SHV-12}$ plasmid showed a conjugation frequency of $6.9x10^{-5}$. In contrast, all *bla*$_{CTX-M}$/IncF plasmids exhibited mean conjugation frequencies ($1.6x10^{-2}$ to $7.9x10^{-1}$), indicating highly efficient horizontal gene transfer.

Conclusion: The prevalence of MDRO in general and of ESBL-producing Enterobacterales in dogs was lower than reported in humans (5.5%) (Symanzik et al., 2022) and substantially lower than in livestock in Germany (32.3 %–56.5 %) (EFSA & ECDC, 2026). The identified ST95, ST405, and ST69 belong to major, globally distributed ExPEC-associated lineages in human medicine. This suggests that dogs serve as reservoirs and transmission vectors of clinically relevant antimicrobial resistance genes.

PS01.67

Contribution of the novel class D β-lactamase OXA-944 to carbapenem resistance in *Acinetobacter guillouiae*

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Background: Environmental bacteria are important reservoirs of antibiotic resistance genes that can be transferred to human pathogens. The acquisition of carbapenemase genes can lead to limited therapy options and represents a threat to human health. *A. guillouiae* encodes an intrinsically OXA-274-like class D β-lactamase, yet its contribution to carbapenem resistance remains poorly understood. This study investigated the antimicrobial susceptibility profiles of *A. guillouiae* isolates from multiple hospitals, with a particular focus on clinical isolates carrying novel OXA-274-like β-lactamases.

Methods: Whole genome sequencing (WGS) of five clinical *A. guillouiae* strains collected at the hospitals of Frankfurt or Cologne was performed, followed by the identification of new OXA-274-like variants in WGS data and NCBI RefSeq. To test carbapenemase activity, *bla*$_{OXA-274}$ from *A. guillouiae* DSM 590 and *bla*$_{OXA-944}$ from the carbapenem-resistant clinical isolate *A. guillouiae* 2348 (ACGU_2348) were cloned into *A. baumannii* ATCC® 19606^{T}, *A. baumannii* ATCC® 19606^{T} Δ*bla*$_{OXA-98}$ and *A. guillouiae* DSM 590. Antimicrobial susceptibility testing was performed with disk diffusion testing and the minimal inhibitory concentration (MIC) was determined by antibiotic gradient strips and with broth microdilution for the carbapenems imipenem, meropenem and doripenem.

Results: Most clinical *A. guillouiae* isolates (59/62) were recovered from polymicrobial cultures or alongside normal skin and mucosal flora. Microbiological routine diagnostics often failed to identify *A. guillouiae*, suggesting massive underreporting. The monoculture isolate ACGU_2348 recovered from a wound infection exhibited unusually high carbapenem MICs (meropenem, 24 µg/ml; imipenem, 12 µg/ml). Whole-genome sequencing of ACGU_2348 identified *bla*$_{OXA-944}$, a novel OXA-274-like β-lactamase sharing 97.5 % amino acid identity with OXA-274, while resistome analysis revealed no acquired resistance genes explaining the phenotype. Comparative genomics of 62 genomes revealed a highly diverse OXA-274-like family with 25 variants exclusive to *A. guillouiae*. Antimicrobial susceptibility testing of clinical *A. guillouiae* expressing distinct OXA-274-like β-lactamases showed that carbapenem resistance was unique to ACGU_2348 and independent of non-β-lactamase-mediated mechanisms. Expression of OXA-944 in *A. guillouiae* DSM 590 resulted in a 3-fold higher MIC in broth microdilution for all tested carbapenems compared to OXA-274, indicating carbapenemase activity. In the clinically relevant species *A.*

baumannii, the expression of OXA-944 lead to carbapenem resistance with MICs of 8 to 32 µg/ml, respectively.

Summary: The intrinsic OXA-274-like β-lactamase family in *A. guillouiae* is highly diverse. Minor sequence variations, as exemplified by OXA-944 can lead to substantially increased carbapenem MICs limiting therapy options significantly.

PS01.68

A sample-to-results workflow for agnostic or targeted pathogen sequencing from wastewater

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Question: Cost-effective population-level pathogen surveillance can be achieved through agnostic or targeted sequencing of wastewater samples. Agnostic pathogen detection via metagenomic sequencing supports surveillance for unknown and novel pathogens. Targeted sequencing permits the monitoring of whole genomes or key genomic regions to track variants for a pathogen of interest. However, wastewater is a challenging substrate that requires optimized enrichment, extraction, and sequencing protocols. Here we present an effective and streamlined sample-to-results workflows for agnostic or targeted pathogen sequencing from wastewater.

Methods: We employed Nanotrap® Microbiome Particles to concentrate intact viruses and bacteria from contrived wastewater samples in a semi-automated, high-throughput method. Nucleic acids were then isolated using the magnetic bead-based Monarch® Mag Viral DNA/RNA Extraction Kit. From these total nucleic acid extracts, we ran agnostic or targeted pathogen sequencing.

Results: When applying an agnostic sequencing approach, we detected expected control pathogens and organic population compositions consistent with wastewater substrates. For the targeted pathogen sequencing workflows, we focused on influenza A (Flu A) and respiratory syncytial virus (RSV). The targeted sequencing of viral pathogens like Flu A and RSV from wastewater is technically challenging due to low viral titers, fragmented RNA, and high background from environmental and host nucleic acids. However, sequencing data following these robust workflows showed high genome coverage and accurate strain identification.

Conclusion: These workflows provide scientists and public health labs with streamlined and cost-effective solutions for monitoring pathogens from population-level wastewater sources.

PS01.69

Tracing SARS-CoV-2 variants and recombinant lineages in Mecklenburg-Western Pomerania, Germany (2022–2025)

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Global SARS-CoV-2 genomic surveillance has transformed pathogen tracking, yet its reliance on consensus genome sequences imposes a fundamental resolution limit, obscuring intra-sample diversity and hindering the detection of recombination and co-infection. Here, we analyzed 3,360 SARS-CoV-2 genomes from a high-resolution regional surveillance dataset in northeastern Germany (Mecklenburg–Western Pomerania) spanning 2022–2025.

We reconstructed the local spatio-temporal dynamics of variant circulation, revealing substantial lineage diversity, region-specific deviations from global trends, and phases of intense co-circulation that create conditions for recombination.

Using a two-stage framework combining high-sensitivity consensus-based screening (REcombination BARcode detector REBAR) with targeted raw-read validation, we identifed 61 candidate recombinants, of which 21 were supported by read-level evidence, including 17 previously undescribed genomes. Despite frequent co-infections, only a minority of recombinants (n=2) showed signatures of onward transmission, indicating strong constraints on their establishment. Breakpoints cluster in functionally relevant genomic regions, while recurrent non-canonical patterns suggested a broader, incompletely resolved recombination landscape.

Together, our results demonstrate that regional genomic surveillance can uncover fine-scale evolutionary dynamics missed at global scale and that scalable integration of consensus-based screening with focused validation provides a practical path to resolving recombinant SARS-CoV-2 diversity.

PS01.70

Repurposing of nitroxoline for treatment of high-priority multidrug-resistant Gram-negative bacteria

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Background: New antibiotics are urgently needed to combat antimicrobial resistance, yet their development faces significant economic and research challenges. Repurposing of already approved drugs offers a promising route to accelerate the deployment of new antibacterial therapies. Nitroxoline (NTX) is an 8-hydroxyquinoline antibiotic which is recommended as the first-line therapy for uncomplicated urinary tract infections in Germany. Here, we investigated the activity spectrum of NTX against 35 multidrug-resistant Gram-negative species, its mode of action and resistance mechanisms.

Methods: Antimicrobial susceptibility to approx. 800 clinical isolates was assessed by microdilution, agar dilution, and disc diffusion. Nitroxoline interactions with other antibiotics were

evaluated via checkerboard assays and the *Galleria mellonella* infection model. Protein expression and stability upon NTX exposure in *Escherichia coli* was analysed using two-dimensional thermal proteome profiling. Intracellular metal content was measured by X-ray fluorescence imaging. Experimentally evolved resistant isolates and patient isolates were analysed by whole-genome sequencing and subsequent SNP profiling.

Results: Nitroxoline was highly active against many multidrug-resistant species, including carbapenem-resistant *E. coli*, *Klebsiella* spp. and carbapenem-resistant *Acinetobacter spp* respectively. NTX synergized with outer membrane-targeting antibiotics, including colistin, and restored colistin susceptibility in resistant *E. coli* and *K. pneumoniae* strains *in vitro* and in *G. mellonella* larvae *in vivo*. Proteomics revealed decreased abundance and stability of outer membrane proteins like OmpDF and LPS biosynthesis proteins like LptDEFG or LpxH. Deletion of mutants catalysing downstream LPS biosynthetic reactions, beginning with *waaF*, were more sensitive to nitroxoline. Direct measurements of metals in intact *E. coli* showed that NTX increases intracellular levels of copper, manganese and zinc. Experimental evolution of NTX resistance in *E. coli*, *A. baumannii* and *K. pneumoniae* identified mutations in repressors of RND- and MFS-type efflux pumps, resulting in their upregulation. A recurrent *K. pneumoniae* G60_L67dup mutation, also detected in clinical isolates, emerged as a resistance hotspot. Efflux pump inhibitors restored NTX susceptibility across species, although effects were less pronounced in *A. baumannii*.

Summary: Nitroxoline acts as a metallophore, increasing intracellular copper, manganese and zinc levels and promoting metal intoxication. It further disrupts outer membrane integrity through interference with lipopolysaccharide biosynthesis and trafficking. Resistance is mediated by MFS- and RND-type efflux pumps, whereas the marked susceptibility of *A. baumannii* likely depends on additional, yet unidentified, factors.

PS01.71
MSSA ST398 from Austria: Insights into antimicrobial resistance and phylogeny

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Introduction: Animal-independent, human-associated methicillin-susceptible *Staphylococcus aureus* (MSSA) from the pandemic sequence type (ST) 398 has been linked to severe infections. Isolates from this lineage are frequently resistant to erythromycin and clindamycin due to the presence of the *ermT* gene, and their genomes contain an immune evasion cluster (IEC) encoding *scn* (a marker of human adaptation), which is thought to play a role in its increasing prevalence in the community.

Objectives: To characterise an MSSA ST398 isolated from a household in Austria in terms of antimicrobial resistance and phylogenetic relationships.

Methods: Whole genome sequencing (WGS) was performed using Illumina. WGS was bioinformatically analysed for ST, *spa* type and the presence of antimicrobial resistance genes (ARGs), mobile genetic elements and phylogenetic relationships with other 273 MSSA ST398 strains. Vitek 2 was used for antimicrobial susceptibility testing against antibiotics. Susceptibility to disinfectants was determined by broth microdilution.

Results: An MSSA ST398 strain belonging to a novel *spa* type (succession of repeat codes: 08-83-34-25) was phenotypically resistant to clindamycin and erythromycin and susceptible to increased exposure of levofloxacin according to EUCAST. The isolate showed a reduced susceptibility to disinfectants benzalkonium chloride and chlorhexidine. WGS revealed the presence of several ARGs, including *ermT*, which was located on a plasmid. IEC contained *scn* and hemolysin genes and was found on the StauST398 prophage. Phylogenetic analyses showed that the isolate was most closely related to human and animal-derived isolates from France, almost all of which contained the *scn* gene.

Summary: Here, we report that MSSA ST398 was isolated from the community in Austria for the first time. The isolate was resistant to clinically important antibiotics and had a reduced susceptibility to disinfectants, which may complicate mitigation efforts. Phylogenetic analysis hinted at its relatedness to both human and animal-derived isolates, almost all of which encoded *scn*, calling for the One Health approach to surveillance.

Reference:

Tóth AG and Makarova O (2026) Resistance to clinically important antibiotics and reduced susceptibility to disinfectants in the pandemic ST398 methicillin-susceptible *Staphylococcus aureus* from Austria. Front. Microbiol. 17:1734430. doi: 10.3389/fmicb.2026.1734430 (original paper, published) https://doi.org/10.3389/fmicb.2026.1734430

PS01.72
Macrolide and quinolone resistance of *Mycoplasma genitalium* strains in Germany from 2021 to 2026

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Mycoplasma genitalium is a sexually transmitted bacterium and currently the smallest independently reproducible pathogen in human medicine. Its genome is highly reduced (580 kbp), and the pathogen lacks a classic bacterial cell wall. Culturing the bacterium is extremely difficult, and serological tests are not available; therefore, nucleic acid amplification techniques are used in clinical laboratories to detect an infection. A causal link to cases of urethritis in men and cervicitis and PID in women has been confirmed. The association with other clinical presentations (proctitis, prostatitis, miscarriages, and preterm births) is under discussion. In the general population without a risk profile, the prevalence is estimated to be approximately 3%, which can rise to 20% or higher in risk groups (MSM, sex workers, HIV-positive patients). Treatment of infections follows international guidelines using macrolides or fluoroquinolones. The pathogen"s limited epidemiological significance is offset by its pronounced ability to acquire antibiotic resistance, which will lead to significant treatment challenges in the future if no countermeasures are taken.

To assess the current resistance situation on a national scale, the results of samples submitted to the reference laboratory between January 2021 and February 2026 were analyzed. The samples came from all over Germany. In the vast majority of submissions, no additional information (e.g., risk group, sexual orientation) was provided, so that a distinction can only be made between the total number of samples and those from women. The presence of resistance was determined by detecting mutations in the 23S rRNA (macrolides) and in the topoisomerase ParC (quinolones). A total of 778 submissions with complete resistance profiles were evaluated. Among all samples, resistance rates were 61.6% (macrolides) and 27.4% (fluoroquinolones). The prevalence of dual-resistant strains was 24.9%. With rates of 20.6% (macrolides) and 11.8% (fluoroquinolones), resistance among the identified female patients is significantly lower, but has now also reached a high level. Based on the data collected, it can be concluded that treatment in accordance with guidelines without prior resistance testing makes little sense. In particular, the prevalence of double-resistant strains is cause for concern, as the alternatives listed in the guidelines have a lower clinical eradication rate (doxycycline) or are currently scarcely available (pristinamycin, minocycline). The real risk of symptomatic cases becoming untreatable can only be addressed through coordinated measures, based primarily on a consistent resistance-guided therapy regime.

PS01.73
Diagnostic options for suspected neurosyphilis using treponemal antibody tests

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Background: The number of infections with the spirochaete *Treponema pallidum ssp. pallidum* has shown an increase since 2010, with the exception of the pandemic years 2020 and 2021, and has currently reached a new high in Germany and all over the world. Neurosyphilis is a typical late consequence of previously inadequately treated syphilis, but it can also appear as syphilitic meningitis within the first year after infection.

Following the discontinuation of the gold standard Treponema pallidum particle agglutination assay (TPPA), a new recommended procedure is required for the diagnosis of neurosyphilis in Europe. Two Treponema pallidum hemagglutination assays (TPHA) based on avian erythrocytes were investigated and in addition three monovalent Enzyme Immunoassays (EIA).

Material and Methods: Both TPHAs were tested for the detection of T. pallidum-specific antibodies in cerebrospinal fluid (CSF) and serum followed determination of serum CSF ratios for detection or exclusion of neurosyphilis with 100 sample pairs. Two different methods for determining the antibody index were compared, the parallel titration of serum and CSF versus the calculation of the ITpA index (Intrathecal *Treponema pallidum* Antibody).

Furthermore, monovalent EIAs from three different companies were compared for the detection of Treponema-specific IgG and IgM antibodies in serum and CSF and the resulting antibody indices using 65 sample pairs.

Results: Using the parallel titration method, both tests correctly recognize eight of the 12 pathological samples, using the ITpA index nine of 12. Of the 38 non-pathological samples, both tests recognized 21 correctly. No antibodies can be detected in 16 samples. Overall, both tests show identical results with the method of parallel titration. The TPHA tests examined show high specificity (95-100%). However, their sensitivity is only 63-67%, which is far below that of the TPPA.

The IgM EIAs used show a sensitivity of only 0-80% compared to the 19S-IgM-FTA-abs (fluorescence Treponema pallidum antibody absorption test). For the detection of treponema-specific IgG antibody indices, the sensitivity ranges between 42.3 and 90.5% (Tab.1). The IgG and IgM EIA can detect pathological and non-pathological samples when calculating an AI. However, many non-pathological samples are classified as negative, especially by IgM EIA.

Conclusions: The results of the present study show that TPHAs can also be used for the diagnosis of neurosyphilis but with significantly lower sensitivity. Antibody index calculation for IgG and IgM antibodies is also possible with monovalent EIA, even in these tests, there is a limited sensitivity. However, in the absence of alternatives, the tests will still have to find their way into routine diagnostics so that the diagnosis of neurosyphilis can be maintained - albeit with restrictions.

Fig. 1

	Demeditec		Euroimmun		Mikrogen	
	IgG	IgM	IgG	IgM	IgG	IgM
	[DU/mL] < 0.69	[DU/mL] < 0.52	[RE/ml] < 1.0	[RE/ml] < 5.0	[U/mL] ≲ 0.19	[U/mL] < 0.17
sensitivity [%]	42.3	0.0	90.5	80.0	65.2	66.7
specificity [%]	100	-	90.9	100	96.8	100

PS01.74
Surveillance of invasive meningococcal disease in Germany and new vaccination recommendations

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Introduction: In Germany, invasive meningococcal disease (IMD) must be notified to the Robert Koch-Institute, whereas submission of meningococcal isolates and clinical samples to the German National Reference Laboratory for Meningococci and Haemophilus influenzae (NRZMHi) is voluntary. Overall, 80-90% of the notified cases are submitted to the NRZMHi.

IMD is a vaccine-preventable disease The available vaccines are serogroup specific. In 2006, routine MenC vaccination was recommended for toddlers and in 2024 routine MenB vaccination for infants.

Goal: To present laboratory surveillance data of IMD in Germany 2025

Materials and Methods: The IMD data of the NRZMHi were analysed according to serogroup, patients" age, antimicrobial susceptibility and whole genome sequencing (cgMLST).

Results: Since 2024, IMD case numbers are above the pre-pandemic level of 2019 with 292 cases in 2024 and 288 cases in 2025. Since 2023, MenY increased significantly. In 2025,

44% of the cases were MenB, 41% MenY, 10% MenW and less than 2% MenC.

Infants, adolescents, young adults and the elderly aged 60 years and older were still most affected by IMD. MenY was less prevalent than MenB among infants and toddlers but most prevalent among the elderly.

Only one of 228 meningococcal isolates was resistant to cefotaxime, ciprofloxacin and rifampicin, respectively, whereas 28 isolates (12%) were resistant to penicillin. Penicillin resistance was at the same level as in 2024. Few isolates harboured a beta-lactamase or were resistant to two antibiotics.

Whole genome sequencing was not done in real-time but retrospectively. Preliminary results of 179 isolates showed that MenY isolates (n=84) were assigned to five clonal complexes of which cc23 (n=74) dominated. In contrast, MenB isolates (n=65) were assigned to 13 clonal complexes of which cc213 comprised 14 isolates and cc41/44 ten isolates.

Due to the significant increase of MenY cases since 2023, the German Standing Committee on Vaccination (STIKO) evaluated the implementation of quadrivalent MenACWY vaccines in 2025. Based on a mathematical model, STIKO recommended routine MenACWY vaccination for adolescents aged 12-14 years instead of MenC vaccination of toddlers.

Summary: Since 2023, MenB and MenY are the most prevalent serogroups in Germany. MenW cases increased on a low level from 4% in 2023 and 2024 to 10% in 2025. STIKO recommended routine MenACWY vaccinations for adolescents aged 12-14 years in 2025.

Due to the increase of penicillin resistant meningococcal isolates ongoing surveillance is important.

PS01.75
Laboratory surveillance of invasive *Haemophilus influenzae* infections in Germany 2025

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Introduction: The National Reference Laboratory for Meningococci and *Haemophilus influenzae* (NRZMHi) performs nationwide surveillance of invasive *H. influenzae* (Hi) infections in Germany. Data from 2025 provide insight into the current post-pandemic epidemiology.

Materials and Methods: Epidemiological data on invasive Hi infections from 2025 were analyzed. Isolates from blood or cerebrospinal fluid (CSF) are mandatorily notifiable in Germany, whereas submission to the NRZMHi is voluntary. All submitted isolates were subjected to species confirmation, serotyping and antimicrobial susceptibility testing for ampicillin and cefotaxime.

Results: In 2025, invasive Hi infection was confirmed in 1239 cases, including 1186 blood isolates, 38 CSF isolates, and 4 from both materials. Surveillance coverage was estimated at 82%.

Nontypeable Hi (NTHi) predominated (1040 isolates; 84.7%). Among capsulated strains, Hif was most frequent (94; 7.7%), followed by Hib (47; 3.8%), Hia (25; 2.0%), and Hie (22; 1.8%); Hic and Hid were not detected.

Most cases occurred in patients aged >40 years (1077; 87.7%). Ampicillin resistance remained an important characteristic.

Notably, Hib increased to the second most frequent capsular type in 2025. In parallel, a regional cluster of invasive Hib infections in Hamburg predominantly affected homeless persons and people with substance use, with 13 confirmed cases and three fatalities, representing an unusual occurrence in adults.

Discussion: The epidemiology of invasive Hi infections in 2025 remained largely stable compared to recent years. Disease burden continues to be driven by NTHi infections in older adults, whereas capsulated strains play a minor role overall. The rise in Hib and its occurrence in vulnerable populations underline the importance of continuous surveillance and targeted public health interventions.

PS01.76
Identification of Aspergillus species using MALDI-TOF MS

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Introduction: *Aspergillus* is an opportunistic fungal genus threatening immunocompromised patients. Invasive aspergillosis often results in fatale outcomes. Conventional identification of *Aspergillus* cultures relies on microscopy of long-term-growth-cultures, requiring well experienced users, or molecular methods. This process is time-consuming and especially microscopic identification brings a high potential for misdiagnosis, worsening the patient`s outcome. MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) is a fast and well-established method for the routine identification of bacteria and yeasts. However, the identification of moulds has been possible only to a limited extent due to a lack of reference spectra.

Objective: Since we had addressed *Aspergillus* species of the sections Fumigati and Nigri in a previous study this study explores, how the MALDI Biotyper can be used to discriminate the remaining clinically relevant *Aspergillus* species.

Material and Methods: Seventy clinical *Aspergillus* strains belonging to 19 species of the sections Aspergillus, Circumdati, Clavati, Flavi, Nidulantes, Terrei and Usti from the German National Reference Center for Invasive Fungal Infections were included. All isolates were identified molecularly by beta-tubulin or calmodulin sequences. Isolates were cultivated on Sabouraud agar. To gain a homologous biomass, liquid culture was incubated for 16-24h followed by a standard extraction-protocol of the Filamentous Fungi Module provided by Bruker. Extracts were measured with the MALDI Biotyper sirius System. Quality control and re-calibration was performed in the FlexAnalysis software. Reference Spectra ("Main-Spectra-Projection" - MSP) were created using the

Compass Explorer Software. Intra-database-comparison and visual analysis were applied.

Results: Cultivation and extraction routine for reference spectra measurement as well as a process for analysis of MSPs was implemented. After quality control, 70 isolates were included for further analysis. Intra-database comparison showed 100% correct identification rate at the section and series level. On species level, overall correct identification rate was 87%. Within the different sections, we found varying rates of correct species identification in the sections Circumdati (83%), Flavi (81%), Nidulantes (96%), Terrei (60%). Furthermore, 100% correct identification rate on species level was achieved for the sections Aspergillus, Clavati and Usti.

Conclusion: Our study shows that it is possible to identify *Aspergillus* strains belonging to the sections Aspergillus, Circumdati, Clavati, Flavi, Nidulantes, Terrei and Usti to the species level using MALDI-TOF MS. Prerequisites for a MALDI-TOF MS based species identification are a high number of isolates per species and a comprehensive reference library. By including more strains, we are confident that finally all species within these sections can be identified in this way.

PS01.77

Antimicrobial resistance in *Salmonella* and *Campylobacter* in Germany: Trends from phenotypic and genomic surveillance

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Background: *Campylobacter* spp. and *Salmonella enterica* are the two most frequently notified bacterial intestinal pathogens in Germany and Europe. Although infections caused by both pathogens are usually self-limiting, antimicrobial therapy is required in severe disease and particularly in immunocompromised patients. According to current guidance, azithromycin is the preferred option for *Campylobacter* infections, whereas ceftriaxone or azithromycin are recommended for systemic *Salmonella* infections; fluoroquinolones should only be used according to susceptibility testing.

Methods: Human isolates submitted to the National Reference Centre for *Salmonella* and other bacterial enteric pathogens, Germany, were analysed by broth microdilution. *Salmonella* isolates collected between 2018 and 2025 and *Campylobacter* isolates collected between 2020 and 2025 were included. Selected isolates were further characterised by whole-genome sequencing using an Illumina Nextera platform.

Results: In enteric *Salmonella*, ampicillin resistance showed a slight decline over time, whereas cefotaxime resistance remained low and largely stable. Ciprofloxacin resistance fluctuated over the study period, with a moderate increase towards the end of the observation period. Pronounced serovar-specific differences were observed; resistance trends for *S.* Typhimurium, *S.* Enteritidis, *S.* Infantis, *S.* Kentucky, *S.* Typhi and *S.* Paratyphi B will be presented and discussed separately. For *Campylobacter*, ciprofloxacin resistance remained consistently high throughout 2020–2025 in both *C. jejuni* and *C. coli*. Erythromycin resistance remained low in *C. jejuni* but was higher and more variable in *C. coli*, whereas tetracycline resistance was high in both species, particularly in *C. coli*.

Conclusions: The data indicate a substantial and persistent resistance burden in the two most important bacterial enteropathogens in Germany, with particularly concerning resistance to fluoroquinolones. Continued phenotypic and genomic surveillance is essential to detect emerging trends, support treatment recommendations, and improve risk assessment for clinically relevant lineages.

PS01.78

Exposure to *Leptospira* in Munich's urban river surfers

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Leptospirosis is a zoonotic disease found worldwide that is caused by pathogenic bacteria of the genus *Leptospira*. Small mammals, particularly rodents, are the primary reservoir for the pathogen. Transmission to humans occurs through skin lesions, conjunctiva or mucous membranes following direct contact with infected animals or more often indirect contact with soil or water contaminated with the urine of infected animals. Known risk factors for leptospirosis include contact with rats and participating in recreational activities involving water (Baharom et al. 2024, Walker 2018).

The consultant laboratory for leptospirosis took part in a prospective controlled cross-sectional study that was conducted following severe leptospirosis cases that occurred among urban river surfers in Munich, Germany.

EDTA-Blood and serum samples of 102 surfers and 100 controls were investigated. Serum samples were screened for anti-leptospiral IgG and IgM antibodies using an in-house enzyme-linked immunosorbent assay (ELISA). Samples that tested positive for IgG were further analyzed using a microscopic agglutination test (MAT). EDTA blood samples and 15 environmental samples collected near the Eisbach River wave and adjacent areas were analyzed by a PCR targeting the LipL32 gene to detect the presence of pathogenic *Leptospira*.

Seven surfers tested positive, three tested borderline, and 92 tested negative for IgG. In the control group, two participants tested positive, one tested borderline, and 97 tested negative. A higher seroreactivity of IgG antibodies was observed among Eisbach surfers than among the control group (9.8 % vs. 3.0%; one-sided p=0.041). Similar seroreactivity of IgM antibodies was observed in both groups. Among the surfers, 11 (10.8 %) participants tested positive and 9 (8.8 %) tested borderline for IgM. In the control group, 10 (10 %) tested positive and 13 (13 %) tested borderline. All IgG-positive samples that were further analyzed using MAT tested negative. *Leptospira* DNA was not detected in any of the investigated blood samples. One out of

the 15 soil samples, collected from a puddle near the surfer exit point, tested positive for *Leptospira* DNA by PCR.

These findings demonstrate that urban freshwater environments may pose a risk of exposure to and infection with *Leptospira*. Increased awareness following exposure to urban freshwater could help prevent misdiagnosis and enable prompt identification of infection.

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PS01.79

Generation and characterization of a set of murine monoclonal IgG-antibodies against *Bartonella henselae*

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Here we describe a set of murine monoclonal immunoglobulin-G (IgG) antibodies directed against *Bartonella henselae* generated by hybridoma technology. Mice were immunized intraperitoneally with heat-killed *B. henselae* (strain Marseille) according to standard immunization protocols. Hybridoma cell lines were generated by fusing AG8 cells with spleen lymphocytes. Subsequent clonal selection was performed in hypoxanthin-aminopterin-thymidin (HAT) medium and hybridomas were expanded in RPMI-1640 medium (with appropriate supplements). Supernatants were collected and pretested for reactivity in immunofluorescence (pure, 1:10 dilution) and western blotting (1:100 dilution). If reactive, hybridomas were further expanded (VKS1-VKS-34), frozen in five stock vials each and monoclonal antibodies (mAbs) were purified using HiTrap® protein-G coupled sepharose columns. If there is interest in these hybridomas or mABs, collaboration with industry partners will be based on a contractual framework whereas material transfer agreements may be established with academic institutions.

PS01.80

An optimized 16S–23S rRNA intergenic spacer region PCR for the detection and identification of *Bartonella* spp

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PCR-based methods are indispensable diagnostic tools for the laboratory detection of *Bartonella* infections, primarily because these fastidious pathogens exhibit extremely slow growth. In this study, we present an optimized ITS-PCR assay designed to improve the detection and differentiation of clinically relevant *Bartonella* species, including recently identified species and those for which genome sequences have recently become available. This PCR assay is highly specific, demonstrates a sensitivity comparable to previously described ITS-PCR protocols for *Bartonella* spp. and, therefore, constitutes a valuable tool for broadening the laboratory diagnostic capabilities for *Bartonella* infections.

PS01.81

An outbreak of non-toxigenic, multidrug-resistant (MDR) *Corynebacterium diphtheriae* infections with sequence type (ST)-103 in Germany: An imminent health threat?

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Background: Since 2022, Germany faces an outbreak of non-toxigenic *Corynebacterium (C.) diphtheriae* strains affecting mostly socially disadvantaged persons. The genomic epidemiology of these strains reveals an alarming prevalence of MDR ST-103 isolates. This study aims to raise awareness for this ongoing health threat in Germany and beyond.

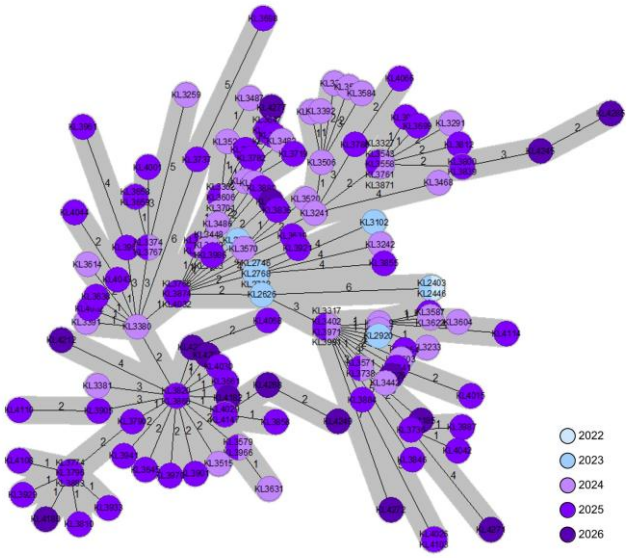
Methods: The national consiliary laboratory for diphtheria receives >700 potentially toxigenic Corynebacterium strains from medical laboratories all over Germany per year. All strains are routinely cultured and identified by MALDI-TOF MS. Toxigenicity is verified by PCR and Elek test. Antimicrobial resistance testing is performed according to the EUCAST guidelines. Next-generation sequencing (NGS) is conducted on all MDR strains to reveal their molecular fingerprints via (core genome) multilocus sequence typing for genomic outbreak investigation.

Results: By now, a total of 141 patients harbouring ST-103 isolates were detected with the first case occurring in 2022 (see Figure 1). Since then, the amount of cases increased exponentially from 7 (5%) in 2023 via 42 (30%) in 2024 to 76 cases (54%) in 2025. The year 2026 revealed already 15 new cases (11%). Men were more often affected 113; 80%) than women (28; 20%) and had an higher average age [47 years] than women [43 years]. Clinical data were available for 134 patients (95%): The majority of these isolates [83; 62%] originated from wound infections [46; 34%] including deep wounds [9; 7%], intraoperative sites [15; 11%], patients" blood [10; 8%] or bones [3; 2.0%] reflecting ST-103"s invasiveness. Notably, 49 isolates (35%) were phenotypically resistant against Penicillin, Cotrimoxazol, Erythromycin, Clindamycin, and Meropenem. Many isolates [90; 66%] phenotypically pretended Meropenem susceptibility, but sequencing data revealed the presence of *PBP2m* and *ermx* genes as well as other resistance markers. The geographical focus of the ST-103 outbreak is mainly located in metropolitan areas with cases occurring predominantely in the federal states Berlin [80; 57%], North Rhine-Westphalia [29; 21%] and Hamburg [13; 9%], so far.

Conclusion: The current ST-103 outbreak poses a major threat to public health due to its increasing prevalence and medical invasiveness. Only antibiotics of last resort (e.g. linezolid, vancomycin and rifampicin) are still able to tackle underlying infections. Urgent efforts are needed to clarify the extent of the current outbreak, associated risk factors and epidemiological links to stop its transmission. Joint efforts are

inevitable to assess the global prevalence of ST-103 to mitigate its threat.

Fig. 1



PS01.82
Comparison of the early immune response of macrophage-like THP-1 cells against *L. pneumophila* serogroup 1 strains

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Background: Legionnaires' disease (LD) is a severe pneumonia with a high mortality rate of ~8%. *Legionella pneumophila* serogroup 1 (SG1) is the primary causative agent, with so-called mAb 3/1-positive strains being particularly associated with increased virulence and major outbreaks. These MAb 3/1-positive strains posses a specific epitope on its lipopolysaccharide (LPS) that enhances bacterial attachment and potentially modulates host immunity. The genetic basis of this epitope-variability is closely linked to the presence of the *lag-1 gene*.The objective of this study is to provide a comparative evaluation of how distinct SG1 subtypes (mAb 3/1 positive and negtive) influence the intensity of macrophage activation and proinflammatory cytokine release.

Method: Human THP-1 monocytes are differentiated into macrophage-like cells using PMA (10ng/ml) for 24 h, followed by 24 h resting on media. The macrophages are infected with *L. pneumophila* strains (moi=20) for 0,5; 1; 2; 6; 24 and 48 h. The immune response is evaluated by cell surface marker analysis using flow cytometry and the cytokine release into the supernatant using Legendplex (BioLegend).

Results and Conclusion: Within the first 6 h of infection, all *L. pneumophila* SG1 strains failed to trigger significant proinflammatory M1 polarization in THP-1 macrophages. A subtype-dependent progression toward an activated state was observed at 24 h and 48 h, evidenced by increased CD86 expression and cytokine release. Specifically, the strain 'Corby' (mab-Type Knoxville) and its mutant TF3/1 (loss of function *lag-1* gene, thus mAb 3/1-negative) induced a higher secretion of TNF-α, IL-6, and IL-1β than other mAb 3/1-positve strains. These findings suggest that strain-specific factors beyond the *lag-1* and its LPS-associated epitope play a decisive role in modulating host immunity. This differential activation of the innate immune system may be a key determinant underlying the varying clinical severity and significant mortality rates observed in Legionnaires' disease.

PS01.83
Studies on the comparability of inhibition zone diameters and minimum inhibitory concentrations for *Clostridioides difficile*

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Introduction and objectives: *Clostridioides difficile* infection (CDI) is a major nosocomial infectious disease in Germany. First line treatment recommendations for CDI are fidaxomicin, vancomycin, and with certain restrictions, metronidazole. While the empirical treatment of CDI with the first two antibiotics can be considered safe for Germany, given the resistance profiles of *C. difficile* isolates circulating in the German hospital setting, this is not the case for metronidazole, where a resistance rate of ~1% can be seen in an active surveillance study performed in this setting. The first identification of fidaxomicin-resistant clinical isolates in German hospitals indicates that increasing numbers may also be expected for this antibiotic in the future, which increases the demand for routine resistance testing for this bacterial pathogen. However, the resistance testing method recommended by EUCAST for *C. difficile* is agar dilution, a resource-intensive method that requires large amounts of antibiotics, since fresh agar plates supplemented with antibiotics must be prepared for each test, a scenario hardly feasible for routine microbiology laboratories. To address this issue, we investigated here whether the disk diffusion method, which is commonly used in routine microbiological diagnostics, can also be used to prescreen for resistance to these antibiotics.

Methodology: A total of 200 *C. difficile* isolates representing the major ribotypes circulating in the German hospital setting were tested for the above mentioned first line treatment antibiotics and antibiotics thought to serve as drivers for CDI, such as rifampicin, clarithromycin and moxifloxacin using disk diffusion, epsilometry, and agar dilution (fidaxomicin). The minimal inhibitory concentration (MIC) results were compared to the corresponding zone diameter.

Results: For most of the antibiotics tested, a strong inverse correlation was observed between the MIC and the diameter of

the inhibition zone (Spearman correlation coefficient r < -0.65), except for fidaxomicin (r = -0.36).

Conclusions: Our data suggest that the disk diffusion method can be generally considered as a reliable resistance testing method for *C. difficile*, which could be integrated into the routine resistance testing procedures of microbiology laboratories without greater efforts. Whereas the correlation between agar diffusion and epsilometry/agar dilution was high for vancomycin and metronidazole, there was not a clear correlation for fidaxomicin urging the need for further investigations.

PS01.84
Molecular detection of *Pseudomonas aeruginosa* in respiratory secretions from people with cystic fibrosis

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Pseudomonas aeruginosa (PA) is one of the major pathogens that can cause respiratory tract infections in people with cystic fibrosis (pwCF). Chronic lung infection can lead to progressive decline in lung function, an increased hospitalization frequency and a reduced life expectancy. Thus, regular microbiological examinations are a cornerstone of CF respiratory disease management, while culture-based methods are the standard in CF microbiology. Notably, highly effective CFTR modulators improve airway hydration and mucociliary clearance in CF, dramatically reducing sputum production. Consequently, the reliability of culture-based results is challenging, whereas it is assumed, that more sensitive, molecular-based diagnostic methods may overcome this limitation. For this reason, we started a comparative evaluation of published qPCR tests (target genes: *ecfX*/*gyrB*/*oprL*/*regA*) and a commercially available PCR method (Biofire, Pneumonia Panel plus) for the detection of PA in respiratory secretions in pwCF. For qPCR-tests, cut-off values based on the lower limit of detection (LOD) and the matrix effect using artificial sputum medium (ASM) were determined. Moreover, we reexamined the specificity of qPCR using 41 non-target bacterial strains. We collected sputum samples from 68 pwCF who were either chronically infected with PA, intermittently positive for PA (<50% PA positivity of at least six samples collected over 12 months) or free from PA infection (for at least 12 months prior sampling) based on microbiological culture results. In total, 134 samples were analysed using *ecfX*, *gyrB*, *oprL*, and r*egA* qPCR, and 44 samples were tested in parallel with the commercially PCR method. In the culture-negative samples (free of PA infection), PA could not be detected by any qPCR, whereas 91.9% or 89,2% of the culture-positive samples (chronically infected) were positive in at least two PCR tests of a three-gene approach (*oprL*, *ecfX/gyrB* versus *regA*/*ecfX/gyrB*), respectively. In case of pwCF intermittently PA-positive, PA was detected in all culture-positive samples and in 81,6% or 73,5% of culture-negative samples, respectively, indicating that as expected qPCR is more sensitive. Of note, PCR results that are only borderline were excluded (9,7% or 13,4%). Comparison with the commercial PCR method showed complete agreement for culture-positive samples (from chronically and intermittently infected pwCF) and for culture-negative samples (free of PA infection), while only 12,9% of culture-negative samples from intermittently infected pwCF were tested positive. These data suggest that a three gene-qPCR approach is more suitable to verify the PA status in intermittently infected pwCF than the commercially PCR array (developed to detect nosocomial PA pneumonia) or likely even in pwCF that convert to culture negativity during modulator therapy.

PS01.85
Genomic surveillance of carbapenem-resistant *Acinetobacter baumannii* revealed multiple clusters in Germany

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Carbapenem-resistant *Acinetobacter baumannii* (CRAb) is an important healthcare-associated pathogen with high transmission potential and increasing relevance in nosocomial outbreaks worldwide, particularly in vulnerable populations such as burn victims. However, the diversity and distribution of CRAb lineages in Germany remain poorly characterized. Recent national surveillance data reveal an increasing occurrence of carbapenemase-producing *A. baumannii* isolates. This highlights the need for genomic characterization of circulating lineages to track regional, national, and international transmission events, thereby supporting outbreak detection and infection prevention and control strategies.

To investigate the diversity and distribution of genetically related CRAb lineages in Germany, whole-genome sequencing (WGS) was performed on selected isolates collected between 2025 and 2026 from different German federal states. Isolates were selected based on resistance and carbapenemase profiles, as well as epidemiological data, including prior travel or hospitalization abroad. Genetic relatedness was assessed using multilocus sequence typing (MLST) and core genome MLST (cgMLST), complemented by single nucleotide polymorphism (SNP)-based and phylogenetic analyses.

These analyses revealed considerable genetic diversity among CRAb isolates, including multiple genetically distinct clusters and several previously unrecognized smaller clusters. High-risk lineages such as ST2 and ST19 were identified among the investigated isolates and were associated with carbapenemases including OXA-23 and OXA-72. Several isolates showed close genetic relatedness despite geographic separation, indicating possible missed transmission events and/or circulation of related lineages across Germany. Interestingly, some ST19 isolates clustered closely with previously described international isolates. For several isolates, epidemiological information indicated geographical links to Ukraine prior to admission to German hospitals, suggesting possible cross-border dissemination and ongoing circulation of related lineages.

These analyses demonstrate the co-circulation of multiple CRAb lineages in Germany and highlight the utility of WGS-

based genomic surveillance for identifying transmission clusters and improving our understanding of CRAb distribution in Germany and Europe.

PS01.86
Association of specific *Klebsiella pneumoniae* sequence types with the production of two or more carbapenemases in Germany

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Background: The accumulation of more than one carbapenemase gene in carbapenemase-producing *Klebsiella pneumoniae* increasingly limits treatment options and complicates infection prevention measures. However, the distribution of two or more carbapenemases per isolate across different epidemic lineages remains largely unknown.

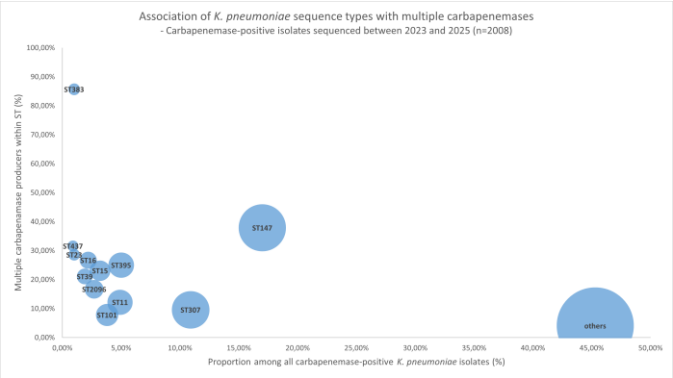
Methods: A nationwide collection of carbapenemase-positive *K. pneumoniae* isolates submitted to the National Reference Centre for multidrug-resistant Gram-negative bacteria between 2023 and 2025 was analysed within the Integrated genomic surveillance (IGS) using Illumina-based whole genome sequencing. Sequence types (STs) and carbapenemase genes were identified *in silico*. To minimize bias caused by clonal expansion, isolates were deduplicated using a stringent cgMLST threshold of ≤7 allelic differences, retaining one representative isolate per cluster, which reduced the number of isolates from 5155 to 2008.

Results: A total of 2008 epidemiologically unrelated carbapenemase-positive *K. pneumoniae* isolates were included. More than one carbapenemase was identified in isolates belonging to several internationally disseminated high-risk lineages; however, marked lineage-specific differences were observed.

ST383 demonstrated a particularly high proportion of isolates carrying two carbapenemases (18/21, 85.7%) despite low overall prevalence. Other common high-risk lineages, including ST11, ST307 and ST147 showed comparatively lower proportions of isolates carrying two or more carbapenemases. ST147 represented the most prevalent lineage among all isolates and showed a proportion of 37,9% isolates carrying several carbapenemases (130/343). Frequently observed carbapenemase combinations included NDM-like together with OXA-48-like enzymes.

Conclusions: Our findings demonstrate lineage-specific differences in the accumulation of more than one carbapenemase among carbapenemase-positive *K. pneumoniae* isolates in Germany. While some globally disseminated high-risk clones were primarily associated with single carbapenemases, lineages such as ST383 and ST147 showed marked enrichment for two or more carbapenemase genes, suggesting differential capacities for the acquisition and maintenance of multidrug resistance determinants.

Fig. 1



PS01.87
Experimental evolution of phage K for enhanced targeting of *S. aureus* USA300 in lung infection models

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Staphylococcus aureus (*S. aureus*) USA300 is a prominent community-associated methicillin-resistant *S. aureus* (CA-MRSA) strain, posing a serious public health concern due to its aggressive pathogenicity and association with necrotizing infections, including pneumonia. Bacteriophages, owing to their high host specificity and potent lytic activity, offer a promising alternative therapeutic strategy.

In this study, the well-studied lytic phage K was adapted through experimental evolution to infect *S. aureus* USA300 at 37 °C. The resulting mutant phage KJ25 was characterized using growth inhibition assays, time-kill kinetics, metabolic profiling, genomic comparisons with the parental phage and transcriptomic analyses of the bacterial response to infection. Phage KJ25 exhibited significantly improved killing of USA300, achieving faster bacterial clearance and sustained suppression of regrowth. Genomic analysis identified a loss-of-function mutation in *gp102* encoding a predicted DNA-binding protein implicated in transcriptional regulation. RNA sequencing revealed that KJ25 infection to USA300 induced a slower and less disruptive host transcriptional takeover than wild-type phage K. Furthermore, the efficacy of KJ25 was validated *in vitro* using A549 lung epithelial cells and *ex vivo* in a murine precision-cut lung slice (PCLS) infection model. In both systems, phage treatment reduced bacterial burden while preserving lung tissue integrity supporting its therapeutic potential.

In conclusion, host adaptation of phage K to USA300 yielded the novel variant KJ25 with enhanced lytic activity against multidrug-resistant *S. aureus* in pulmonary infection models. This work highlights the value of experimental evolution for tailoring therapeutic phages and support phage adaptation as a promising strategy for developing interventions against antibiotic-resistant bacterial infections.

PS01.88
Analysis of staphylococcal enterotoxin variants across public genomic databases
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Introduction: Staphylococcal enterotoxins (SE) are a major global cause of foodborne illness. Their high genetic variability makes precise molecular characterization crucial for tracing outbreaks. Therefore, comparative genomic database analyses are essential to ensure sequence consistency and reliable detection.

Objectives: This study aimed to compare nucleotide and amino acid sequences of classical (SEA, SEB, SEC, SED, SEE) and non-classical (SEG, SEH, SEI) enterotoxins retrieved from public genomic databases. Sequence conservation, variability and phylogenetic relationships were evaluated to assess inter-database consistency and annotation discrepancies.

Materials and Methods: Sequences of SE were obtained from BVBRC and NCBI databases, complemented with the BakCharak pipeline. Data curation included removal of duplicates, ORF length filtering and exclusion of truncated or inconsistent sequences. Coding sequences were translated and aligned to generate database-specific consensus sequences. Phylogenetic reconstruction and BLASTp analyses were performed to evaluate sequence similarity and divergence among SE and databases.

Results: Consensus sequences of SEA, SEB, SEC, SED, SEG, SEH, and SEI were highly conserved across the databases, with amino acid identity ranging from 95.8% (SEG) to 99.6% (SED/SEH). BVBRC assemblies incorporated a larger number of sequences, yielding extended alignments but increased ambiguity at certain positions, consistent with greater sequence heterogeneity. In general, larger input sets (181 sequences for SEI and 128 for SEG from BVBRC) were associated with increased variability while maintaining stable identities (95-97%), whereas smaller collections (3-5 sequences for SEE/SED from NCBI-BAK) produced either very high similarity (99.6% for SED) or reduced robustness when diversity was insufficiently captured (SEE at 61.2%). SEA and SEB exhibited only minor ambiguous sites, and SEC remained highly conserved across the databases. SED and SEH showed highest conservation (>99%), while SEG and SEI displayed moderate variability. By contrast, SEE showed marked divergence between databases. The BVBRC-derived sequence presented substantial ambiguity and lower identity (61.2%) relative to the NCBI-BAK counterpart (98.0%), suggesting either annotation inconsistency or genuine underlying heterogeneity. Phylogenetic reconstruction confirmed clustering primarily by enterotoxin type, with most consensus sequences grouping tightly within their respective clades. Notably, the BVBRC-derived SEE formed a distinct branch, consistent with its reduced similarity and increased variability.

Summary: This study shows that most SEs are highly conserved, but SEE exhibits marked variability due to annotation issues or high genetic diversity. These findings highlight the need for rigorous database selection and sequence curation in genomic studies. Understanding this variation is essential for accurate *S. aureus* detection and control in food safety.

PS01.89
Emergence of *mecC*-MRSA across hedgehogs and humans: A One Health perspective
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Introduction: In 2011, the *mecC* gene, an alternative determinant of beta-lactam resistance, was identified in *Staphylococcus aureus* and published independently from 2 different groups. Subsequent studies suggested a zoonotic origin of *mecC*-MRSA. These strains are strongly associated with European hedgehogs (*Erinaceus europaeus*), where they are thought to have evolved under selective pressure from penicillin-producing dermatophytes (*Trichophyton erinacei*). In Europe, *mecC*-MRSA comprises six distinct clonal lineages (CC49, 130, 425, 599, 1943, 2616). The aim of our study was to investigate the occurrence of *mecC*-MRSA in hedgehogs in and around Jena and to identify human isolates in cooperating hospitals.

Material and Methods: Swabs were obtained from hedgehogs admitted for veterinary care (due to illness or injury) or kept for assisted hibernation. They were collected between 2024 and 2026 in the Jena region. Additionally, some road-killed hedgehogs were sampled. Isolates were screened using DNA microarray based typing and selected isolates underwent Oxford Nanopore sequencing. Human isolates originated from routine diagnostics and screening at the university hospitals in Dresden and Regensburg. Dresden isolates (2000–2020) and Regensburg isolates (2000–2019) were analyzed by DNA microarray, while more recent Regensburg isolates (2020–2024) were subjected to Illumina sequencing.

Results: Among 65 systematically sampled hedgehogs, seven (10.8%) carried *mecC*-MRSA. Additionally, three *mecC*-MRSA-positive hedgehogs were identified from random sampling in preceding years. Five hedgehogs carried CC599-MRSA-XI, four carried CC130-MRSA-XI and one animal harboured both lineages.

Among 1,761 non-duplicate MRSA isolates from Dresden, seven (0.40%) were identified as CC130-MRSA-XI.

Among 3,049 MRSA isolates collected in Regensburg (2010–2019), twelve (0.39%) were *mecC*-positive: seven CC130 (0.23%) and five CC599 (0.16%). In a second sampling period (2020–2024; n = 518), eleven isolates (2.12%) were *mecC*-

positive, including five CC130 (0.96%) and six CC599 (1.16%). Other known *mecC* lineages (CC49, CC425, CC1943, CC2616) were not detected, neither in hedgehogs nor in humans.

Conclusions: Hedgehogs in Thuringia represent a relevant reservoir of *mecC*-MRSA, although the prevalence appears to be lower than reported in some Scandinavian studies. In humans, *mecC*-MRSA remains rare but may be increasing, as suggested by recent data from Regensburg. Continued surveillance is warranted to better understand geographic distribution, transmission dynamics, and potential public health implications.

PS01.90

Presentation of the serum opacity factor of *Streptococcus canis* as multifunctional virulence factor mediating cell attachment under flow

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Introduction: Wound infections with the zoonotic pathobiont *Streptococcus canis* (*S. canis)* are associated with a certain risk of developing severe systemic infections in dogs, cats, and humans. Hereby, infection starts with bacterial adherence to the vascular endothelium thereby inducing inflammation, cell damage and bacterial spreading into deeper tissues. Cell culture infection analyses with a *S. canis* transposon library under defined shear stress identified a protein with strong homology to streptococcal serum opacity factors as noval surface-bound adhesin of *S. canis*.

Objectives: A comparison of sequence homology suggested that the serum opacification factor may possess multifunctional virulence traits. Specific experimental analyses were conducted to confirm the serum opacification activity and to elucidate further functional activities of this protein which are associated with the infection potential.

Materials and Methods: In addition to serum opacification analysis, binding activity to human fibronectin was determined by FACS-analyses. Endothelial and epithelial cell culture infection analyses under defined flow conditions were performed using wild type *S. canis* and an isogenic knock-out mutant. These analyses were expanded to cell separation migration analyses (CSMA) and to differential assessment of bacterial effects on cell migration versus cell proliferation. Moreover, surface exposure was analysed by electron microscopic visualization.

Results: The new adhesion factor of *S. canis* represents an LPxTG-anchored surface protein with serum opacification activity and was therefore named *S. canis* serum opacification factor (ScSOF). It facilitates adhesion to endothelial and epithelial cells even under mechano dynamical simulation of blood flow, prevents endothelial wound closure, contributes to the streptococcal surface architecture, binds fibronectin, and inhibits β-haemolytic activity.

Summary: Our findings identified ScSOF as a surface-bound adhesion that displays multifunctional virulence traits with crucial activities in different infection mechanisms of *S. canis*. Therefore, ScSOF represents an important streptococcal virulence factor which harbours high potential as molecular target for new immune prevention strategies and therapeutical interventions.

PS01.91

Nasal carriage and milk contamination by Staphylococcus in Algerian camels: Species diversity and antimicrobial resistance profiles

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Staphylococci are major pathogens capable of colonizing and infecting humans and various animal hosts. The emergence and spread of antimicrobial resistance (AMR) among several staphylococcal species cause a severe threat to both human and animal health. However, evidence regarding the distribution and AMR profiles of *Staphylococcus* species associated with camel husbandry is limited. This study was conducted to (i) determine the nasal carriage rate by staphylococci in dromedaries; (ii) identify the spectrum of *Staphylococcus* species involved in subclinical mastitis in female dromedaries; and (iii) characterize the AMR patterns of the isolates obtained from both milk and nasal samples.

The study was conducted across eight camel farms including 145 dromedaries located in three distinct regions of southern Algeria: El-Meniaa, Laghouat, and Oued Souf. A total of 74 milk samples from all udder quarters (29 female dromedaries) was screened for subclinical mastitis using the California Mastitis Test (CMT), followed by the collection of 93 milk samples for bacterial isolation. Simultaneously, swabs were collected to evaluate concurrent nasal colonization. Bacterial isolation was performed on blood agar and species identification was performed by MALDI-TOF mass spectrometry. Finally, antimicrobial susceptibilities were determined using the VITEK 2 system, with resistance profiles interpreted according to the EUCAST 2025 guidelines.

The prevalence of subclinical mastitis was 51.7% (15/29) at the animal level and 26.9% (25/93) at the udder quarter level. Staphylococcal species were detected in dromedaries across all farms surveyed, accounting for 33 (35.8%) milk isolates and 11 (37.9%) nasal swab isolates. The most prevalent species in milk samples was *S. simulans* (39%), followed by *S. epidermidis* (18%) and *S. microti* (15%). In contrast, nasal isolates were predominantly identified as *S. simulans* (18%) and *S. delphini* (18%), alongside two *Mammaliicoccus [S.]*

sciuri isolates and one *S. aureus* isolate. Four female dromedaries harboured staphylococcal isolates simultaneously in both milk and nasal samples. Of these, three carried identical species in both sites (*S. simulans*; n=2 and *S. delphini*; n=1), while one animal harboured distinct species (*S. saprophyticus* in milk; *S. delphini* in nasal swab). Antimicrobial susceptibility testing demonstrated low resistance rates to agents most commonly used in human and veterinary medicine. This study highlights a diverse distribution of staphylococcal species in Algerian dromedaries with low resistance profiles. Integrating a One Health approach into surveillance and intervention strategies is essential to mitigate the broader risks of staphylococcal dissemination and AMR emergence. Furthermore, continuous molecular monitoring of camel bacterial isolates is recommended to track potential zoonotic transmission and detect early shifts in AMR.

PS01.92
Screening of bats blood microbiome for novel and reoccurring zoonoses

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Introduction: Caves offer an environment with extreme conditions – lack of light, fewer nutrients, high humidity, low temperature, and others, which implies a high level of adaptation of the organisms. In Europe 47 bat species are encountered and 33 of these are found in Bulgaria. Bats have been identified as an important reservoir hosts for numerous emerging infectious viral, bacterial, fungal and protozoa pathogens.

Objectives: Aim of our study was to monitor bats and environmental samples from Bulgarian caves for novel or reoccurring zoonoses.

Materials and Methods: In 2022-2024 we analysed bats of Vespertilionidae and Rhinolophidae families inhabiting three Bulgarian caves – Parnitsite, Orlova chuka and Devetashka cave. A 30 µl blood sample of 200 bats was collected. Soil, water and guano samples were also collected. DNA was extracted and PCR, 16S and ITS targeted, and shotgun whole-genome sequencing analysis was applied for microbial biodiversity analysis. Taxonomic identification of the cleaned raw reads was performed by Kraken2 tool with Refseq NCBI database for prokaryote, protozoa, fungi and DNA viruses in www.usegalaxy.eu environment. OTU table was generated with Bracken tool and visualized by Pavian software.

Results: A significant number of microbial pathogenic species were identified in the bat"s blood. Sequencing reads exceeding 10K were identified for Mycobacteria, Bartonella, Plasmodium and Leishmania species. Soil, water and guano samples demonstrated high abundancy of mycobacterial presence. Other pathogenic species such as Toxoplasma spp. have also been identified but less evidently according to the number of reads.

Summary: While tuberculosis, bartonelosis and leishmania are rare diseases, malaria is almost absent in Bulgaria. Our results demonstrated that bats in Bulgaria are a hidden or potential reservoir for bartonella and atypical mycobacterial species.

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PS01.93
Integrated poultry production as a reservoir of *mcr-1.1* and *tet(X4)* encoding *Escherichia coli* in Pakistan: Divergent plasmid and transposon dynamics along the farm-to-fork chain

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Question: Plasmid-borne colistin (*mcr-1.1*) and tigecycline (*tet(X4)*) resistance threatens two last-resort antibiotics, yet how these genes move and evolve along the poultry food chain is poorly resolved. We asked whether *mcr-1.1* and *tet(X4)* share dissemination routes from breeder to retail meat, and whether their mobile-element contexts differ.

Methods: From a vertically integrated broiler operation in Punjab, Pakistan (200 samples across breeders, day-old chicks, day-30 broilers and retail meat; n=50/stage; 2023), we recovered 18 *mcr-1.1* and/or *tet(X4)*-positive *E. coli*. Isolates underwent susceptibility, biofilm and CHIC-8E11 intestinal-cell adhesion assays, Illumina sequencing, and Nanopore long-read sequencing of three strains to resolve plasmids. Genomes were placed in a 231-genome core-genome phylogeny incorporating 213 *E. coli* (2011–2024) from Pakistan.

Results: *tet(X4)* occurred in 11/18 (61%) and *mcr-1.1* in 7/18 (39%); one ST398 breeder co-carried both on separate plasmids. Phylogeny resolved two clades: Clade I carried *mcr-1.1* on conserved IncI2 plasmids, Clade II *tet(X4)* on IncFIB(AP001918)-IncFII megaplasmids. Within Clade II, ST1011 spanned breeder→day-30 broiler→retail meat, direct evidence of clonal farm-to-fork transmission. The determinants diverged: *mcr-1.1* showed post-mobilisation stabilisation (complete ISApl1 loss; 93% IncI2 conservation), whereas *tet(X4)* stayed actively mobile in IS26-bounded transposons, co-mobilising floR and fosA4 and driving a significantly higher resistance-gene burden. This gap tracked plasmid size: *tet(X4)* IncF megaplasmids (134–148 kb) dwarfed the streamlined ~60 kb IncI2 vectors. Across 231 genomes (87 STs), *mcr-1.1* reached 80.5% and *tet(X4)* 32.5%. All isolates adhered to intestinal epithelium, strongest among *tet(X4)* carriers. *tet(X4)*- and *mcr-1.1*-positive *E. coli* from wild black kites (*Milvus migrans*) shared STs with poultry (ST-69, -206, -1431), evidencing wildlife spillover. In an ongoing layer survey (three farms, 75 samples, minimal antibiotic use), n =16 and n=15 samples were *mcr-1.1*- and *tet(X4)*-positive respectively, mirroring the broiler pattern despite low selection pressure.

Conclusions: *mcr-1.1* and *tet(X4)* disseminate by distinct mechanisms: endemic conjugation versus active transposition. yet both reach consumer-level retail meat and wildlife. Because *tet(X4)* is carried on mobile elements that continually accrete co-resident resistance genes (*floR*, *fosA4*), poultry are becoming a self-sustaining gene reservoir poised to assemble

a broadly transmissible, near-pan-resistant *E. Coli* in effect a poultry-borne "superbug" able to broadcast resistance to last-resort drugs across bacterial populations. Integrated One Health surveillance spanning production, retail, wildlife and clinical settings is urgently needed.

PS01.94
LukAB promotes *Staphylococcus aureus* escape from macrophages by triggering an unknown cell death pathway

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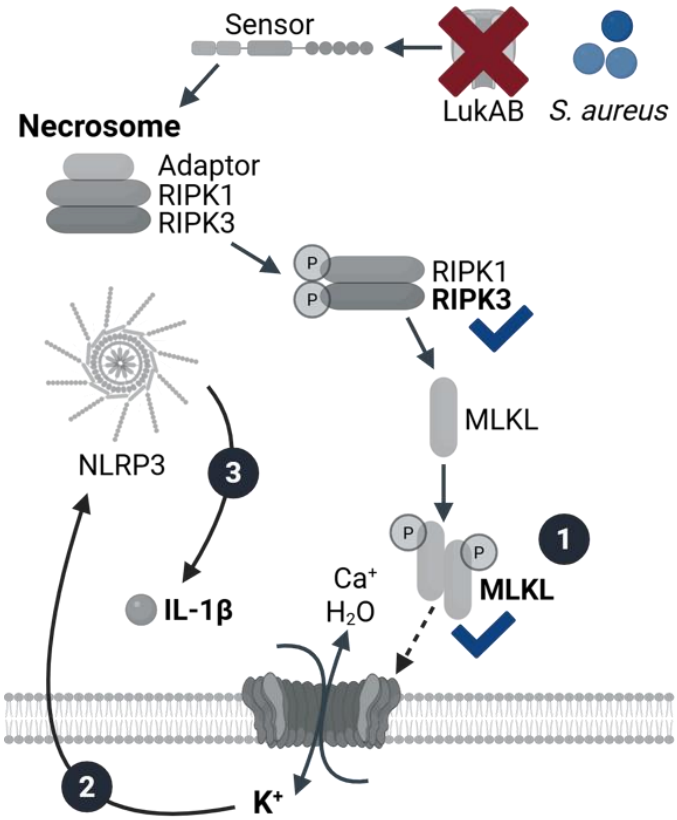
Staphylococcus aureus is a facultative pathogen that causes diverse diseases worldwide, some life-threatening. To evade host immunity, S. aureus expresses virulence factors including the pore-forming Leukocidin A/B (LukAB). Secreted LukAB is cytotoxic for various immune cells, acts via cell surface integrins, CD11b/CD18, and a hydrogen voltage channel, HVCN1, and potently triggers NLRP3 inflammasome activation, IL-1β secretion and pyroptosis. Although LukAB is critical for S. aureus killing of infected phagocytes and consequent bacterial exit, the receptors, signaling and cell death pathways activated by LukAB "from the inside" are unknown. Using genetically modified bacteria and host cells we found that CD11b, CD18 or HVCN1 KO cells were protective against LukAB-expressing S. aureus, implicating these factors in intracellular LukAB killing. Infection also triggered NLRP3 activation and IL-1β release, albeit independently of LukAB or gasdermin D, and was hence non-pyroptotic. Apoptosis was also ruled out but RIPK3 and MLKL phosphorylation were observed 3 h after infection. MLKL KO THP-1 cells were partially resistant to S. aureus cytotoxicity. However, both effects were again observed in LukAB-negative bacteria, showing that LukAB-mediated cell death is also independent of conventional necroptosis. Interestingly, the necroptosis inhibitor, nec-1, and extracellular K+ blocked MLKL phosphorylation and IL-1β secretion, respectively, suggesting that K+ efflux via MLKL pores, despite not mediating LukAB-related cell death, activates the inflammasome. Collectively, S. aureus dodges conventional cell death pathways to enact the demise of the phagocyte via LukAB. A LukAB-producing THP-1 cell line is being established to investigate the cytosolic cell death pathway.

Fig. 1



PS01.95
Development of a stable microbial community for the study of plasmid-mediated antimicrobial resistance in a continuous culturing device

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Question: Antimicrobial resistance (AMR) is a pressing societal problem whose study requires experimental models that are simple yet plausible. While AMR acquisition via mutation is well characterised in laboratory evolution, horizontal gene transfer (HGT) lacks robust experimental systems, largely due to the absence of stable model communities and population bottlenecks and significant labor imposed by batch culture. We asked whether a stable synthetic community could be used to track, in near real-time, how clinically relevant conjugative AMR plasmids spread and reshape community composition under antibiotic pressure.

Methods: We constructed a three-member obligate syntrophic community (TriComm) in an *E. coli* BW25113 background, in which each member carries two amino-acid biosynthesis knockouts (*metA*, *lysA*, and *pheA*) and depends on the others for growth. Two members were fluorescently labelled (GFP and mScarlet) to enable continuous tracking in Chi.Bio bioreactors, cross-validated by plate counting and flow cytometry. We introduced two clinically relevant plasmids into the community: pOXA-48 (conferring penicillin/carbapenem resistance; conjugative variant PVI and non-conjugative variant PVB) and PN23 (colistin resistance via *mcr-1*), and monitored community dynamics with and without the corresponding antibiotic.

Results: TriComm members could not grow in isolation without supplementation, confirming obligate cross-dependency. Grown continuously for up to 500 hours, TriComm showed reproducible oscillatory dynamics, with each member fluctuating around a stable level, establishing a limit of detection near 1% across all three tracking methods. Under antibiotic pressure, plasmid-free communities declined, whereas communities carrying conjugative plasmids (PVI, PN23) survived: selective plating and whole-genome sequencing confirmed plasmid transfer from the LM strain to PL and PM. The non-conjugative variant PVB failed to rescue the community under ampicillin, and PN23 collapsed at 2×MIC colistin, showing that plasmid spread to all members is required for survival. In the absence of selection, plasmid-carrying colonies declined log-linearly, reflecting fitness cost. A rare colistin-resistant plasmid-free replicate acquired resistance by chromosomal mutation. Lastly, we could recapitulate these community and plasmid dynamics using a mathematical model.

Conclusions: TriComm provides a stable, continuously monitored model community for dissecting plasmid-mediated AMR dynamics. Our data underscore the predominant role of HGT over spontaneous mutation in community survival and demonstrate how conjugation, plasmid fitness cost and selective pressure jointly determine resistance spread.

PS01.96

When genomic similarity does not imply transmission: Limitations of molecular typing in a homogeneous VREfm population

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Question: Core genome multilocus sequence typing (cgMLST) is widely used to identify clusters and transmission events of vancomycin-resistant Enterococcus faecium (VREfm). However, the discriminatory value of genomic similarity depends strongly on the underlying population structure. If stable, closely related genotypes are already widespread within the background population, low allelic distances may occur frequently even in the absence of recent transmission. We therefore investigated whether the homogeneous population structure of hospital-associated VREfm limits cgMLST-based transmission inference and whether clustering based predominantly on fixed allelic-distance thresholds may overestimate the true extent of transmission.

Methods: We analysed routinely generated cgMLST profiles of 7,130 multidrug-resistant bacterial isolates collected from inpatients between 2016 and 2023, comprising 2,787 MRSA, 1,719 MDR *E. coli*, and 2,624 VREfm isolates. First, the species-specific distributions of all pairwise cgMLST distances were compared to characterise the underlying genomic population structure and determine how frequently close genomic relatedness occurred within each population. The cgMLST distances were then linked to patients" time-resolved locations to assess whether close genomic relatedness was enriched among epidemiologically linked patients and therefore informative for recent transmission. The resulting genomic–epidemiological patterns were compared across all three species.

Results: The species-specific cgMLST distance distributions differed markedly. MRSA and MDR *E. coli* showed highly diverse background populations, with 80% and 75% of isolate pairs differing by more than 1,340 and 1,900 alleles, respectively. In contrast, 99.6% of all VREfm isolate pairs differed by fewer than 500 alleles, demonstrating a far more genomically homogeneous population and consequently reduced discriminatory contrast between potentially transmission-linked and unrelated isolates. Consistent with this population structure, VREfm comprised 4,074 genetically identical isolate pairs, yet only 38.9% (1,583/4,074) could be epidemiologically linked under direct-contact assumptions.

Conclusions: In a homogeneous VREfm population, genomic similarity alone may be insufficient to distinguish recent transmission from background relatedness. Even genetically identical cgMLST profiles should therefore not automatically be equated with recent transmission. Particularly when investigating outbreaks or defining transmission clusters, care must be taken to avoid creating apparent clusters that arise primarily from background genomic similarity. Our findings highlight the need to consider population structure and to integrate detailed spatiotemporal epidemiology and, where appropriate, additional genomic or contextual information before inferring VREfm transmission.

PS01.97

Zetoro: An interactive web application for data-driven derivation of pathogen-specific cgMLST thresholds

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Question: Core genome multilocus sequence typing (cgMLST) is increasingly used for hospital transmission surveillance, yet thresholds for defining genomic relatedness are often adopted from previous studies or outbreak settings and may not be transferable across pathogens, institutions, or underlying population structures. We therefore developed a data-driven framework to derive pathogen-specific cgMLST thresholds from routinely generated genomic and epidemiological data and implemented it in Zetoro, an interactive browser-based web application.

Methods: The framework integrates cgMLST profiles with time-resolved patient-location data. Pairwise cgMLST distances are related to epidemiological proximity under a parameterised spatiotemporal contact model, and pair-connectivity is calculated as the proportion of epidemiologically linked isolate pairs at each genomic distance. Thresholds are inferred by comparing connectivity at low genomic distances with species-specific background connectivity using two complementary baseline-referenced criteria. We evaluated the framework using routine data collected from 2016 to 2023 at a 1,500-bed tertiary-care hospital, comprising 7,130 isolates: 2,787 methicillin-resistant *Staphylococcus aureus* (MRSA), 1,719 multidrug-resistant *Escherichia coli* (MDR *E. coli*), and 2,624 vancomycin-resistant *Enterococcus faecium* (VREfm).

Results: Under the default parameterisation, MRSA and MDR *E. coli* yielded stable and concordant thresholds of 8 and 3 allelic differences, respectively. In contrast, VREfm showed elevated background connectivity, a compressed cgMLST distance distribution, and less stable threshold inference, illustrating how strongly threshold reliability depends on pathogen population structure. The complete workflow is available in Zetoro, allowing users to upload their own cgMLST and patient-location data, adjust epidemiological parameters, visualise distance distributions and connectivity curves, and interactively explore resulting threshold estimates.

Conclusions: Combining genomic similarity with explicit epidemiological proximity enables transparent, data-driven, and context-specific derivation of cgMLST thresholds for hospital transmission surveillance. In principle, Zetoro can be applied to any pathogen for which sufficient cgMLST profiles and time-resolved epidemiological data are available. By making epidemiological assumptions, population structure, connectivity patterns, and threshold estimates directly explorable, Zetoro provides an accessible framework for investigating how genomic and epidemiological characteristics influence transmission inference.

PS01.98
Disinfectants, cleaners, or biocidal active substances: What inactivates microorganisms in biofilms?

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Introduction: Biofilms in water-bearing systems are a critical reservoir for pathogenic and antibiotic-resistant bacteria. They not only protect embedded microorganisms from chemical and physical stress but also frequently serve as sites for the exchange of antibiotic resistances. Consequently, outbreaks originating from biofilms, for example in drains or toilets, are particularly difficult to control. Although biofilm formation in these systems cannot currently be prevented, the microorganisms residing within biofilms can be inactivated. During outbreak situations, the replacement of contaminated pipework often remains the only option if effective chemical interventions are unavailable. In addition, any chemical solution intended for frequent use and discharge into wastewater systems should be environmentally compatible.

Objectives: The aim of this study was to evaluate the efficacy of disinfectants, cleaners, and biocidal active substances against *Pseudomonas aeruginosa* embedded in biofilms. Furthermore, the environmental compatibility of the tested formulations was assessed to identify effective and sustainable approaches for biofilm control.

Methods: The inactivation of *P. aeruginosa* in biofilms was investigated using the *Bead-Assay* published by the RKI. Biofilms were cultivated on glass beads under constant shaking and subsequently exposed to the respective test solutions. After treatment, the biofilms were detached from the glass beads using an ultrasonic bath. Subsequently, the number of viable cells was determined to assess the antimicrobial efficacy of the tested substances. In addition, the ingredients of the test solutions were evaluated with regard to their environmental impact.

Results: Among the tested cleaners, only one showed an effect on the survival of *P. aeruginosa* at the highest tested concentration, irrespective of whether "active chlorine" was present as an active ingredient. For disinfectants and compounds, particularly high efficacy was observed for those based on reactive oxygen species, as well as for the quaternary ammonium compound benzalkonium chloride.

Summary: Effective measures for the inactivation of bacteria within biofilms are urgently needed to protect patients and to eliminate reservoirs of antimicrobial resistance. Existing disinfectants and compounds based on reactive oxygen species appear to be the most promising options, provided that their application does not generate environmentally harmful by-products. Although benzalkonium chloride showed high efficacy, its use for this purpose should be discouraged because of its contribution to resistance development and its negative impact on wastewater treatment systems.

PS01.99
Metagenomic analysis of gut microbiome composition dynamics on species and strain level in humans: Implications for microbiome therapies

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The gut microbiome confers colonization resistance against antibiotic resistant bacterial pathogens (ARBPs). Infections by ARBPs requires antibiotic treatment accompanied by increased selection pressure promoting spread of antimicrobial resistances. Novel microbiome-based strategies are needed to enhance resilience against ARBPs, including strain displacement by microbiome intervention therapies.

Host-intrinsic and extrinsic factors can influence the dynamic gut microbiome composition associated with profound changes leading to dysbiosis with the potential to contribute to exacerbation of non-communicable diseases (NCD) such as inflammatory bowel diseases or irritable bowel syndrome in which the microbiome is considered disturbed with a quantitative or qualitative imbalance in microbial composition (dysbiosis). Dysbiosis in NCD lack reference standards highlighting the need for investigations concerning dynamics of the gut microbiome composition and microbiome intervention studies. To this end, investigation of the gut microbiome on species and strain level is needed since disease development is influenced by specific strains and their traits rather than by a genus or species.

To investigate species- and strain-level dynamics of microbiomes, we used a Swiss traveler cohort in which fecal samples were collected before travel and for up to one year thereafter. Samples were analyzed by metagenomic shotgun sequencing (MSGS; 50-100 Gb/sample) to resolve microbiome composition across taxonomic levels up to species and strain level.

Focusing on *Escherichia coli* (*E. coli*), MSGS data were compared with whole-genome sequencing (WGS) data from antibiotic-resistant and susceptible *E. coli* isolates.

Sequencing at 50 and 100 Gb yielded sufficient numbers of *E. coli* reads correlating with CFU measurements. Bioinformatic analyses enabled partial reconstruction of *E. coli* metagenome-assembled genomes (MAGs), corresponding to the WGS of corresponding *E. coli* sequence types (ST).

Together, MSGS combined with MAG reconstruction provided insights into *E. coli* displacement dynamics in the gut microbiome and established a ground truth for extending this approach to other species across different phyla. Our approach and pipeline may provide the basis for future disease-specific microbiome interventions.

PS01.100
Influence of hand rub volume on hand coverage, spillage and user preference

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Introduction: Hand hygiene is the most important measure for the prevention of healthcare-associated infections. However, there is a gap between how it is performed vs. how it should be performed. Current standards for testing the efficacy of an alcohol-based hand rub (ABHR) are performed mostly with a volume of 3 mL. Nevertheless, the volume used in daily practice is often below the recommendations and some ABHRs require larger volumes (> 3 mL) to pass the required standards.

Objectives: An initial study evaluated factors such as coverage, spillage, drying times, and satisfaction with the handling of ABHRs in different formats – liquid, gel and foam - when rubbing according to manufacturers' instructions. In a next step, the influence of volume on hand coverage was investigated.

Methods: For all analyses, ABHRs mixed with a fluorescent dye (Visirub®) were used.

Evaluation of the different ABHR formats was performed with 3 different ABHRs containing 85% (w/w) ethanol. Participants were asked to rub their hands on 3 different days using the respective ABHR formats and the volume specified by the manufacturer. Rubbing was performed above an A3 sheet of paper to detect spillage and the time was measured until the volunteers felt their hands were dry.

To investigate the influence of ABHR volume on hand coverage, volunteers were asked to rub their hands with a propanol-based hand rub (75% w/w), using a specified volume ranging from 0.5 to 3 mL on 6 days (e.g. 3 mL day 1, 1.5 mL day 2, 0.5 mL day 3).

In both studies, the volunteers' experience of hand rubbing ranged from novice to experienced.Hand coverage was measured using a Semmelweis Scanner and volunteers were asked to rate their satisfaction with the product (scale of 1-5, 5=very satisfying).

Results: Regardless of the format, good coverage was achieved with all three formats and the liquid rub dried significantly faster than the foam or gel. The foam rub spilled significantly less than the others. Volunteers rated the foam and liquid as easier to use than the gel.

Volumes of 1 mL were sufficient to cover 95% of the hands within approximately 20 seconds. Volumes below 2 mL were rated worse than higher volumes with 2 mL resulting in the best evaluations.

There was a moderately strong but significant correlation between hand coverage and hand rub volume or rub-in time, respectively.

Conclusion: The results of the study provide more insights into the requirements for an effective hand disinfection to bridge the gap between theory and daily practice. The volumes that yielded in sufficient hand coverage are to be further analysed regarding their antimicrobial efficacy by additionally probing the hands before and after disinfection.

PS01.101
Macrophage renewal in the small intestine governs early-life control of streptococci

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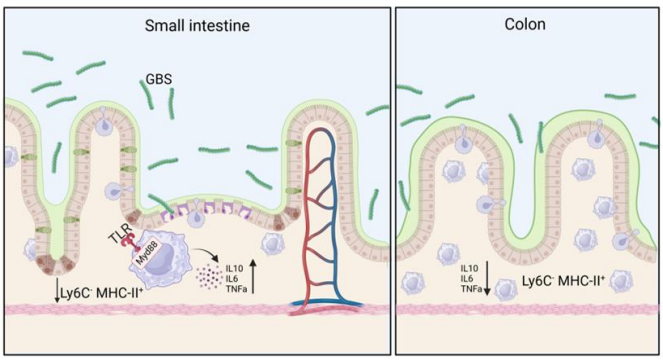
Group B Streptococcus (GBS) is both a common intestinal colonizer that is transmitted intergenerationally, and a primary cause of neonatal sepsis worldwide. However, the innate immune mechanisms that restrict the pathogen at the intestinal barrier early in life remain poorly understood. Using an enteral GBS-colonization model in infant mice, we found that lamina-propria (LP) macrophages controlled both GBS-colonization densities and invasion in an age-dependent fashion. LP macrophages turnover and differentiation were strongly impacted by topology. In the small intestine, monocytes infiltration of the LP occurred from birth on in response to perinatally acquired microbiota, whereas in the colon it was driven by the weaning reaction. Moreover, macrophages of the small intestine mounted a robust, MyD88-dependent inflammatory response to GBS, while those of the colon remained largely unaffected. Together, these findings demonstrate that region-specific macrophage dynamics early in life critically influence host–pathogen-interactions during GBS-colonization.

Fig. 1



PS01.102

Cross-species infection by two closely related *Acinetobacter* bacteriophages reveals candidate determinants of host-range specificity

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Question: *Acinetobacter pittii*, a member of the *Acinetobacter calcoaceticus–baumannii* complex, is increasingly recognized as an opportunistic hospital pathogen but remains underdiagnosed because routine diagnostics frequently misidentify it as *A. baumannii*. The emergence of multidrug-resistant (MDR) *A. pittii* further limits treatment options, highlighting the need for alternative therapeutic strategies. Bacteriophages represent promising antibacterial agents, yet phages infecting *A. pittii* remain poorly characterized. This study aimed to isolate and characterize lytic *A. pittii* phages and investigate genomic features associated with differences in host range.

Methods: Clinical *Acinetobacter* isolates were differentiated using the *gyrB* multiplex PCR described by Higgins *et al.* Species identification of non-*A. baumannii* isolates was confirmed by 16S rRNA gene sequencing. Antimicrobial susceptibility testing and whole-genome sequencing were performed for *A. pittii* isolates. Environmental wastewater samples were screened for lytic bacteriophages. Phages were characterized by host-range analysis, efficiency of plating (EOP), transmission electron microscopy, whole-genome sequencing, genome annotation and comparative genomic analysis.

Results: Among 53 clinical isolates initially identified as *A. baumannii*, five (9.4%) were reclassified as non-*baumannii* species, including three *A. pittii*. All *A. pittii* isolates exhibited multidrug resistance, and one isolate carried a plasmid harbouring two copies of $bla_{\text{OXA-72}}$. Two closely related myovirus-like phages were isolated from environmental samples. Despite an average nucleotide identity of 83% and broadly conserved genome architecture, the phages differed in host range: phage E infected both *A. pittii* and *A. baumannii*, whereas phage F remained restricted to *A. pittii*. Both genomes lacked integrases, antimicrobial resistance genes and virulence factors, supporting a lytic lifestyle. Comparative genomic analysis identified divergence in receptor- and adsorption-associated structural proteins, including tail fibers, collar fibers and neck whiskers, as candidate determinants of host-range differences.

Conclusions: Our findings highlight *A. pittii* as an underrecognized MDR pathogen and identify two genomically related lytic phages with distinct host ranges. Comparative analysis of these phages provides a valuable model for investigating molecular determinants of phage host specificity and supports the further development of phage-based therapeutic approaches against emerging *Acinetobacter* pathogens.

PS01.103

Combining the MeMed BV® with microbiological diagnostics – A prospective observational study to enhance antimicrobial stewardship in children

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Reliable differentiation between bacterial and viral etiology remains a key challenge in pediatric care, often leading to inappropriate use of antibiotics.

This prospective, non-interventional study evaluates the clinical utility of the MeMed BV® (MMBV), a test quantifying TNF-related apoptosis-inducing ligand, interferon-γ-induced protein 10 and CRP, and calculating an algorithm based score indicating the likelihood of a viral or other non-bacterial etiology (0 to 35) versus bacterial (co-) infection (66 to 100); 36 to 65 indicating an equivocal finding.

Children aged ≥ five months presenting to the pediatric emergency department at the University Hospital of Bonn with suspected respiratory tract infection or fever without source were recruited between September 2025 and July 2026. Patients for whom the score was requested formed the test group, those without formed the control group.The primary endpoint was the number of antibiotic treatments initiated per group.

Of 201 children, 106 received the MMBV (44.3% female) and 95 formed the control group (33.7% female; $p = 0.122$). The median age was 41.5 months in the test group (interquartile range, IQR, 23.2–74.8) and 30 months in the control group (IQR 14–60; $p = 0.018$). Diagnoses included respiratory tract infections (test group: 54/106; 50.9% / control group: 53/95; 55.8%), unspecified viral infections (21/106; 19.8% / 22/95; 23.2%), fever without source (12/106; 11.3% / 4/95; 6.8%) and urinary tract infections (4/106; 3.8% / 5/95; 5.3%) with no difference between the groups ($p = 0.376$). In the test group, less children were treated with antibiotics (47/106; 44.3%) compared to the control group (54/95; 56.8%; $p = 0.103$); the median duration of treatment was similar (test group: 7 days; IQR 5–8 / control group 7.5 days; IQR 5–9.75; $p = 0.394$).

The median duration of antibiotic treatment was longer in children with a bacterial MMBV (8 days; IQR 7–10) compared to those with a viral MMBV (6 days; IQR 5–7.5; $p = 0.011$). The initiation of antibiotics differed significantly according to the MMBV ($p < 0.001$): 20 of 23 children with a bacterial MMBV were treated with antibiotics, compared to 15 of 55 children with a viral MMBV. 33 children in the test group received positive microbiological or viral tests (Figure 1). The median MMBV within the positive viral PCR group (16/106) was 11 (IQR 4 – 40.5), and 1.5 (IQR 0 – 37.5) within the positive viral rapid test group (11/106). In 3 children with positive urine culture, the median MMBV was 98 (IQR 53.5 – 98.5). Of note, MMBV yielded bacterial scores in 2/17 patients in positive viral rapid testing. In an exploratory analysis of repeat measurements in 14 children, the majority of scores

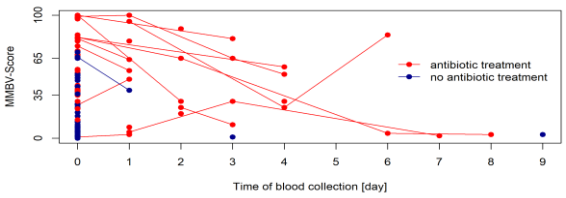
decreased, shifting from a high likelihood of a bacterial towards a viral infection (Figure 2).

Fewer antibiotics were prescribed when the score indicated a viral infection, indicating the potential use of MMBV, which seems to meaningfully complement existing microbiological tests.

Fig. 1

detected	Total (n)	no MMBV obtained (n)	viral MMBV (n, %)	equivocal MMBV (n, %)	bacterial MMBV (n, %)
Blood Culture					
Candida albicans	1	1	*na*	*na*	*na*
Enterococcus faecalis	1	1	*na*	*na*	*na*
Dermacoccus nishinomiyaensis	1	1	*na*	*na*	*na*
Microbacterium spp.	1	1	*na*	*na*	*na*
Staphylococcus haemolyticus	1	1	*na*	*na*	*na*
Streptococcus pneumoniae	3	3	*na*	*na*	*na*
Bacterial Rapid Test					
Group A Streptococcus	4	3	*na*	*na*	1 (100)
Urine Culture					
E. coli	4	2	*na*	*na*	2 (100)
Proteus mirabilis	1	1	*na*	*na*	*na*
Serratia marsescens	1	1	*na*	*na*	*na*
Stenotrophomonas maltophilia	1	*na*	1 (100)	*na*	*na*
Viral PCR					
Adeno	5	4	*na*	1 (100)	*na*
Boca	4	2	2 (100)	*na*	*na*
EBV	5	4	1 (100)	*na*	*na*
Entero/Rhino (no differentiation)	4	3	1 (100)	*na*	*na*
Entero	3	2	1 (100)	*na*	*na*
hCoV	2	*na*	1 (50)	*na*	1 (50)
hMPV	4	3	1 (100)	*na*	*na*
Parainfluenza	2	2	*na*	*na*	*na*
Rhino	9	4	4 (80)	1 (20)	*na*
RSV	1	1	*na*	*na*	*na*
Viral Rapid Test					
Influenza A	26	18	6 (75)	1 (12,5)	1 (12,5)
Influenza B	4	2	1 (50)	*na*	1 (50)
hCoV	2	*na*	2 (100)	*na*	*na*
RSV	13	8	4 (80)	1 (20)	*na*

Fig. 2



PS01.104
Contamination of injection valves in peripheral intravenous catheters

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Background: Bloodstream infections associated with peripheral venous catheters (PVC) are considered rare in relation to the number of days of use. The problem becomes clinically relevant due to the frequent use of PVCs. (1) Integrated injection hubs can serve as entry points for pathogens. (2) Residues of propofol used for induction of anaesthesia could promote bacterial growth. (3)

(Fig.1) PVC with integrated injection hub

Research Question: How often are structurally integrated injection hubs of PVCs, which were inserted for anaesthesia management, bacterially contaminated after 24 hours under routine clinical conditions, and which microorganisms are detected?

Methodology: In a prospective quality assurance project, a total of 146 PVKs were examined. For 100 PVCs, swabs were collected from the injection valves at three time points: immediately after placement (before medication administration), at the end of the operation (end of suturing), and after 24 hours. For 46 PVKs, no 24-hour swabs were available. The samples were analysed at the Landesuntersuchungsamt Rheinland-Pfalz with the request for "culture and identification." The culture media used were ready-to-use plates containing Columbia agar with sheep blood and casein-soy-peptone broth, which enable the cultivation of fastidious (gram- positive and gram-negative) bacteria, fungi, and microbial biofilms.

Results: No microbial growth was observed in the "before" samples. Three injection valves tested positive at the "end of sewing" time point; however, none of these tested positive after 24 hours, suggesting that contamination most likely occurred during sample collection. After 24 hours, bacteria were detected in two cases: Streptococcus salivarius and Staphylococcus xylosus. The contamination rate after 24 hours was thus 2%. The Clopper-Pearson confidence interval (95%) was calculated with a lower limit of 0.24% and an upper limit of 7.05%.

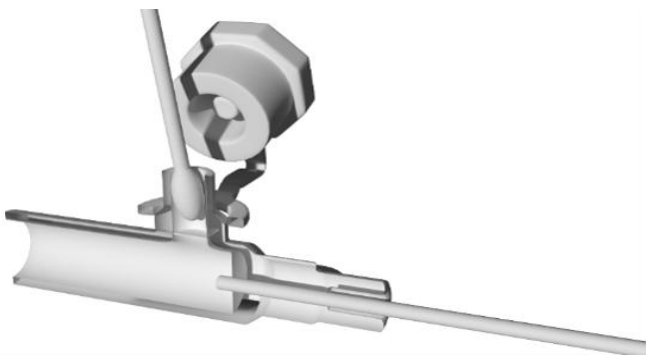
Discussion: Under routine conditions, low bacterial contamination of PVK integrated injection hubs was observed after 24 hours. Studies addressing a similar issue reported significantly higher rates with longer times in use. (1)(2) The results suggest that there is no high risk of infection during anaesthesia, when the hubs are handled properly.

1. Mermel LA. Short-term Peripheral Venous Catheter-Related Bloodstream Infections: A Systematic Review. Clin Infect Dis. 2017;65(10):1757-62.

2. Juhlin D, Hammarskjöld F, Mernelius S, Taxbro K, Berg S. Microbiological colonization of peripheral venous catheters: a prospective observational study in a Swedish county hospital. Infection Prevention in Practice. 2021;3(3):100152.

3. Tessler M, Dascal A, Gioseffini S, Miller M, Mendelson J. Growth curves of Staphylococcus aureus, Candida albicans, and Moraxella osloensis in propofol and other media. Can J Anaesth. 1992;39(5 Pt 1):509-11.

Fig. 1



PS01.106
Distinct epithelial entry and translocation mechanisms of *Salmonella* Typhimurium and Paratyphi A in human intestinal models

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Salmonella enterica serovars Typhimurium (STm) and Paratyphi A (SPA) cause predominantly gastrointestinal and systemic infections, respectively. However, the cellular mechanisms underlying their distinct interactions with the human intestinal epithelium remain poorly understood. Here, we used primary human intestinal epithelial monolayers and three-dimensional (3D) colonoids to compare the invasion and translocation strategies of STm and SPA.

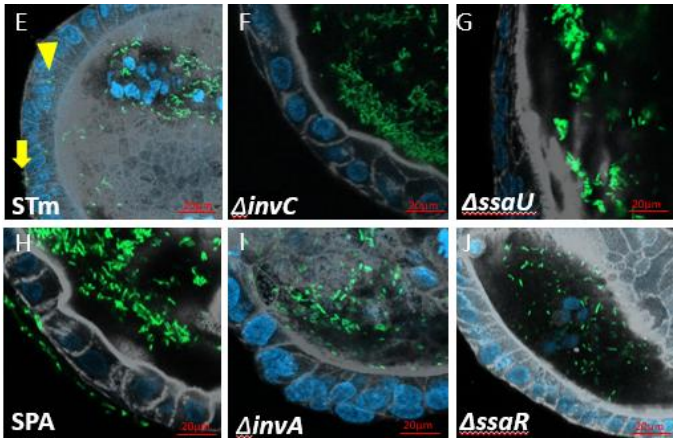
In polarized epithelial monolayers, SPA displayed significantly higher invasion and translocation rates than STm. Deletion of either SPI-1- or SPI-2-encoded type III secretion systems (T3SSs) markedly impaired translocation of both serovars. These findings were further supported by experiments usingin differentiated human colonoids, where luminal microinjection demonstrated of bacteria resulted in translocation of wild-type bacteria across the epithelium, whereas T3SS-deficient mutants showed strongly reduced translocation.

Confocal and scanning electron microscopy revealed distinct spatial interactions with the intestinal epithelium. STm was broadly distributed across epithelial surfaces and mucus, whereas SPA preferentially associated with MUC2-positive cells and mucus-rich regions, indicating serovar-specific epithelial tropism.

To investigate the cellular pathways involved in bacterial translocation, we applied pharmacological inhibitors of host endocytic mechanisms. Dynasore, an inhibitor of dynamin-dependent endocytosis, significantly reduced STm translocation without affecting SPA. In contrast, inhibiton of the Ras-Arf6 pathway by rRasarfin selectively impaired SPA translocation while having no significant effect on STm.

Together, our findings demonstrate that STm and SPA employ distinct strategies to interact with and cross the human intestinal epithelium. The data are consistent with a dynamin-dependent pathway for STm and a Ras–ARF6-associated, dynamin-independent pathway for SPA. These serovar-specific mechanisms may contribute to their distinct infection phenotypes and provide potential targets for intervention.

Fig. 1



PS01.107
Host-pathogen interactions of *Burkholderia thailandensis* E264: Functional analysis of the T3SS Effector BipC and its host cell interactome

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Introduction: Human pathogenic bacteria within the *Burkholderia pseudomallei* group are partly responsible for causing infectious diseases such as melioidosis. These pathogens are characterized by multi-drug resistance and a diverse virulence mechanism, complicating effective treatment. A key factor in their pathogenicity is their intracellular lifecycle, involving host cell invasion and phagosomal escape into the cytosol. Cytosolic bacteria evade the host's LC3-associated autophagy, enabling survival and replication. The bacteria utilize actin-based motility to invade adjacent cells via membrane protrusions and membrane fusion, allowing the bacteria to infect neighboring cells with minimal immune response. The Bsa Type III secretion system, including *Burkholderia* invasion proteins (Bip), is a major virulence factor. The translocator BipC is homologous to Salmonella SipC and Shigella IpaC; it likely forms a translocon pore within the host cell membrane and may act as a translocated effector, probably promoting intracellular survival through uncharacterised interactions with the host cell membrane.

Methods: Bioinformatics analyses were performed to predict protein structure. *In vitro* binding assays with recombinant protein identified new interaction partners from the host cell. CRISPRi-mediated gene knockdown strains of the model organism *B. thailandensis* were used in macrophage infection assays, supplemented by proteomics analyses.

Results: Bioinformatics analysis revealed that BipC and its homologs have only ~40% sequence similarity and limited structural resemblance, with no clearly defined conserved motifs or transmembrane regions. In vitro pulldown assays and

affinity purifications from primary human macrophage lysates showed that BipC interacts with F-actin and other unidentified binding partners. The interactome of BipC reveals a complex host protein network containing endocytosis proteins and membrane-associated host proteins. However, BipC also demonstrated a strong affinity for negatively charged lipids such as phosphorylated membrane phosphoinositides (PIPs). Macrophage infection with B. thailandensis bipC knockdown strains had no significant impact on bacterial invasion, but significantly impaired the bacterium's ability to escape from the phagosome.

Conclusion: These findings indicate that BipC plays a crucial role in phagosome escape and likely interacts within a local microdomain at different host cell membrane systems, potentially involving host membrane contact sites. Although the BipC sequence and structure show limited similarity to its homologs, its interactions imply a different function, possibly modulating host phagocytosis pathways, in contrast to its homologs.

PS02.01
Personalized bacteriophage therapy – A consensus-based guideline

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Bacteriophages (phages) – viruses that selectively infect bacteria – are a promising option for personalized therapy of difficult -to-treat bacterial infections. Clinical implementation in many countries worldwide, however, faces multiple hurdles, including a lack of consensus on general principles for phage therapy, infrastructural requirements, procedures for quality-assured phage selection and preparation, clinical administration, monitoring, and documentation. Existing guidance provides limited practical direction across the entire translational pathway and lacks inspection-ready specifications to support both pharmacies and clinical sites. These gaps impede safe and transparent clinical use and effective regulatory oversight. Likewise, there are no established processes to identify research questions that will be key to advancing clinical phage research in the future. To address these needs, this consensus-based guideline was developed within the methodological framework of the Association of the Scientific Medical Societies of Germany. It was created through a collaborative effort involving 20 professional societies, 18 international experts, patient advocacy groups, and regulatory authorities. The guideline provides over 60 recommendations on core principles, infrastructure, preparation and quality control, administration, and future research. Recommendations have been endorsed by leading international societies and stakeholders. By providing clear and practice-oriented recommendations, this consensus statement paves the way for the safe and standardized use of personalized phage therapy.

PS02.02
A laboratory chair as the source of persistent *Candida parapsilosis* contamination in a clinical microbiology laboratory

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Introduction: The *Candida parapsilosis* complex is an opportunistic fungal pathogen, commonly associated with healthcare-associated transmission via contaminated hands and environmental surfaces due to its biofilm-forming capacity. In 2025, an increased rate of *C. parapsilosis* contamination in clinical specimens was observed in our microbiology laboratory, prompting an extensive investigation of a suspected laboratory contamination source. While environmental persistence in healthcare settings is well described, persistent contamination linked to specific laboratory workflow surfaces has rarely been reported.

Methods: Contamination was defined as either (i) a single detection per specimen with no growth on primary culture but growth detected after enrichment, and negative repeat culture from a retained specimen, if available; or (ii) a single colony-forming unit detected on primary culture with a negative secondary smear after enrichment. A detailed investigation was conducted over a period of four weeks and was expanded stepwise according to interim findings. Initial investigations included swab samples and sedimentation plates from biosafety cabinets, as well as hand swabs collected before and after specimen processing. Subsequently, specimen processing workflows were systematically observed to identify potential sources of contamination, followed by targeted sampling of disposable gloves, and high-touch surfaces, including centrifuges, equipment switches, and laboratory chair handles.

Results: Between May 1, 2025, and May 1, 2026, *C. parapsilosis* was detected in 114 clinical specimens, of which 42 (37%) were classified as contamination. Contaminated specimens included swabs (n=12), foreign body material (n=4), tissue samples (n=18), and aspirates (n=8). Biosafety cabinets showed no growth of *C. parapsilosis. C. parapsilosis* was detected on hand swabs before and after specimen processing and on the uppermost glove in the box, suggesting contamination of the glove box opening during glove removal. Initial intervention via reinforced hand hygiene and glove use training did not reduce contamination rates. Workflow-guided environmental sampling identified only the height-adjustment lever of a laboratory chair as positive for *C. parapsilosis*, alongside other skin commensals on surrounding surfaces. After inclusion of chair disinfection in routine cleaning, contamination rates decreased.

Conclusion: The height-adjustment lever of a laboratory chair was identified as the source of persistent *C. parapsilosis* contamination. As *C. parapsilosis* is an environmentally persistent biofilm-forming organism and not considered a typical skin commensal, it may represent a relevant source of laboratory contamination. Such events may delay diagnosis and reporting, increase workload, and generate additional costs. Thorough assessment of all workflow-associated surfaces is essential in investigating persistent laboratory contamination.

PS02.03

Sepsis due to two GES-27/GES-5-type carbapenemase producing *Pseudomonas aeruginosa* in a patient with chronic necrotizing pancreatitis and multiple liver abscesses

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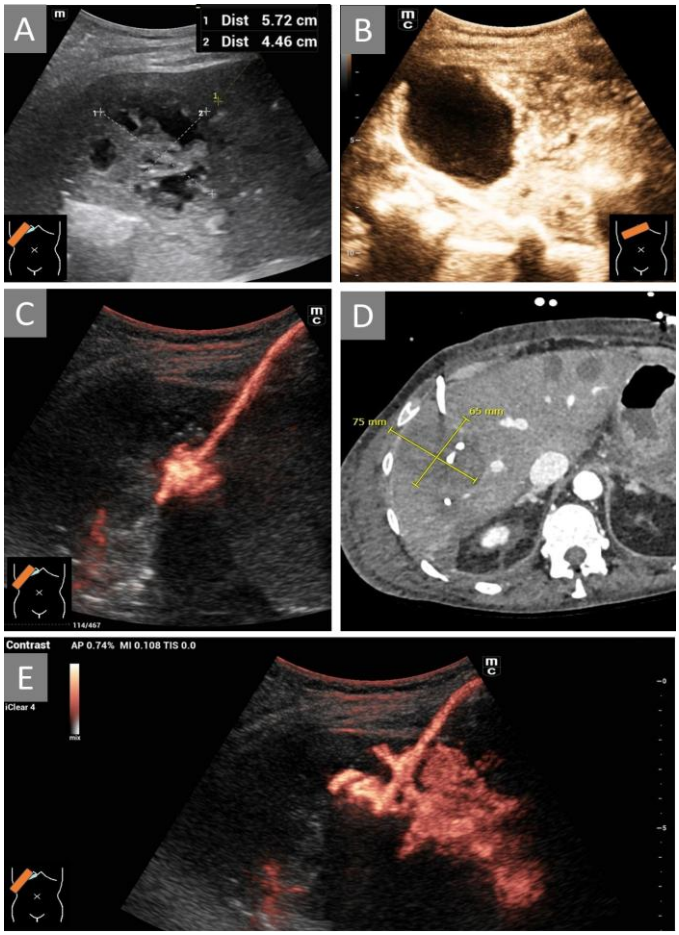
Introduction: *Pseudomonas aeruginosa* is a gram-negative rod known for its ability to develop multiple antimicrobial resistance pathways, among which carbapenemase production results in reduced treatment options.

Method: We report the isolation of two different Guiana extended spectrum (GES)-carbapenemase-producing *Pseudomonas aeruginosa*, GES-5 and GES-27, from a 63-year-old woman treated for alcohol-induced necrotizing pancreatitis complicated by multiple liver abscesses. *P. aeruginosa* strains, cultured on blood agar plates for 24h at 37°C and 5% CO2, were isolated from urine. liver abscess and blood Identification was done using MALDI-TOF mass spectrometry (VITEK MS®, bioMérieux, France); routine antimicrobial susceptibility testing was performed with an automated testing system (VITEK 2 XL®, bioMérieux, France). Testing of last resort antibiotics Cefiderocol, Ceftazidime/Avibactam, Ceftolozan/Tazobactam, and Imipenem/Relebactam was done using E-test (Liofilchem, Italy); microbroth dilution was used for colistin.

Results: *P. aeruginosa* strains were classified as multi-drug resistant with carbapenem resistance. Optimized carbapenemase testing targeting IMP, IMI, GES, and GIM carbapenemases (eazyplex SuperBug complete C®, Amplex Diagnostics, Germany). With this test we were able to confirm the presence of these GES carbapenemases in our laboratory. Both strains were further analyzed at the German National Reference Centre for multidrug-resistant gram-negative bacteria in Bochum, confirming the GES-5-type and the GES-27-type *P. aeruginosa.* Modified Hodge tests for Imipenem, Meropenem, and Ertapenem were positive in the GES-5 / GES-27 strain, whereas MBL gradient test was negative for Imipenem and Meropenem in GES-5 isolate and MBL gradient test was positive for Imipenem and Meropenem in the GES-27 strain. From the group of reserve antibiotics, only Cefiderocol (MIC 1,5 µg/ml; E.test) and colistin (MIC 0,5 µg/ml; microbroth dilution) were tested susceptible.

Conclusions: Guiana extended spectrum (GES)-carbapenemase-producing *Pseudomonas aeruginosa* is associated with substantial morbidity and mortality. Therefore, rapid and accurate detection is essential for the timely initiation of appropriate therapy and the implementation of effective infection control measures. Despite comprehensive treatment of all identified clinical conditions, the patient"s condition progressively deteriorated, ultimately leading to death. This represents the second documented infection with GES-type-carbapenemase-producing *Pseudomonas aeruginosa* at our institution. Although the patient most likely died due to complications related to her underlying liver disease, it should not be overlooked that an infection caused by this type of bacterium could have been a decisive factor in the fatal outcome.

Fig. 1



PS02.04

Truncated *tox* gene by IS1132 in non-toxigenic toxin gene-bearing (NTTB) *Corynebacterium diphtheriae*

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Introduction: *Corynebacterium diphtheriae* (*C. diphtheriae*) outbreaks have been reported recently in Germany and other European countries, in part fuelled by armed conflicts [1]. Both infections and transmission events are not limited to toxigenic strains, but can also occur in non-toxigenic tox-bearing (NTTB) *C. diphtheriae* [2]. A positive *tox* gene PCR-result identifies potentially toxigenic strains of *C. diphtheriae*. Toxin production needs to be verified by testing for diphtheria toxin (DT). For this reason, the definitive diagnosis of diphtheria, which requires toxigenicity according to WHO, is notoriously difficult.

Objectives: Accurate and timely laboratory diagnostics, that allows differentiation between toxigenic and non-toxigenic *C. diphtheriae*, directly affects patient management and infection control measures. *C. diphtheriae* with toxin expression requires immediate public health response. Whole-genome sequencing (WGS) offers added value to routine testing and helps to understand potentially discordant results of molecular and phenotypic assays.

Methods: Culture and *tox* PCR were performed according to the WHO manual [3]. DT expression was determined by Elek test. DNA of the clinical *C. diphtheriae* isolate was submitted to short-read WGS for a 2 x 250bp paired end sequencing on Illumina® MiSeq (Illumina, Inc., San Diego, California, USA). Long read-sequencing was performed on a MinION Mk1B device (Oxford Nanopore Technologies, Oxford, UK) using an R10.4.1 Flow Cell (FLO-MIN114) as previously described [4]. The genomes from the recent outbreak observed in Germany [5] were imported and compared to our strain by core genome multilocus sequence typing (cgMLST) using the Bruker MBioSEQ Ridom Typer.

Results: *C. diphtheriae* was cultivated from an intraoperative swab. Although the strain tested PCR positive for *tox* gene, it lacked DT expression in Elek test and was thus categorized as non-toxigenic tox-bearing (NTTB) *C. diphtheriae*. Short- and long-read WGS revealed a truncated tox gene. At nucleotide position 19, the insertion sequence IS1132 was inserted in the reverse direction. The isolate had a novel sequence type, ST-1111. In core genome MLST analysis the isolate turned unrelated to any other of the previously described *C. diphtheriae* outbreak strains in Germany.

Conclusion: We report a novel ST of NTTB *C. diphtheriae* with interruption of its *tox* gene by IS1132 at nucleotide position 19. Our findings highlight the value of WGS in investigating cases that may be DT positive, as well as in reliably assessing the epidemiological context, which is an essential prerequisite for infection control measures and public health interventions.

[1] PMID: 37344769

[2] PMID: 37384376

[3] https://iris.who.int/handle/10665/352275. Licence: CC BY-NC-SA 3.0 IGO

[4] PMID: 38397011

[5] PMID: 36398576

PS02.05

EHEC/HUS outbreak Germany 2025: Molecular epidemiology, phylogenetic classification and genetic diversity of the outbreak strain

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Introduction: In autumn 2025, an outbreak of shigatoxigenic *Escherichia coli* (STEC) including cases of haemolytic uraemic syndrome (HUS) occurred in Germany. At the beginning of the outbreak, the majority of cases were concentrated in northeastern Germany, later shifting to the south-west. According to the case definition, more than 400 cases were associated with the outbreak, of which ~200 were confirmed by molecular methods. A total of 62 patients -mainly children- developed HUS and three individuals died.

Results: The outbreak strain was identified as STEC O45:H2, ST301, *stx2*a and *eae*-xi positive and exhibits the following antibiotic resistances: ampicillin, chloramphenicol, kanamycin, nalidixic acid, trimethoprim/sulfmethoxazole, trimethoprim and tetracycline. More than 140 isolates could be assigned to the outbreak cluster by genome analysis using cgMLST. Pairwise allele distances ranging from 0 to 10 and above within the cluster indicate an unusual mutation rate. Long read sequencing revealed four plasmids, one of them carrying resistance genes. Further genetic, molecular and biological analyses are ongoing to determine the virulence factors and characteristic of the outbreak strain.

Summary: We present another example where STEC of a rare serovar, caused a large outbreak in Germany. Prior to the outbreak, 2015 to 2025, the German Reference Center for Salmonella and Other Bacterial Enteropathogens detected only 13x O45:H2 strains among over 10 000 analysed samples in the genome-based national surveillance of clinical STEC, four associated with HUS. This demonstrates, the relevance of rare STEC serovars and the power of integrated genomic surveillance to gain an overview on national and global levels.

PS02.07

Discrimination of cereulide-producing *Bacillus cereus sensu stricto* by MALDI-TOF mass spectrometry

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Introduction: *Bacillus cereus sensu stricto* is one of the major etiological agents of foodborne intoxications (REF). It is ubiquitous and can contaminate a wide range of food matrices (cereal products, dairy products, spices, and ready-to-eat foods, etc.). The clinical syndromes associated with *B. cereus* include a diarrheal form and an emetic form. The latter is linked to the production of cereulide, a heat-stable peptide toxin encoded by the *ces* gene cluster. Rapid identification of emetic strains is crucial to prevent foodborne outbreaks and for microbiological risk assessment. Molecular methods allow accurate characterization of *ces*-positive strains; however, they

are relatively time-consuming and costly. In this study, we evaluated the discriminatory power of MALDI-TOF MS in differentiating *B. cereus s.s.* strains carrying the *ces* gene from those lacking this gene.

Materials and Methods: N=30 *B. cereus s.s.* strains (n=10 *ces*-positive and n=20 *ces*-negative), isolated from different food and environmental matrices, were included in the study. The presence of the *ces* gene cluster was previously assessed by molecular methods (PCR and WGS).

MALDI-TOF MS analysis was performed using the MALDI Biotyper® system (MBT - Bruker Daltonics GmbH & Co. KG, Germany), using pure bacterial cultures on Columbia sheep blood agar incubated overnight at 37 °C. Samples were prepared using the standard protein extraction protocol with formic acid/acetonitrile. One µl of extract was spotted onto an MBT target plate in 8 replicates, and once dried, the spots were covered with 1 µl of α-cyano-4-hydroxycinnamic acid (HCCA) matrix. Spectra were acquired in linear positive mode over a mass range of 2–20 kDa, using default settings. Spectra visualization and analysis was performed by the flexAnalysis software (Bruker Daltonics). Discrimination between *ces*-positive and *ces*-negative strains was performed by assessing reproducible differences in spectral patterns.

Results: MBT analysis revealed significant reproducible differences between the protein profiles of *ces*-positive and *ces*-negative strains, in high concordance with results of molecular methods. All the isolates carrying the *ces* cluster exhibited homogeneous spectral patterns that were clearly distinct from those of non-emetic strains, which also displayed their own distinct profiles. Comparative analysis of spectra enabled the identification of specific peaks patterns and features associated with emetic strains, allowing a clear separation between the two groups.

Discussion: Although the number of strains included in this study is limited, the results of this study demonstrate a promising potential of MALDI-TOF MS for the rapid discrimination of *B. cereus s.s.* emetic strains. Further studies including a larger number of isolates will be necessary to validate the identified spectral biomarkers, to establish an automated subtyping approach, and to standardize analytical protocols for routine diagnostic use.

PS02.08

Deletion of the deubiquitinating enzyme OTUD7B in astrocytes protects against cerebral malaria by inhibiting microvesicle-induced neuroinflammation

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Background: Cerebral malaria is a severe neurological complication of *Plasmodium falciparum* infection which can result in the death of the infected individual. Disruption of the blood–brain barrier (BBB) and endothelial dysfunction have been implicated in the disease pathology; however, whether astrocytes, a major component of the BBB, influence disease outcome remains yet to be uncovered. Therefore, using the murine model of experimental cerebral malaria (ECM), we investigated the role of the deubiquitinating enzyme OTUD7B in astrocytes during cerebral malaria.

Methods: GFAP-Cre *Otud7b*$^{fl/fl}$ mice lacking OTUD7B in astrocytes and control *Otud7b*$^{fl/fl}$ mice were infected with 1 × 10^6 *Plasmodium berghei* ANKA (PbA)-infected red blood cells. Disease progression was assessed using the Rapid Murine Coma and Behaviour Scale. BBB integrity was evaluated by Evans blue assay, and parasitemia by Giemsa-stained blood smears. Brain pathology was examined by immunohistochemistry. Transcriptomic profiling was performed on ex vivo–isolated astrocytes. Microvesicles were inhibited in vivo using pantethine. Astrocytes were stimulated with microvesicles, and signaling pathways were analyzed by Western blot. Intracerebral *Otud7b* or *Cxcl10* was silenced using siRNA.

Results: We show for the first time that astrocytes critically regulate experimental cerebral malaria (ECM) and astrocytic OTUD7B promotes the disease. Mice lacking astrocytic OTUD7B showed reduced brain pathology and were protected from ECM. Transcriptomic analysis revealed decreased pro-inflammatory chemokine and cytokine expression in OTUD7B-deficient astrocytes. Plasmodium-derived microvesicles induced OTUD7B-dependent inflammatory responses. Mechanistically, OTUD7B removed K48-linked ubiquitin chains from TRAF3 and TRAF6 following microvesicle stimulation or TLR3/TLR9 activation, leading to sustained NF-κB and p38 MAPK signaling and increased CXCL10 expression. Therapeutic silencing of CNS *Otud7b* or *Cxcl10* after disease onset protects against ECM.

Conclusion: Deletion of OTUD7B in astrocytes protects against cerebral malaria by inhibiting microvesicle-induced TRAF3/TRAF6 *Cxcl10* mediated neuroinflammation.

PS02.09

Serum IgA elicited by vaccination with full-length MSP1 vaccine engages myeloid effector pathways and is shaped by prior malaria exposure

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Background and Question: While the role of IgG in malaria immunity is well-documented, systemic Immunoglobulin A (IgA) remains relatively understudied in the context of vaccination. IgA is the second most abundant serum isotype and mediates distinct Fc receptor-dependent functions through the FcαRI (CD89) receptor on myeloid cells. This study aims to characterize the magnitude, kinetics, and functional profile of IgA induced by a full-length MSP1 malaria vaccine (SUM-101) in individuals with varying malaria exposure histories.

Methods: We systematically evaluated IgA responses in malaria-naïve and semi-immune (previously exposed) individuals following a multi-dose vaccination regimen. MSP1-specific IgA was purified from serum to assess its ability to recognize native parasites and to measure Fc-mediated effector functions, including antibody-dependent respiratory burst (ADRB) in neutrophils, opsonic phagocytosis by THP-1 monocytes and neutrophils, Natural Killer (NK) cell activation, and complement deposition.

Results: Vaccination induced robust MSP1-specific IgA in naïve individuals, with titers peaking after the third dose. In contrast, semi-immune individuals exhibited significantly attenuated responses, with peak titers approximately ten-fold lower than those in the naïve cohort, suggesting that prior exposure may "imprint" and constrain IgA expansion. Functionally, purified vaccine-induced IgA demonstrated strong ADRB activity in neutrophils and promoted opsonic phagocytosis in monocytes. While the IgA did not exhibit direct Growth Inhibition Activity (GIA) or classical complement fixation (C1q), it successfully facilitated C3b deposition, particularly in high-titer individuals. Interestingly, pre-existing immunity modulated these functions differently: high pre-immunity limited further boosting of neutrophil activity but was associated with stronger NK cell activation.

Conclusions: Full-length MSP1 vaccination elicits a functionally distinct IgA response that robustly engages myeloid effector pathways. The magnitude and cellular targeting of these responses are profoundly shaped by host immune history, emphasizing the need for isotype-specific functional analyses in malaria vaccine development to better understand and optimize protective immunity.

PS02.10
NLRC4-inflammasome activation by *Y. enterocolitica* phagosome disruption in primary human macrophages

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Introduction: Pathogenic *Yersinia* spp. activate canonical and non-canonical inflammasomes in macrophages, causing cytokine release and pyroptosis. Inflammasome activation is initiated by distinct *Yersinia* activities, including disruption of phagosomal membranes and sensing of needle components of the bacterial type 3 secretion system (T3SS).

Aim: We investigated the spatiotemporal dynamics of NLRC4 inflammasome activation in macrophages following phagocytosis of mutant *Yersinia* strains that overproduce the T3SS but contain no effectors. These strains cause strongly increased phagosome disruption and inflammasome activation.

Material and Methods: NLRC4 inflammasome dynamics and structural organisation were analysed on a single cellular level with live and super-resolution fluorescence microscopy.

Results: Formation of ASC-containing specks, dense condensates of multiple inflammasomes, occurred only after phagosome disruption by the bacterial T3SS. NAIP, which recognizes T3SS needle proteins and activates NLRC4, but neither NLRC4 nor ASC were detected at the bacteria-containing disrupted phagosomes. In comparison, NAIP, NLRC4 and Pro-Caspase 1 were all found in ASC specks that were located in some distance from the phagosomes. This indicates that NAIP migrates from disrupted phagosomes to the specks after its activation by the bacterial T3SS needle proteins and stimulates NLRC4 there or on its way there. Super-resolution MINFLUX and STED microscopy documented the nanometer scale sub-organisation of NAIP, NLRC4, Pro-Caspase1 and ASC within the specks.

Summary: Our data provide novel information on the spatiotemporal dynamics of NLRC4 activation by the *Yersinia* T3SS as well as the organisation of inflammasomes and their components within primary human macrophages.

PS02.11
Comparison of two immunoassays for Anti-SARS-CoV-2 Spike IgG quantification: Evaluation of cross-platform alignment

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Question: Quantification of Anti-SARS-CoV-2-Spike IgG remains essential for monitoring immune responses after COVID-19 infection or vaccination. Method transitions between commercial immunoassays are often complicated by the lack of harmonised conversion to the WHO International Standard Binding Antibody Levels / mL (BAU/mL).(1) The SERION ELISA agile SARS-CoV-2 IgG (Institut Virion\Serion GmbH, Würzburg, Germany) provides established quantification of Anti-SARS-CoV-2-Spike IgG levels with validated BAU/mL conversion.(2, 3) This assay was compared with Elecsys® Anti-SARS-CoV-2 S (ECLIA, Roche Diagnostics International AG, Rotkreuz, Switzerland).(4, 5) The objective was to evaluate approaches for cross-platform harmonisation.

Methods: As part of the ARIPro study, a longitudinal, prospective cohort study, examining acute respiratory infection immunity and epidemiology, randomly collected serums were analysed using the Serion ELISA and the Roche ECLIA assay. To visualise trends a Bland-Altmann plot was created and Locally Estimated Scatterplot Smoothing (LOESS) was performed. Spearman rank correlation, Deming, weighted Deming, and logarithmic Deming regression analysis were performed comparing both assays.

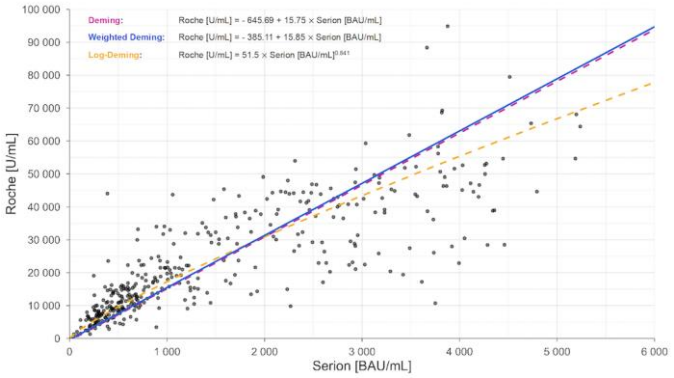
Results: In total, 367 serum samples collected in 2025 from healthy adults were analysed demonstrating a strong positive linear relationship. Scatterplot analysis revealed dense clustering at lower IgG levels, with increasing dispersion at higher titres and a strong positive correlation between both assays (Spearman"s r=0.87, 95% CI 0.78-0.85; p<0.0001). Relative Bland-Altmann analysis showed that Roche results were approximately 175.9% with Lower of Agreement (LoA) ranging from 152.48-199.31%. Deming, weighted Deming, and logarithmic Deming regression analyses demonstrated strong linear relationships between the Serion and the Roche Anti-SARS-CoV-2-Spike IgG assays. The estimated slopes were 0.064 (95% CI 0.058-0.069) for Deming, 0.063 (95% CI 0.058-0.068) for weighted Deming, and 1.188 (95% CI 1.119-1.259) for logarithmic regression.

Conclusions: Despite strong correlation quantitative discrepancies across the measurement range underscore that both platforms are not interchangeable. The observed slope pattern may indicate proportional bias, with Roche results tending to increase more strongly across concentration range. The findings suggest that cross-platform harmonisation may benefit from regression-based conversion approaches. However, heteroscedasticity - particularly at high

concentrations - indicates that caution is still required when combining quantitative results from different immunoassay platforms. Within the current analysis, weighted Deming regression showed the most consistent fit.

Figure 1: Scatterplot of paired Anti-SARS-CoV-2-Spike IgG levels with fitted Deming regression (magenta dashed line), weighted Deming regression (blue solid line), and logarithmic Deming regression (orange dashed line).

Fig. 1



PS02.12

Coxiella burnetii outer membrane vesicles (OMVs) aid host cell infection

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Introduction: *Coxiella (C.) burnetii* is a Gram-negative, obligate intracellular bacterium and the causative agent of the zoonotic disease Q fever. As an intracellular pathogen, it requires the manipulation of its host cell for the establishment of its intracellular replicative niche, the so-called *C. burnetii*-containing vacuole (CCV). Bacterial outer membrane vesicles play an important role in host-pathogen interactions. Although recent studies have elucidated the function of OMVs during infections with other Gram-negative bacteria, our understanding of their role in *C. burnetii* infection remains limited. It is currently unknown whether *C. burnetii* produces OMVs, and, if so, what functions these OMVs play during host-pathogen interactions.

Methods: *C. burnetii* was grown in axenic culture for 7 days. The supernatant was collected and concentrated and subsequently subjected to density gradient ultracentrifugation and size exclusion chromatography to purify *C. burnetii*-OMVs. Size and concentration of the OMVs were assessed by nanoparticle tracking analysis as well as electron microscopy analysis. The effect of *C. burnetii*-OMVs on the infection of host cells was assessed by immunofluorescence and colony forming unit assay. In addition, *C. burnetii*-OMVs were subjected to proteomic analysis to identify possible effector cargo.

Results: OMVs with a size of ~ 70 – 80 nm and a concentration of ~7.5 x 10^9 particles/mL could be isolated from *C. burnetii* in axenic culture. Electron microscopy could confirm the presence of intact OMV-like structures. Upon addition of *C. burnetii*-OMVs during the infection process, an initial increase of CCVs per cell could be detected via immunofluorescence microscopy. CFU assays confirmed the presence of a higher bacterial burden in cells treated with *C. burnetii*-OMVs. Proteomic analysis identified different proteins in *C. burnetii*-OMVs, including cytosolic proteins and proteins involved in signal transduction.

Conclusion: *C. burnetii*-derived OMVs were shown to benefit *C. burnetii* in the initial phases of the infection, as evident by an increase in CCVs per cell and a higher bacterial burden in infected cells. Through initial proteomic analysis, several proteins could be identified in *C. burnetii*-OMVs, including proteins involved in signal transduction and uncharacterized proteins. Further proteomic analysis and bioinformatic characterization is required to determine the exact protein(s) responsible for the observed increase in *Coxiella* infectivity.

PS02.13

O_2 availability might influence the induction and regulation of intracellular _Coxiella burnetii_ persistence by altering the host cell metabolism

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Coxiella (C.) burnetii is an obligate intracellular bacterium and the causative agent of Q fever in humans. While the acute form of the disease typically presents as a flu-like illness, or pneumonia, approximately 3% progress to chronic Q fever, which most commonly manifests as endocarditis. Notably, clinical symptoms can arise months or even years after the primary infection, indicating long-term persistence of *C. burnetii* within the human host. However, the intracellular niche and the mechanisms governing its transition to persistence remain poorly understood.

Our previous work showed that hypoxia-induced (0.5% O_2) citrate limitation, mediated by stabilization of HIF-1α and inhibition of STAT3 activation, prevents *C. burnetii* replication in bone marrow-derived macrophages (BMDM) without affecting bacterial viability. Importantly, supplementation with trimethyl citrate restored bacterial replication. Furthermore, *C. burnetii* replicates equally well at 2.5% and 0.5% O_2 under axenic culture conditions, indicating that the O_2 availability does not directly influence bacterial growth. Instead, these findings suggest that the host cell metabolism might play a critical role in shaping the intracellular lifestyle of *C. burnetii*. Characterization of *C. burnetii* under 0.5% O_2 revealed a small cell variant (SCV)-like phenotype. In addition to altered morphology, these bacteria exhibited increased infectivity, enhanced resistance to antibiotics, and reduced susceptibility to clearance by IFN-g-activated BMDM. These data indicate that intracellular hypoxic conditions trigger *C. burnetii* persistence.

To determine whether induction of hypoxia-mediated *C. burnetii* persistence is cell type-specific, we extended our analysis to the endothelial cell line EA.hy926, a well-established model for endocarditis. Under severe hypoxic conditions (0.5% O_2), *C. burnetii* replication was prevented. In contrast, at 1% and 3% O_2, bacterial replication was less impaired. Additionally, distinct morphological changes were observed. Thus, also in EA.hy926 cells hypoxia promoted the formation of the SCV-like phenotype of *C. burnetii*, characterized by a reduced diameter, an electron-dense cell wall, and condensed chromatin. Importantly, preliminary metabolomic analyses of *C. burnetii*-infected EA.hy926 cells cultured under varying O_2 concentrations revealed oxygen concentration-dependent metabolic signatures. Importantly, citrate levels were significantly reduced at 0.5% O_2 compared to 1% and 3% O_2. Given the importance of citrate for *C. burnetii* replication, these findings provide a plausible metabolic explanation for the observed persistent phenotype of *C. burnetii* in severe hypoxic EA.hy926 cells. Together, these results support our hypothesis that *C. burnetii* persistence may be driven by the host cell metabolism.

PS02.14
The interferon-stimulated gene product MX2 restricts intracellular replication of *Salmonella enterica* serovar Typhimurium (STM)

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Introduction: *Salmonella enterica* serovar Typhimurium (STM) is an important human pathogen that causes a range of illnesses, from mild gastroenteritis to severe systemic infections. A key factor in *Salmonella*"s ability to cause disease is its capacity to survive and replicate inside host cells, particularly within a compartment called the *Salmonella*-containing vacuole (SCV). This allows the bacterium to avoid many of the immune defenses that would normally eliminate it. The innate immune system, specifically type I interferons (IFN-I) and interferon-stimulated genes (ISGs), is critical in defending against intracellular pathogens. However, the role of IFN-I and ISGs in restricting *Salmonella* remains elusive.

Objective: Given the enigmatic role of IFN-I and ISGs in *Salmonella* infections, this study aimed to investigate whether IFN-I responses influence the ability of STM to invade and replicate in epithelial cells.

Materials and Methods: Specifically, STM"s ability to invade and replicate intracellularly within HeLa cells was assessed under different conditions by gentamicin protection assays, followed by colony count or immunofluorescence assays. Next, a select group of ISGs was screened by overexpression in HeLa cells to evaluate their effects on STM infection. From the screen, a promising ISG candidate was selected to determine the stage of STM infection that it affects - invasion, replication, or bacterial release.

Results: Our findings indicate that IFN-I treatment reduces intracellular STM replication after an overnight incubation, while bacterial invasion remains unaffected. Among the ISGs tested, ectopic overexpression of MX2 emerged as a potential inhibitor of STM infection. Further experimentation confirmed that MX2, a well-characterized antiviral ISG, exhibited antibacterial properties by limiting STM intracellular replication after an overnight incubation. MX2 overexpression had minimal to no effect on bacterial invasion, release, or cytosolic bacterial populations.

Summary: These findings support a role for IFN-I signaling in restricting intracellular STM replication and identify MX2 as a potential antibacterial effector. Although MX2 is primarily recognized for its antiviral activity, our results suggest that its function extends beyond viral infection and may contribute to cell-intrinsic defense against intracellular bacterial pathogens. Collectively, these observations further support the concept that ISGs can exert broader antimicrobial activities than previously appreciated.

PS02.15
The influence of lysosomal ion channels on intra-macrophage replication of high consequence bacterial pathogens

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Facultative intracellular pathogens such as *Francisella tularensis, Yersinia pestis,* and *Brucella* spp., the causative agents of tularemia, plague, and brucellosis, are characterized by low infectious doses and high virulence, underscoring both their bioterrorism potential and clinical importance. A key aspect of their pathogenicity is their ability to survive and replicate within macrophages, evading host immune responses.

Intracellular localization poses a major therapeutic challenge, as bacteria within endosomal or phagosomal compartments are often poorly accessible to antibiotics due to restricted penetration and the possible acidic environment of these vesicles. While endosomal ion channels contribution to antiviral defense has been shown, their role in bacterial infections remains insufficiently characterized. Given that these pathogens interact closely with the endo- lysosomal system, which controls vesicular maturation and degradation, we aim to elucidate how ion flux across these compartments influences bacterial survival and replication.

We established robust *in vitro* infection models using differentiated THP-1 derived macrophage-like cells to mimic the human host environment. Infection dynamics were analyzed using fluorescence microscopy and flow cytometry (FACS), enabling quantitative assessment of intracellular bacterial burden.

To investigate the functional role of endosomal ion channels, cells were treated with specific pharmacological modulators, including channel antagonists that interfere with vesicular acidification, a critical step in phagosomal maturation. We evaluated the therapeutic potential of combining ion channel antagonists with conventional antibiotics, comparing their efficacy to monotherapies in reducing intracellular bacterial load.

Our data confirm that *Y. pestis* and *Brucella* spp. depend on early phagosomal acidification to induce virulence-associated pathways, whereas *F. tularensis* rapidly escapes from the phagosome prior to acidification and is largely independent of this process.

Quantitative FACS analysis revealed that modulation of endosomal ion channels significantly alters intracellular bacterial replication. The effect of combination treatment with antibiotics and ion channel antagonists is presented for these three intracellular bacteria compared to monotherapies.

These findings demonstrate that intracellular pathogens employ distinct strategies to exploit or evade endo lysosomal processes. Targeting ion channel–mediated regulation of vesicular environments represents a promising approach to enhance antibiotic efficacy against intracellular bacteria. Combination therapies that improve drug accessibility within infected cells may provide a novel avenue for treating infections caused by high-risk pathogens.

PS02.16

Bactericidal/permeability-increasing protein synergistically enhances the perception of immunostimulatory DNA

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Bactericidal/permeability-increasing protein (BPI) is known for its bactericidal activity towards Gram-negative bacteria, its neutralization capacity towards lipopolysaccharide and, as recently described, its ability to potently activate bone marrow-derived dendritic cells (BMDCs) in an NFAT-dependent manner. As a highly cationic protein pre-stored in neutrophils, BPI can be found in chromatin-containing neutrophil extracellular traps (NETs). Since immunogenic interactions between antimicrobial peptides and nucleic acids have been reported previously, we investigated binding interactions between BPI and immunostimulatory DNA and the effect of co-administration on murine BMDCs. Remarkably, combination of BPI and the synthetic TLR9 ligand CpG-oligodeoxynucleotide 1826 (CpG) induced a pronounced synergistic interleukin (IL)-12 response and enabled significantly enhanced Th1 differentiation from naïve T cells compared to up to 50-fold higher concentrations of CpG alone. Mechanistically, binding of CpG by BPI enabled cellular co-localization of both molecules. However, in contrast to CpG, the TLR7/8 agonist R848 did not bind to BPI as demonstrated by solid-phase binding assays and thermophoresis. Yet, replacement of CpG with R848 revealed that binding is not an essential requirement for the synergism. In our ongoing work, we investigate a convergence of signaling pathways initiated by both BPI and CpG. In conclusion, BPI enhances the induction of high IL-12 levels and a consecutive Th1 response in the presence of immunostimulatory DNA implicating relevance in the immune defense against intracellular microbes.

PS02.17

Evaluation of recombinant human milk compounds as anti-infective agents against respiratory syncytial virus (RSV) and *Staphylococcus aureus* (*S. aureus*)

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Staphylococcus aureus (*S. aureus*)-induced sepsis and Respiratory syncytial virus (RSV) infection represent major global health burdens, particularly affecting vulnerable populations such as infants and the elderly. The rise of antimicrobial resistance and the lack of effective RSV treatments underscore the urgent need for novel preventive and therapeutic strategies. Leveraging host-derived protective factors offers a promising approach to overcome these challenges by utilizing natural evolved defense mechanisms. Human breast milk is a key source of nutrition and immune protection for newborns and infants. Its bioactive components, such as lactoferrin and human milk oligosaccharides, exhibit potent antimicrobial, anti-inflammatory, and immunomodulatory properties both *in vitro* and *in vivo*. Emerging evidence suggests these compounds may interfere with pathogen–host interactions by serving as decoy receptors or altering receptor binding through structural changes.

Here, we investigate the anti-infective potential of recombinantly produced and structurally optimized human milk compounds against RSV and *S. aureus*. To evaluate antiviral activity, compounds were screened against RSV using serial dilutions, and viral infection was quantified by immunocytochemical analysis of viral replication. Preliminary *in vitro* screening identified human milk compounds that induced a dose-dependent reduction in RSV infection, primarily through inhibition of viral attachment. Interestingly, compounds that initially showed only limited antiviral effects in their native form exhibited enhanced activity after targeted structural modification by up to 40-fold.

To evaluate the antibacterial potential of these human milk compounds against *S. aureus*, a physiologically relevant *in vivo* environment is required to capture their proposed anti-infective and immunomodulatory mechanisms, including inhibition of bacterial adhesion to host cells and enhancement of host cellular defenses. Therefore, we established an advanced *S. aureus* infection model in the wax moth larva *Galleria mellonella* (*G. mellonella*). Moving beyond conventional survival readouts, this platform integrates host gene expression analysis and longitudinal infection tracking using an auto-bioluminescent *S. aureus* strain, enabling non-invasive assessment of bacterial containment after treatment with human milk compounds.

These findings support the therapeutic or prophylactic potential of human milk compounds as promising anti-infective agents. Future research will focus on investigating the compounds' molecular mechanisms and evaluating their protective and immunomodulatory efficacy against *S. aureus in vivo* using the validated *G. mellonella* infection model. Compounds demonstrating significant anti-infective potential will undergo further evaluation in murine infection models of RSV and *S. aureus*-induced sepsis, providing critical validation of their efficacy under physiologically relevant conditions.

PS02.18
High-throughput TCR construct assembly for functional discovery of therapeutic TCRs

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Background: Difficult-to-treat infections, including opportunistic fungal infections, chronic viral infections and infections in immunocompromised patients, remain a major clinical challenge despite advances in antimicrobial therapy, requiring new approaches such as TCR-based immunotherapies. T cells play a central role in protective immunity against many of these pathogens by recognizing antigen-derived peptides through their T cell receptors (TCRs). Each T cell clone expresses a unique TCR that defines its antigen specificity and functional potential. Identifying pathogen-reactive TCRs could therefore improve our understanding of protective immune responses in infection and support the development of diagnostic tools, immune monitoring strategies, and TCR-based cellular therapies. However, discovering clinically relevant TCRs requires functional screening of large numbers of candidate receptors for antigen specificity, potency, and safety. Current approaches often depend on de novo synthesis of complete TCR constructs, which is costly, time-consuming, and limits throughput. As a result, only relatively small numbers of TCRs can typically be evaluated, restricting the discovery of rare but therapeutically relevant pathogen-specific TCRs. Therefore, scalable and cost-efficient approaches for TCR construct assembly are needed to enable high-throughput identification and characterization of TCRs targeting difficult-to-treat infections.

Methods: We established Golden Gate cloning-based approaches directly amplifying and cloning DNA fragments from existing cDNA libraries containing sequenced TCR α and β chains or utilizing DNA oligo-based approaches cloning constructs via conserved germline components and variable CDR3 regions in bulk (in collaboration with the Birnbaum laboratory based on their recently developed approach, *Immunity 2026*) and single TCR level not only suitable for viral transduction but also orthotopic TCR replacement (OTR).

Results: All TCR assembly approaches substantially reduced turnaround time compared with conventional de novo synthesis, making screening of hundreds or even thousands TCRs possible by additionally lowering costs per construct 10- to >100-fold (depending on amount of synthesized TCRs in oligo-based approaches). Moreover, efficiency is high and also in bulk-based approaches >95% of correctly assembled TCR sequences could be retrieved. Additionally, we could show that introducing TCRs via OTR in bulk is feasible at high efficiency potentially overcoming challenges of multiple integrations of conventional viral transduction.

Conclusion: We show that Golden Gate Cloning-based TCR assembly approaches allow for highly efficient cloning, majorly reducing costs while accelerating the whole process and making subsequent functional screening of hundreds or even more TCRs possible, enabling discovery of clinically relevant TCRs at a high throughput.

PS02.19
The innate-adaptive immune crosstalk in the postnatal liver

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Immunostimulatory molecules from bacteria colonialising the intestinal mucosa after birth are drained via the portal vein and reach the liver tissue. Here, immune cells, such as liver-resident macrophages, namely Kupffer Cells (KCs) are situated in the liver´s periportal regions. In early life, these cells are important for the clearance of bacteria in the bloodstream. Also, the number of T cells is especially high in the neonatal liver while adaptive immune responses are yet to be established. Previously, it was shown in adult mice that KCs play a role in the differentiation of CD8+ T cells into effector cells as well as in the promotion of T cell tolerance. Furthermore, chronic HBV specific CD8+ T cells are directly primed in the adult liver. However, the role of KCs in the differentiation of liver resident CD4+ T cells remains largely unknown.

We hypothesise that T cells and their influence on KCs create a tolerogenic environment in neonatal liver tissue. Experiments conducted in mice *in vivo* show that following toll-like-receptor (TLR) 4 signalling and under the influence of gut derived immunostimulatory molecules, T cells proliferate prior to acquiring a state of anergy. Opposite to that, a pro-inflammatory response of myeloid cells, particularly monocytes and KCs to the TLR4 signalling has been observed. Additionally, a PD1-PDL1 mediated interaction between neonatal KCs and T cells has been identified consistent with the predicted interaction between neonatal KCs and T cell by single cell RNAseq. However, depletion of KCs *in vivo* does not change the neonatal liver T cell phenotypes while *in vivo* blockage of PDL1 does. We therefore aimed to establish an *ex vivo* system to analyse the interaction between neonatal hepatic T cells and KCs. We isolated KCs from seven-day old and adult mice and co-cultured them qith CD3/CD28-prestiulated T cells to examine the role of KCs to provide a secondary signal.

The result of this project could set the basis for a better understanding of the establishment of postnatal hepatic immune cell homoeostasis and the influence of microbiota-derived signals. Besides, it could help to assess the challenges that the neonatal immune system faces to combat invasive pathogens. This may allow the development of future interventional strategies to reduce mortality from often lethal early childhood diseases such as early onset sepsis.

PS02.20

Gender-specific aspects of *Staphylococcus aureus* PVL-mediated infection

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Background: Sex-specific differences in immune response and disease progression have been described for a variety of infectious and non-infectious diseases. *Staphylococcus aureus*, particularly Panton–Valentine leukocidin (PVL)-positive strains, is associated with severe skin and soft tissue infections as well as invasive disease. However, the influence of biological sex on the clinical presentation and course of PVL-mediated *S. aureus* infection remains insufficiently understood.

Methods: To investigate the influence of biological sex on PVL-mediated cytotoxicity, the effects of selected sex hormones and inflammatory mediators on PVL-induced toxicity in HL-60-derived neutrophil-like cells were analyzed. Differentiated HL-60 cells were incubated with hormones estradiol, testosterone, dihydrotestosterone (DHT), progesterone as well as lipopolysaccharide (LPS) and prostaglandin in combination with PVL, followed by flow cytometry-based cytotoxicity assays. In addition, the hormone-dependent gene expression of the cellular receptors of PVL, C5aR and CD45, was assessed using reverse transcription quantitative PCR (RT-qPCR).

Results: All investigated sex hormones increased PVL-mediated cytotoxicity in HL-60-derived neutrophil-like cells, with estradiol and progesterone showing the strongest effects. Testosterone and dihydrotestosterone induced only a modest increase in cell mortality. The inflammatory mediators LPS and prostaglandin exerted opposing effects on PVL-mediated cytotoxicity, with LPS reducing cytotoxicity whereas prostaglandin markedly enhanced it. These findings were accompanied by reduced C5aR expression following LPS treatment and increased C5aR expression after treatment with prostaglandin, although both mediators decreased CD45 expression. Among the sex hormones investigated, progesterone and DHT increased the expression of both C5aR and CD45, whereas estradiol and testosterone reduced CD45 expression without markedly affecting C5aR expression.

Conclusion: Our findings suggest that sex hormones and inflammatory mediators influence PVL susceptibility in HL-60 cells, potentially through altered receptor expression. Further analyses are required to determine whether exposure to serum factors induces broader transcriptional changes in neutrophils or affects post-translational receptor regulation.

PS02.21

Measurement of anti-PVL antibody levels and neutralization efficacy in commercially available immunoglobulin products

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Background: *Staphylococcus aureus* is a versatile pathogen causing recurrent skin and soft tissue infections as well as severe invasive disease. Panton–Valentine leukocidin (PVL), a pore-forming toxin produced by certain *S. aureus* strains, targets neutrophils and macrophages, leading to cell lysis, tissue destruction, and, in rare cases, necrotizing pneumonia with mortality exceeding 50%. As patients with PVL-mediated necrotizing pneumonia rarely report prior PVL-SA infections, severe disease is thought to occur during first contact or recent colonization following respiratory viral infection, potentially in the absence of protective PVL-neutralizing antibodies. Intravenous and subcutaneous immunoglobulin (IVIg/SCIg) may contain PVL-neutralizing IgG antibodies and improve outcomes. This study evaluated European immunoglobulin products for therapeutically relevant levels of PVL-neutralizing antibodies and their ability to inhibit PVL-mediated cytotoxicity in human neutrophils.

Methods: To determine the concentration of PVL-neutralizing antibodies, at least 15 batches of IVIg/SCIg preparations from four manufacturers comprising seven products were analyzed. Anti-PVL antibody levels were quantified by ELISA using immobilized recombinant PVL subunits and an anti-human IgG detection antibody. Antibody titers were calculated from a commercial human IgG standard curve.

To assess PVL-neutralizing capacity, freshly isolated human neutrophils were exposed to recombinant PVL following pre-incubation with serial dilutions of IVIg/SCIg preparations. Cytotoxicity was quantified by flow cytometry using propidium iodide staining, and half-maximal inhibitory concentrations (IC_{50}) were determined by non-linear regression analysis of dose-dependent inhibition.

Results: Anti-PVL antibody titers of all batches ranged from 1.8±0.4 AU to 4.9±0.4 AU, while mean titers per manufacturer ranged from 2.6±0.3 AU to 3.2±0.8 AU. Among the four manufacturers, only the comparison between pooled batches with the lowest and highest titers reached statistical significance ($p = 0.02$). The batches comprised seven products with titers ranging from 2.4 ± 0.3 AU to 4.0 ± 0.5 AU. The product with the highest titer differed significantly from all other products ($p < 0.001$), while the lowest-titer product differed significantly from the three highest-titer products ($p = 0.03$).

Preliminary data on neutralization capacity of IgG products indicate that a 1.5-fold difference in anti-PVL antibody titer achieved a 2.5-fold increase in neutralizing potency.

Conclusion: Commercially available IVIg/SCIg products contain measurable anti-PVL antibodies with variable titers and demonstrate corresponding differences in neutralization potency against PVL-mediated cytotoxicity. These findings support the concept that IVIg/SCIg may contribute to functional PVL neutralization, although product-dependent variability should be considered in therapeutic applications.

PS02.22

Megakaryocyte responses to pneumococcal infection and pneumolysin exposure

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Introduction: *Streptococcus pneumoniae* is a leading cause of community-acquired pneumonia and invasive disease, particularly in children and the elderly. While the role of classical immune cells in pneumococcal defense is well established, the contribution of non-classical immune players remains underexplored. Megakaryocytes, the precursors of platelets, have recently been identified in the pulmonary niche (Livada et al., 2023), where they may participate in both thrombopoiesis and immune regulation. However, their interaction with *S. pneumoniae* and its key virulence factor, pneumolysin, remains unknown.

Aim: This study aimed to characterize the interaction between human megakaryocytes and *S. pneumoniae*, including responses to bacterial binding, phagocytosis, and exposure to pneumolysin.

Methods: Megakaryocytes were differentiated from hematopoietic stem cells (HSCs) isolated from healthy donors. Cells were exposed to *S. pneumoniae* TIGR4 or D39 strains, and bacterial binding was assessed by flow cytometry. Phagocytosis was quantified using colony-forming unit (CFU) assays. Viability after pneumolysin treatment (6 µg/mL) was evaluated by flow cytometry.

Results: Megakaryocytes efficiently bound and phagocytosed *S. pneumoniae*. Stimulation with TRAP-6 and ADP significantly enhanced both processes. Notably, megakaryocytes exhibited remarkable resistance to pneumolysin, with only a minor, non-significant reduction in viability following exposure.

Conclusion: Human megakaryocytes are capable of recognizing, engulfing, and resisting *S. pneumoniae* despite exposure to pneumolysin. These findings suggest a previously unrecognized role for megakaryocytes in the pulmonary immune response during pneumococcal pneumonia, positioning them as potential contributors to early host defense.

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PS02.24

B-cell lymphoma shapes host immune responses to invasive fungal infection

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Invasive fungal infections such as aspergillosis and disseminated candidiasis remain life threatening, with mortality exceeding 30% despite therapy. Impaired host immunity caused by underlying disease is a major contributor. Here, we examined how B-cell lymphoma stages alter antifungal responses to *Aspergillus fumigatus* at day 7 post infection using the Eμ-myc transgenic lymphoma model.

Both wild type and transgenic immunocompetent mice cleared the infection. In lung and spleen, wild type and pre-lymphoma mice group showed pulmonary $CD45^+$ cells and myeloid expansion, including Ly6Ghi neutrophils, eosinophils, cDC2s, and macrophages, whereas lymphoma mice showed total myeloid cell accumulation only in the BAL fluid. Within lung and spleen macrophage subsets, an increased monocyte-derived macrophage and a proinflammatory M1 phenotype was observed exclusively in pre-lymphoma infected group whereas wildtype infected group showed increase only in the BAL compartment indicating spatially distinct inflammatory programs. Additionally, only wild type infected mice showed a higher proportion of anti-inflammatory $CD206^+$ M2 macrophages. However, no difference in macrophage subsets was observed in lymphoma group revealing divergent macrophage polarization upon disease.

In both lung and spleen, most T cell subsets, including regulatory T cells expanded in wild type infected mice. Th17 cells, however, increased exclusively in the pre-lymphoma infected group. Pre-lymphoma infected mice also showed reduced IFN γ, elevated ENA 78, and increased Ly6Gint/lo neutrophils in the spleen. Additionally, an increase in bone marrow Lin^- progenitors and skew toward MEP was observed together with increased circulating platelets in the blood in pre-lymphoma group. Altogether, this revealed an active tumour primed inflammatory state in pre-lymphoma group over lymphoma group with distorted T cell polarization and biased bone marrow haematopoiesis. These findings demonstrate that lymphoma disease stage alters antifungal immunity and highlight the value of combined infection-disease models for assessing how underlying malignancy and its stages affect immune responses.

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PS02.25

Evaluation of bacterial pathogenic factors as prognostic markers of mortality in *Staphylococcus aureus* bloodstream infection – A WGS based multicenter prospective cohort study

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Background: Mortality in patients with *Staphylococcus aureus* bloodstream infections (BSI) is high [1]. The contribution of specific virulence factors to a fatal outcome has remained largely unclear, as many are strictly human-specific and their relevance cannot be assessed in animal models or laboratory strains [2]. Consistently, only few virulence factors have been directly linked with mortality in clinical cohort studies, but often neglecting potentially confounding clinical factors [3].

Aim: In this study, we sought to identify *S. aureus* genomic traits as predictive factors for in-hospital mortality by integrating an extensive set of clinical data with whole genome sequencing analysis.

Methods: The genomes of 643 *S. aureus* isolates from the multicenter BLOOMY BSI study were sequenced and a vast amount of UniRef100 and UniRef90 traits assigning protein variants were annotated. Univariate and multiple logistic regression analyses together with analysis of variance (ANOVA) were applied in a stepwise manner and a considerable number of clinical variables were incorporated. The Scoary2 tool was used as a confirmatory method.

Results: We identified 116 bacterial traits associated with increased or decreased in-hospital mortality after adjusting for the PBS (Pitt Bacteremia Score), the CCI (Charlson Comorbidity Index) and sex as most impactful clinical factors for survival in *S. aureus* BSI. These traits operate on various levels of *S. aureus* pathogenicity, including immune modulation, metal homeostasis, gene transcription and translation, detoxification, adhesion, metabolic functions and environmental sensing. Moreover, novel, uncharacterized potential virulence factors were identified. Nine predictive proteins were confirmed with an orthogonal statistical approach.

Conclusion: Patient in-hospital mortality was associated with the presence of known and potentially novel virulence factors of *S. aureus* after correcting for confounding clinical parameters in a comprehensive, standardized clinical cohort. These factors need further investigation to gain a better understanding of their function, mode of action and regulation in the context of *S. aureus* BSI and to evaluate their potential as diagnostic and therapeutic targets.

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PS02.26

Aging enhances susceptibility to *Staphylococcus aureus* infection in the lung

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The interaction between aging and bacterial infections, particularly *S. aureus* pneumonia, remains incompletely understood. This study therefore seeks to investigate the impact of aging on both the host response to *S. aureus* infection and the bacterial adaptation.

To investigate the direct influence of aging on *S. aureus*, bacteria were grown in an aged environment, resulting in distinct growth kinetics characterized by earlier entry into the stationary phase, accompanied by transcriptomic adaptations indicating metabolic alterations. The effect on the host was examined in two primary human cell types: lung fibroblasts (IMR-90) and small airway epithelial cells (SAEC). Cells were treated with doxorubicin to induce an aged phenotype. *In vitro* analysis revealed a cell-dependent effect of aging on bacterial burden and host defense. Aged IMR-90 cells displayed increased susceptibility and impaired immune responses, whereas aged SAEC exhibited the opposing pattern, with reduced bacterial load and enhanced defense pathway activation, suggesting that aged lung fibroblasts may drive increased bacterial burden in the aging lung. Finally, the impact of aging was assessed in a murine *ex vivo* precision-cut lung slice (PCLS) model, where aged mice displayed increased bacterial load accompanied by elevated inflammation, confirming that aging affects both host defense

and bacterial physiology, thereby increasing susceptibility to infection.

In summary, this data reveals that aging promotes bacterial infection through a dual mechanism: cell-specific alterations in host defense and transcriptomic reprogramming of *S. aureus*, leading to modified growth kinetics and metabolic adaptations. Collectively, these findings provide novel evidence that aging shapes not only the host response but also the pathogen itself, contributing to the increased severity of bacterial lung infections in the aged host.

PS02.27

Temperature-dependent regulation of persistence and virulence genes in *Pseudomonas aeruginosa* PAO1 on oak leaf lettuce and indications for root internalization

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Increasing evidence of *Pseudomonas aeruginosa* on fresh plant-based foods raises food safety concerns, particularly in the face of changing environmental conditions. Climate change-associated temperature shifts may influence the plant microbiota and the presence of human pathogens on fresh produce. This study investigates the temperature-dependent gene expression of *P. aeruginosa* PAO1 on green oak leaf lettuce as a model system and looks at the capability of *P. aeruginosa* PAO1 for root internalization. For this purpose, temperature-dependent transcriptomic changes of *P. aeruginosa* PAO1 were assessed by analyzing differentially expressed genes following plant inoculation and incubation at 18 and 22 °C, respectively. Moreover, oak leaf lettuce was cultivated in soil inoculated with *P. aeruginosa* PAO1_sfGFP_UHH07, and internalization was analyzed using confocal laser scanning microscopy. Transcriptomic analyses showed that a moderate temperature increase shifts bacterial gene expression, with virulence genes upregulated at 22 °C and persistence-associated genes predominating at 18 °C. Results indicate that *P. aeruginosa* PAO1 is capable of internalizing into the roots of oak leaf lettuce, but a translocation into leaves was not detected. These results show that temperature influences the persistence and pathogenic potential of *P. aeruginosa* present on oak leaf lettuce, highlighting potential impacts of climate change on food safety.

PS02.28

LPS elongation in the attenuated *Coxiella burnetii* Nine Mile II strain only occurs under specific conditions

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Coxiella burnetii is a Gram-negative pathogen and the causative agent of Q fever, a mild flu-like illness, which can develop into an interstitial pneumonia or hepatitis. Furthermore, the infection can become chronic, which is potentially fatal. Lipopolysaccharide (LPS) is an essential *C. burnetii* virulence factor. *In vitro* propagation of *C. burnetii* Nine Mile Phase I (NMI) leads to LPS truncation and the establishment of the avirulent Nine Mile Phase II (NMII). This process involves large chromosomal deletions that irreversibly eliminates several genes, including cbu0678-cbu0698, which are involved in LPS O-antigen biosynthesis. A recent publication reported a 3-bp mutation in the gene cbu0533 of NMII during passaging in ACCM-1 media, which resulted in LPS elongation and increased virulence in infected guinea pigs (Long *et al.*, 2024).

To address potential safety concerns, we screened different genetic modified NMII laboratory strains, *C. burnetii* NMII infected mouse bone marrow-derived macrophages (BMDM) and spleen and liver tissue samples of intraperitoneal infected immunocompromised mouse strains after 7 and 14 days of infection with *C. burnetii* NMII for the reversion in the cbu0533 gene through deep sequencing.

MiSeq analysis of gDNA of different NMII strains across laboratories did not detect reversion in cbu0533. Reversion did also not occur during *in vitro* infections. During *in vivo* infection, *C. burnetii* only survives and replicates in immunocompromised mice. Thus, replication of *C. burnetii* was detected via qRT-PCR and CFU after 7d of infection in Myd88, Acod1, IFNγ and Rag2/γ-chain deficient mice. Importantly, no cbu0533 reversion was found. However, after 14d of infection, cbu0533 reversion was detected in spleen and liver of IFNγ and Rag2/γ-chain deficient mice. Long-term axenic passaging of NMII in ACCM-2 or ACCM-D showed no reversion. However, after 10 weeks of passaging in ACCM-1, cbu0533 reversion was detected.

Finally, we infected immunocompetent Balb/c mice with NMI, NMII or NMII cbu0533 revertant strains for 14d to compare the virulence and cytokine profile by qRT-PCR. Whereas NMII was completely cleared, genomic DNA, but no viable bacteria, of the other strains could be recovered in spleen and liver samples. In contrast to NMII infected mice, the inflammation marker TNFα, IFNγ, IL-6 and Cxcl-1 were transcriptionally upregulated in spleen and liver of NMI infected mice. The NMII cbu0533 revertant displayed in spleen a similar cytokine profile as the NMII strain and in liver an inflammatory response like the NMI strain.

Taken together, it is safe to use NMII under BSL-2 containment in short-term mouse infection models, in cell infection models and under axenic growth in ACCM-2 and ACCM-D media. Nevertheless, a reversion of the cbu0533 gene might occur during *in vivo* infection experiments in certain immunocompromised mouse strains after an infection period of more than 7d or during axenic culture in ACCM-1 media.

PS02.29

Bacteriophage activity in the infected wound milieu: Evaluating antimicrobial efficacy beyond conventional *in vitro* models

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Background: Bacteriophages are gaining renewed interest as antimicrobial agents against multidrug-resistant wound infections. However, phage efficacy is commonly assessed in simplified *in vitro* systems that do not adequately reflect the complex milieu of infected, hard-to-heal wounds. Biofilm architecture, plasma components, immune cells, extracellular matrix components, and tissue barriers may substantially influence phage diffusion, bacterial accessibility, and antimicrobial activity.

Objectives: This study aimed to evaluate the antimicrobial activity of wound-derived bacteriophages against *Pseudomonas aeruginosa* under clinically relevant conditions and to assess their potential as adjuncts to established wound care approaches.

Methods: A translational 3D human biofilm model containing plasma and buffy coat-derived leukocytes was used to mimic key components of the infected wound bed. In parallel, an *ex vivo* human skin model based on abdominoplasty resection specimens was established to assess phage-bacteria interactions in a tissue-based setting. Both models were infected with *P. aeruginosa* DSM19882 and treated with different phage lysates alone or in combination with standard wound care products. Antimicrobial effects were analysed over time using quantitative bacterial assays and microscopic evaluation of biofilm structure and bacterial distribution.

Results: Phage treatment reduced bacterial burden and biofilm volume in both translational models, demonstrating relevant antimicrobial activity under wound-like conditions. Combination treatments with selected wound care products showed enhanced antimicrobial effects, but also revealed challenges associated with combined therapeutic approaches, including potential interactions between phages, bacteria, host-derived components, and topical antimicrobials.

Summary: Bacteriophages show antimicrobial activity against wound-associated *P. aeruginosa* in clinically relevant translational wound models. The observed differences between conventional *in vitro* assays, biofilm models, and *ex vivo* human skin models underline the need for complex test systems before therapeutic application. These models may improve prediction of antimicrobial therapies in infected, hard-to-heal wounds.

PS02.30

The role of free-living amoebae for the persistence of atypical *Brucella* spp. in the environment

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Atypical *Brucella* spp. have been isolated from a wide range of animal hosts and environmental sources. Unlike the classical highly pathogenic *Brucella* species, such as *B. abortus*, *B. melitensis*, and *B. suis*, these novel strains occur not only in mammals but also in amphibians and other ectothermic animals. They display atypical phenotypes that distinguish them from classical *Brucella*, including broader metabolic versatility and increased acid stress tolerance, resembling their environmental relatives formerly classified as *Ochrobactrum*. Sporadic human infections with atypical *Brucella* strains have raised questions regarding their virulence and potential persistence in environmental habitats. Because free-living amoebae can harbor intracellular pathogens such as *Legionella pneumophila*, they may also provide a potential environmental niche for atypical *Brucella* spp.

In this study, the persistence of atypical *Brucella* and *Ochrobactrum* strains in association with the free-living amoebae *Dictyostelium discoideum* and *Acanthamoeba castellanii* was investigated. Amoeba plate assays, plate killing assays, co-cultivation experiments, growth assays in amoeba-conditioned media and its liquid chromatography–mass spectrometry analysis, substrate utilization assays, and gentamicin protection assays were performed to evaluate the role of amoebae as potential environmental hosts. All strains were able to grow under acidic conditions and at different temperatures, which are important prerequisites for survival in amoeba-associated habitats. In amoeba plate assays, *B. microti* and a *Brucella* sp. isolate from an Australian rodent were susceptible to phagocytosis by *D. discoideum*, with *B. microti* showing higher sensitivity in plate killing assays. Co-cultivation with amoebae promoted growth of all tested *Brucella* strains, whereas enhanced growth in amoeba-conditioned media was observed only for *Ochrobactrum* spp. LC–MS analysis of conditioned media revealed increased levels of amino acids, amino acid derivatives, and lipids. Substrate utilization assays further showed that *Ochrobactrum* spp. and a *Brucella* isolate from an African bullfrog utilized a broader range of substrates than *B. microti*, with *Ochrobactrum* strains displaying the highest metabolic versatility. Gentamicin protection assays and fluorescence microscopy confirmed that neither *Brucella* nor *Ochrobactrum* strains replicated intracellularly in amoebae under the tested conditions.

Overall, our findings suggest that amoebae can exert strain-dependent beneficial effects on atypical *Brucella* spp. and *Ochrobactrum* spp. without supporting intracellular replication. Moreover, metabolic versatility may contribute to the environmental persistence of atypical *Brucella* and *Ochrobactrum* strains in amoeba-associated habitats.

PS02.31

Application of a transposon-directed insertion site sequencing approach to identify bacterial factors involved in intracellular survival of *Yersinia enterocolitica*

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Pathogenesis of yersiniosis comprises cell-invasive events in which the bacteria subvert the endocytic-autophagolysosomal pathway of infected cells to create an intracellular niche for bacterial replication. The *Yersinia*-containing, proliferative vacuoles are protected against acidification and display autophagy-related features.

To identify and characterize *Yersinia* factors that may contribute to manipulate the endocytic-autophagolysosomal pathway in epithelial cells, a transposon-based *Yersinia* mutant library was established. High-throughput transposon insertion sites sequencing of bacterial pools before and after cell infection identified conditional essential genes, which are putative virulence factors. The identified genes have metabolic activities in disulfide bond formation (DsbA), glutamine synthesis (GlnA), energy generation (AcnB), or predicted functions in synthesis of carbohydrates of the bacterial outer membrane (Wzx, WebB/C/F). Individual knockout mutants of these genes were generated using a CRISPR Cas12 system and analyzed in *Yersinia*-epithelial cell-interaction. The mutants of the identified genes were impaired in intracellular bacterial survival and replication. Furthermore, these mutants differentially affected autophagy induction, as indicated by LC3 conversion, recruitment of LC3 to the YCVs, and lysosomal acidification of the *Yersinia*-containing vacuoles (YCV) in infected cells. The combined results from these assays indicated for the ΔdsbA mutant the most distinct phenotype. Reconstitution of the ΔdsbA mutant with DsbA restored nearly wildtype phenotypical behaviour in the replication assays. This demonstrates a prominent role DsbA in mediating autophagy and enabling intracellular proliferation of Yersinia. However, further studies are required to characterize target proteins, that are modified and regulated by DsbA, in order to reveal putative novel virulence factors and to specify the importance of DsbA during infection.

Our results in sum suggest that *Yersinia* outer membrane components and metabolic pathways communicate in manifold ways with the host cell to enable efficient intracellular bacterial multiplication.

PS02.32
Strain-dependent pro-inflammatory activation of fibroblast-like synoviocytes by European *Borrelia burgdorferi* sensu lato isolates from Lyme arthritis patients

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Introduction: Lyme borreliosis, caused by spirochetes of the *Borrelia burgdorferi* sensu lato (Bbsl) complex, is the most prevalent tick-borne disease in North America and Europe. A frequent late manifestation is chronic inflammatory Lyme arthritis (LA). In a subset of patients, synovial inflammation persists or worsens despite adequate antibiotic therapy, resulting in antibiotic-refractory LA (ARLA). While LA in North America is caused mainly by *Borrelia burgdorferi* sensu stricto (s.s.), multiple Bbsl genospecies contribute to the disease burden in Europe, suggesting additional complexity in host-pathogen interactions. Although highly inflammatory strains of *B. burgdorferi* s.s. have been associated with ARLA in North America, the inflammatory potential of European Borrelia genospecies and strains remains poorly characterised.

Objectives: This study aimed to investigate the pro-inflammatory potential of European Bbsl strains isolated from LA patients and to characterise the immune responses of human fibroblast-like synoviocytes (HFLS) following infection.

Material and Methods: Seven European Borrelia strains representing the heterogeneity of Bbsl genospecies circulating in Germany were directly isolated from knee joint punctures of patients with LA. HFLS from healthy controls were infected *in vitro* with the different isolates. Host transcriptional responses were analysed by bulk RNA sequencing, followed by principal component analysis and gene set enrichment analysis. In parallel, cytokine and chemokine concentrations in cell culture supernatants were quantified by multiplex bead-based immunoassay.

Results: Distinct Borrelia isolates induced pronounced pro-inflammatory transcriptional responses in HFLS. These responses were characterised by strong upregulation of inflammatory mediators including *IL6, CXCL1, CXCL3, CXCL6, CXCL8, CXCL10* and *CCL2*, accompanied by enrichment of interferon-gamma response pathways and downregulation of MYC target gene sets. Importantly, the transcriptional activation of pro-inflammatory cytokines and chemokines was reflected by increased protein secretion into the cell culture supernatant, as demonstrated by measurements of IL-6, CXCL1, CXCL8, CXCL10 and CCL2. No genospecies-specific differences in the immune response of HFLS were observed. Instead, substantial variability in inflammatory potential was detected at the strain level.

Summary: European Borrelia isolates exhibit marked strain-dependent differences in their capacity to induce inflammatory responses in HFLS, indicating that pathogenicity is primarily driven by strain-specific rather than genospecies-specific characteristics. The induction of an IFN-driven, highly inflammatory phenotype in HFLS may represent an initial step to the pathogenesis of ARLA. Further studies are required to elucidate the underlying mechanisms of host-pathogen interaction, including bacterial adhesion and the potential secretion of additional pathogen-associated molecular patterns.

PS02.33
Decoding the genetic basis of *Listeria monocytogenes* CC1 hypervirulence

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Listeria monocytogenes is a ubiquitous environmental bacterium and the causative agent of severe foodborne infections in humans, associated with high mortality rates. Due to its adaptation to diverse ecological niches, *L. monocytogenes* exhibits high phylogenetic diversity, leading to a broad spectrum of virulence between different sub-lineages, ranging from hypovirulent to hypervirulent subtypes. Previous research on *L. monocytogenes* primarily focused on

hypovirulent reference strains. In a previous project by our group (Fischer *et al.*, 2022), transposon insertion sequencing (Tn-Seq) was used to identify essential genes and virulence-related genes in the hypovirulent reference strain EGD-e. Tn-Seq is based on the depletion of mutants from a transposon mutant library (Tn library) under specific growth conditions and the detection of the transposon insertion sites in the surviving transposon mutants using next generation sequencing (NGS). Since the hypovirulent reference strain EGD-e is poorly suited for the identification of virulence mechanisms, we constructed a Tn library in a hypervirulent clonal complex 1 (CC1) clinical isolate associated with a major listeriosis outbreak with 92,000 unique insertions sites. Cultivation of the CC1 Tn library under standard laboratory growth conditions and analysis of the resulting depletion of transposon mutants led to the identification of 429 essential CC1 genes, which aligns well with the 394 essential genes identified by Tn-Seq in the reference strain EGD-e (Fischer *et al.* 2022). To decode the genetic basis of hypervirulence, we exposed the CC1 transposon library to various infection-mimicking conditions. Infection of mouse macrophages identified 72 genes relevant for intracellular replication in host cells, which include a mixture of known virulence factors, genes for nucleotide and energy metabolism, and some genes of unknown function, which are of value for further analysis. We also infected human hepatocytes to identify genes relevant host cell invasion. The 51 genes we determined as relevant for cell invasion include several surface proteins, most of which have an unknown connection to active cell invasion. Furthermore, the virulence related transcription regulator *prfA* and some genes regulated by PrfA showed severe attenuation in macrophages and hepatocytes. Moreover, we also investigated the growth of the Tn library in human serum, to identify new factors relevant for extracellular survival during human infection conditions. Current results of this project as well as construction and analysis of selected deletion mutants will be presented on this poster.

Fischer MA, Engelgeh T, Rothe P, Fuchs S, Thürmer A, Halbedel S. *Listeria monocytogenes* genes supporting growth under standard laboratory cultivation conditions and during macrophage infection. Genome Res. 2022 Sep 27;32(9):1711-1726. doi: 10.1101/gr.276747.122.

PS02.34

Virulence prediction of *Listeria monocytogenes* isolates using genomic typing data and machine learning

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Listeria monocytogenes is a foodborne bacterium and a facultative pathogen. While immunocompetent patients often only experience non-specific symptoms or remain asymptomatic, immunosuppressed individuals such as the elderly, patients with chronic diseases, newborns and pregnant women may experience symptoms ranging from flu-like illness to sepsis, meningitis or encephalitis [1]. Up to 30% of listeriosis cases in this group are lethal [2].

Different phylogenetic sub-lineages of *L. monocytogenes* show varying degrees of pathogenicity. However, the reason for the observed difference in virulence remains unknown. Therefore, the objective of the GENESIS-LiVe project is to ascertain the differences between strains exhibiting varying degrees of virulence and to develop a pipeline being able to predict virulence potentials of new *L. monocytogenes* isolates based on genomic subtyping data.

340 closed *L. monocytogenes* genomes originating from NCBI and from the Consultant Laboratory for Listeria at the Robert Koch Institute were utilised to create a pangenome multi locus sequence typing scheme (pgMLST). Virulence potentials were determined with reference to the Sequence Type (ST) using literature data. A substantial proportion of the closed genomes along with their associated virulence potential were used as training data for machine learning algorithms (ML). Five different models were used for ML: support vector classifier (SVC) with radial kernel, SVC with linear kernel, simple neural network, gradient boosting classifier and random forest. The remaining closed genomes were used for evaluating the predictive capabilities of the models. Overall, random forest, neural network and gradient boosting classifier led to the best results. Subsequently, genomes from isolates with unknown virulence were annotated using the pgMLST scheme. The trained models were used to predict the virulence of these isolates based on pgMLST data. Three isolates classified as hypovirulent and one classified as hypervirulent were selected for infection experiments to verify the predicted virulence of the models. The infection of hepatocytes demonstrated that the predictions of the models had been accurate. Therefore, the developed ML algorithms are suitable tools for predicting virulence potentials of new *L. monocytogenes* isolates. However, the models continue to demonstrate a certain degree of inaccuracy and will be improved in subsequent iterations.

References:

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PS02.35

***E. faecium* infection induces differential gene regulation in human U937 cells**

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Introduction: Vancomycin-resistant *Enterococcus faecium* (VREfm) is a clinically highly relevant opportunistic pathogen causing healthcare-associated infections, although its interaction with host cells remains poorly understood.

Objectives: In this study, we investigated four recent clinical VREfm isolates (ST80, ST117, and two ST1299 isolates) in human U937 monocytes to analyze bacterial uptake, early host responses, and cytotoxicity.

Materials and Methods: The internalization of the VREfm isolates by U937 cells was examined using imaging flow cytometry. To characterize the early transcriptional response to host-cell contact and internalization, non-internalized bacteria were removed by washing at 1 hour post-infection, and host cell RNA was analyzed at 6 hours post-infection. To assess cytotoxic effects associated with adherent versus intracellular bacteria, viability was examined under the same conditions and in a gentamicin protection assay to eliminate remaining extracellular VREfm.

Results: Imaging flow cytometry demonstrated that all isolates can be internalized by U937 cells. Transcriptome analysis revealed a consistent pattern of differentially regulated genes across all isolates, indicating a shared innate immune and stress-related response. Functional enrichment analyses identified biological processes related to leukocyte activation, cytokine signaling, signal transduction, and RNA stability. At 6 hours post-infection, cell viability was not impaired under either treatment condition. At 24 hours post-infection, moderate cell death was observed under washing conditions, particularly for isolate 7282, whereas gentamicin treatment prevented this effect. In the absence of gentamicin, TNFR-1 deficiency enhanced cell death for three of the four isolates at 24 hours post-infection.

Conclusion: Together, these data show that VREfm triggers an early pro-inflammatory transcriptional response in U937 monocytes without immediate cytotoxicity, while later cytotoxic effects depend on infection condition and bacterial strain.

PS02.36
Novel protein-protein interactions with base proteins of the Helicobacter pylori Cag Type IV secretion system can be involved in the regulation of heptose production

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Initially found as crucial components of the LPS inner core of many gram-negative bacteria, heptose derivatives were recently termed a novel class of microbe-associated molecular patterns (MAMPs). In the host cell, various heptose metabolites, such as ADP-D-glycero-β-D-manno-heptose (ADP-heptose), induce a strong innate immune reaction triggering pro-inflammatory signaling mediated by pattern recognition and activation of the ALPK1-TIFA axis, causing NF-κB activation (1). In *H. pylori*, heptoses can be excessively produced as intermediates of LPS biosynthesis and translocated inside the host cell via the Cag type IV secretion system (CagT4SS) (1,2). Recently we reported evidence, that the carbon storage regulator A (CsrA) plays a role in the regulation of heptose biosynthesis (2). CsrA is a major global post-transcriptional RNA-binding bacterial regulator acting on gene expression by influencing target transcript stability and translation (3). We now hypothesize that novel protein-protein interactions at the base of the CagT4SS are involved in the regulation of heptose production, together with the regulator CsrA.

To define regulatory mechanisms contributing to metabolite production and transport in bacteria and at the interface between host and pathogen, we explored novel protein-protein interactions at the base of the CagT4SS. To that end we use Bacterial Adenylate Cyclase Two Hybrid (BACTH) assays, mutant analyses, transcriptomics and other biochemical and molecular methods. We will present results from the BACTH screen and of transcript regulation in respective *H. pylori* mutants (e.g. Δ*csrA* and *cag*T4SS), revealing evidence for novel protein-protein interactions at the base of the CagT4SS that could exert regulatory effects on heptose production.

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PS02.37
Exploring the effect of sequencial antibiotic exposure on the developlment of antibiotic resistance in E. coli

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Urinary tract infections (UTIs) include any infection of the urethra, bladder, or kidneys. They affect around 400 million people yearly, *Escherichia coli* being the most common pathogen responsible for the infection. Repetitive antibiotic therapies for recurring UTIs promote resistance emergence, accelerating the spread of multidrug-resistant strains. While antibiotic combinations have long been used to treat complicated, multidrug-resistant cases, efficacy remains time- and space-sensitive. Suboptimal combinations risk poor bacterial killing and increased resistance stimulation, which is exactly the opposite desired outcome.

In this study, we integrate evolutionary principles into the in-depth assessment of both simultaneous and sequential antibiotic combinations to identify the optimal protocol that ensures a fast killing of the pathogens with a reduced risk of short- and long-term resistance development. To this end, we screened the effect of combined antibiotic exposures on resistance development to first- and second-line antibiotics used for the treatment of UTIs in hospital settings. We present our preliminary data towards the understanding of the underlying molecular mechanisms behind reduced/increased bacterial killing and potential for resistance development. Ultimately, and beyond UTIs, we aim to establish a framework in which personalized, evolution-aware therapies maximize treatment efficacy, minimize resistance-driven recurrence, and preserve the long-term utility of antibiotics.

PS02.38

Unraveling the role of SecA2-dependent secretion in *Streptococcus pneumoniae*: Molecular and phenotypic characterization of mutants to decipher host-pathogen interactions

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Introduction: The accessory Sec (aSec) system represents a specialized protein export pathway in Gram-positive bacteria, responsible for the secretion of a distinct subset of proteins, such as e.g. serine-rich repeat proteins which are virulence-associated adhesins. In some *Streptococcus pneumoniae* strains, the ATPase SecA2 drives this pathway independently of the canonical Sec machinery. However, the identity and functional relevance of SecA2-dependent effectors during infection remain poorly defined.

Goal: This study aims to systematically identify and characterize SecA2-dependent proteins across clinically relevant pneumococcal strains, including the invasive serotype 4 strain TIGR4 and the colonization-associated serotype 19F strain EF3030.

Material and Methods: Isogenic Δ*secA2* and Δ*secA2*Δ*lytA* mutants are generated, with the latter designed to reduce autolysis-associated artifacts. Comparative analyses of both secreted and cell-associated proteomes are performed. Bacterial cultures are harvested at defined growth stages and subjected to quantitative mass spectrometry to identify differentially abundant proteins linked to SecA2 activity. Candidate effectors are further analyzed for the presence of known secretion signals, with particular focus on proteins lacking canonical motifs, suggesting alternative export mechanisms. To contextualize these findings, bioinformatic analyses leveraging the Global Pneumococcal Sequencing Project (GPSP) database are conducted to associate SecA2-dependent proteins with strain-specific traits related to colonization and invasive disease. Furthermore, an *in vivo Galleria mellonella* model is used to investigate the role of the aSec system in pneumococcal virulence.

Summary: Together, this work will generate a comprehensive characterization of the SecA2-dependent secretome in *S. pneumoniae* and offers new insights into how this specialized secretion system contributes to bacterial adaptation and host infection. These findings are predicted to offer new insights into the mechanistic understanding of the accessory Sec-mediated secretion and may inform future strategies targeting pneumococcal virulence and transmission.

PS02.39

Spoilage in raw milk – The role and effect of lipolytic *Acinetobacter* spp

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Worldwide, 25–30% of economic losses in the dairy industry are attributable to microbial spoilage. The main causes are proteolysis and lipolysis. The lipolytic activity of bacterial strains is attributed to enzymes such as lipases and esterases. Lipases primarily hydrolyze long-chain triglycerides (≥ C10), while esterases hydrolyze short-chain triglycerides (≤ C10). Lipases break down triglycerides in milk, leading to off-flavors that can be perceived as rancid, bitter, or soapy. These enzymatic alterations can lead to a complete loss of the product and thus to massive financial losses. Due to the heat stability of the microbially produced lipases, these enzymes remain a problem even after heat treatment. Research findings show that so-called psychrotrophic microorganisms exhibit lipolytic activity even at low temperatures. Among these psychrotrophic bacteria, strains of the genus *Acinetobacter* exhibit particular strong lipolytic activity even at low temperatures (0–8°C).

In this project, which is being conducted in cooperation with the Max-Rubner-Institute in Kiel, the isolation and characterization of *Acinetobacter* spp. strains in raw milk samples is intended to provide better insight into the lipolytic activity of strains of this genus in raw milk. This is usually done by screening of the hydrolysis of tributyrin in agar. Tributyrin — due to its short fatty acid chain length (C4) — primarily detects esterase activity and not specifically lipase activity. Nevertheless, tributyrin has practical advantages as it is easily emulsifiable and produces clear hydrolysis zones in the agar. Consequently, this test is ideal for a simple and rapid primary screening of general lipolytic activity, but not for a specific assay of lipase activity. Therefore, other substrates such as tricaproin were used.

To address these issues, more specific tests based on changes in fluorescence during the hydrolysis of specific substrates have been developed. Furthermore, using milk as the medium takes into account its specific nutrient composition, resulting in more accurate reflections of natural conditions. Therefore, milk was inoculated and incubated under diverse conditions and enzymatic activity was measured using 4-methylumbelliferyl laurate (4-MUL). Once categorized accordingly, strains most eminent for the dairy industry can be filtered and tested more targeted.

PS02.40

Use of purine adjuvants to resensitize MRSA from the airways of people with cystic fibrosis

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Introduction: *Staphylococcus aureus* remains the most frequently isolated pathogen from respiratory specimens of people with cystic fibrosis (pwCF). Treatment and prognosis are often complicated by the colonization with methicillin-resistant *S. aureus* (MRSA) strains that do not respond to

standard treatment regimens. Development of novel treatments and adjuvants are important measures to combat the rise in antimicrobial resistance. Guanosine (Gua) supplementation was linked to a significant downregulation of intracellular cyclic-di-AMP and showed promising effects in initial testing when combined with beta-lactam antibiotics as well as other substances targeting the thymidine pathway (1).

Goals: To resensitize MRSA isolates from pwCF to beta-Lactam antibiotics, cotrimoxazole (SXT) as well as testing susceptibility to the pyrimidine analogues 5-fluourouracil (5-FU) and 5-fluorouridine (5-FUrd) in combination with Gua.

Methods: Longitudinally isolated MRSA (n=23) from sputum of 4 pwCF as well as 5 reference strains representing different clonal lineages with diverse pheno- and resistotypes were investigated. Disk diffusions, checkerboard assays and time-kill-curves were performed to assess potential synergistic effects between Gua and our tested substances.

Results: 21.7% (5/23) of clinical strains as well as reference strains USA300 and JE2 became susceptible to oxacillin (OX) with the addition of Gua. 5-FU and 5-FUrd showed strong antimicrobial effects on most of our isolates and the effect was further potentiated by Gua. While SXT supplementation did not enhance susceptibility in previously already susceptible isolates, 3/4 resistant strains appeared susceptible upon Gua exposure. Supplementation of Gua decreased OX minimum inhibitory concentration (MIC) in all tested isolates. The magnitude of change varied between strains. When exposed to combinations of 5-FU(-rd) and Gua, the MICs of some strains were reduced while others showed enhanced resistance. Gua was able to enhance synergy between OX and 5-FU, evident from a decrease in fractional inhibitory concentration index.

Conclusion: Gua is able to enhance susceptibilty to our tested substances in some responding strains but has minimal or no effect on others. The association of this phenotypic resistance or susceptibilty to genetic data is necessary, and ongoing work will address this. Gua remains a promising adjuvant and the manipulation of intracellular cyclic-di-AMP levels a potential target for the future development of antimicrobial therapeutics in the treatment of MRSA.

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PS02.41

Immune recognition of the *Pseudomonas aeruginosa* type VI secretion systems during infection

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Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen that occupies a wide range of ecological niches and causes a variety of acute and chronic infections, particularly in the lungs of cystic fibrosis (CF) patients. One factor that helps *P. aeruginosa* to establish and maintain its niche is the type VI secretion system (T6SS), a contractile nanomachine that translocates effector proteins into the extracellular environment as well as into competing bacteria and host cells. The three most prevalent T6SS subtypes, H1-, H2-, and H3-T6SS, fulfil distinct roles in interbacterial competition and host interaction. While their effectors are well characterized, T6SS activity during chronic infection and the adaptive immune response against T6SS components remain poorly understood. To investigate the anti-T6SS immune response, we collected serum from a cohort of CF patients and performed an ELISA to measure anti-T6SS antibody titers. A detailed analysis of the antibody subtypes revealed inter-individual differences between patients and an overall dominant immune response of the subtype IgG in support of a chronic infection and long-lasting immunity. Overall, our findings provide insights into the anti-T6SS immune response during *P. aeruginosa* infection under the physiological conditions of the CF lung and establish links between T6SS-associated immune responses and clinical parameters. These findings may support the identification of novel diagnostic markers and therapeutic targets for chronic *P. aeruginosa* infections in CF patients.

PS02.42

Beyond single species: Establishing a polymicrobial *in-vitro* biofilm model to study infective endocarditis

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Infective endocarditis is a life-threatening infection of the endocardium and heart valves, caused by biofilm-forming pathogens such as *staphylococci*, *streptococci,* and *enterococci.* With a prevalence of 5.9 %, polymicrobial endocarditis is rarely diagnosed and has received little attention in the literature. Existing approaches focus on single-species biofilms and their targeted antibiotic strategies. Since polymicrobial biofilms exhibit increased antibiotic resistance due to synergistic interactions among pathogens, there is an urgent need for suitable *in-vitro* models. We established a static polymicrobial *in-vitro* biofilm model of infective endocarditis. The spatial distribution and community dynamics of the constituent bacterial species were characterized using visual and molecular biological methods. The model will subsequently be translated into a dynamic system and serve as a testing platform for antimicrobial. This work was supported by TAB-funded projects "GUARDIAN" (2024 FGR 0045).

PS02.43

Expression of DPR in *Streptococcus suis* is crucial for survival in blood and protection against reactive oxygen species

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Streptococcus suis (S. suis) is a common porcine pathogen with zoonotic potential causing meningitis, arthritis and septicemia. It expresses a Dps-like peroxide resistance

protein, called Dpr, which prevents production of reactive oxygen species (ROS) by inhibiting the Fenton-reaction (H_2O_2 + Fe^{2+} -> $\cdot OH$ + OH^- + Fe^{3+}), as it is capable of binding free iron. In this study we want to understand the role of Dpr in protection of *S. suis* against oxidative burst generated by neutrophilic granulicytes.

Two *S. suis* strains and their Dpr-mutants were used to compare their sensitivity to H^2O^2. To assess the role of free iron, deferoxamine-mesylate (DFOM) was used as an iron chelator. Furthermore, we investigated the ability of *S. suis* and its isogenic *dpr*-mutant to survive in porcine blood and measured the number of granulocytes associated with the streptococci and their ROS-production by flow cytometry.

The presence of H_2O_2 led to significantly more killing of the Dpr-mutant compared to the wildtype (WT), which was reversed by the addition of DFOM. In porcine blood the WT was able to proliferate while the mutant was killed. The number of granulocytes associated with the bacteria and producing ROS was higher for the mutant than for the WT strain.

In summary, our results indicate an important role of Dpr in protection against H_2O_2 in the presence of iron and for the survival of *S. suis* in porcine blood, putatively by reducing phagocytosis and oxidative burst of granulocytes.

PS02.44
Investigation of the autochthonous microbiota of parchment

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Parchment production involves a series of distinct processing steps. These typically include skinning, soaking, brining, hair removal, stretching, and the final scraping of remaining residue. The animal skins used for parchment are usually derived from cattle, sheep or goats. The structural integrity of parchment is provided by collagen fibers, which, during production, are partially denatured and hydrolyzed, forming a rigid gelatinous network. However, parchment deterioration, and exposure to high temperatures can compromise the integrity of the manuscripts, leading to their decay. In addition to environmental conditions, the influence of microorganisms is one of the main causes of this deterioration. To better counteract microbial decay, it is necessary to gain an understanding of the microbiota of parchment.

The present study was conducted to characterize the autochthonous microbiota of parchment with a focus on microorganisms that may be involved in decay. The parchment manuscript fragments dated from the ninth to 11th centuries CE and were originally part of the library of the Great Mosque of Kairouan and are now housed at the National Laboratory for the Preservation and Conservation of Parchment and Manuscripts in Raqqada, Kairouan, Tunisia. Twelve parchment manuscript fragments showing symptoms of decay were analyzed by amplicon sequencing focusing on the hypervariable regions V3 and V4 of the 16S rRNA gene and the ITS2 region for fungal species. The five most abundant fungal genera were *Aspergillus*, *Alternaria*, *Plectosphaerella*, *Wallemia*, and *Fusarium*, while the five most abundant bacterial genera were *Bacillus*, *Skermanella*, *Streptococcus*, *Metabacillus*, and *Cytobacillus*. The key organisms that can contribute to the decay of written artefacts are those that can form collagenases. The main genera in this context, *Bacillus* and *Clostridium*, were both found within the microbiota of the parchment samples analyzed, with *Bacillus* being the most prevalent genus.

This procedure contains two critical steps which may introduce bias into the results, namely the parchment swabbing for sample acquisition as well as the DNA isolation. Therefore, different swab materials and DNA isolation procedures were compared.

This study underlines the importance of a comprehensive understanding of the microbiota of parchment in order to counteract microbial decay. It can be regarded as a first step in building a database that can be used as an early-warning system detecting potential problematic decay inducing microorganisms on parchment.

PS02.45
Adjuvant-mediated resensitization of clinically derived methicillin-resistant *Staphylococcus aureus* isolates

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Introduction: The rise of methicillin-resistant *Staphylococcus aureus* (MRSA) represents one of the paradigmatic challenges of the antibiotic era. Through decades of excessive antibiotics use, antimicrobial resistance has become increasingly evident and is a major health threat. Recently, it was proven that supplementation of purine analogue can interfere with intracellular metabolic pathways, enhancing bacterial susceptibility towards antibiotics against which resistance has been developed (1). The objective of this research is to enable a renewed use of previously discarded antibiotic due to the development of substantial resistance.

Methods: The purine analogue guanosine (GUA) has been used in the present study as an adjuvant during antimicrobial susceptibility testing of β-lactam oxacillin (OX), antifolate agents trimethoprim (TMP), sulfamethoxazole (SMX) and cotrimoxazole (SXT) as well as the pyrimidine analogues 5-fluorouracil (5-FU) and 5-fluorouridine (5-FUrd). A total of 48 clinically derived MRSA isolates of diverse origins (bronchoalveolar lavage, blood cultures and tissue samples) alongside six reference strains were initially tested via disc diffusion assays, both in the presence and absence of GUA. Selected clinical MRSA and reference strains were further characterized using checkerboards assays to test for potential synergistic effects. Kill curve assays were additionally performed to assess the time-dependent killing kinetics of guanosine-antibiotic combinations.

Results: 29.2% of the clinical MRSA isolates (14/48) responded susceptible to OX by the addition of GUA. 5-FU and 5-FUrd demonstrated an active antimicrobial effect which was increased by GUA. MRSA resistant to TMP (n=17/35.4%) and Cotrimoxazole (n=3/6.3%) did not respond to combined treatment with GUA. On the contrary, the majority of the strains showed a mean reduction in inhibition zone diameter for all tested antifolate antibiotics. A synergistic effect was observed

for OX in combination with GUA with an approximately 4-fold reduction of the OX-MIC. Combination of OX with 5-FUrd resulted in an additive effect regardless of GUA supplementation, except for the reference ATCC-43300, for which a synergistic effect was observed.

Conclusion: The addition of guanosine revealed a potential strategy to resensitize MRSA strains towards oxacillin, providing an insight into a possible alternative to conventional antibiotic therapy and a viable approach to combat the progression of antimicrobial resistance. Prospectively, the tested antibiotic spectrum could be expanded and mechanistic analyses through whole-genome sequencing could further delineate correlations between genotypic alterations and phenotypic resistance under adjuvant supplementation.

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PS02.46

Determination of neuroinvasiveness of clinical *Borrelia* isolates using a human blood-brain barrier model

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Introduction: Neuroborreliosis is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex. While some *Borrelia* spp. exhibit pronounced neurotropism, the mechanisms underlying their ability to invade the central nervous system (CNS) remain incompletely understood. Since traversal of the blood-brain barrier (BBB) is a prerequisite for CNS entry, endothelial adhesion and barrier transmigration are likely key determinants for pathogenic processes.

Material and Methods: An *in vitro* BBB model was established using human brain microvascular endothelial cells (HBMECs) cultured in a 2D transwell system. *Borrelia* patient isolates originating from skin or cerebrospinal fluid (CSF) were used to assess bacterial adhesion and endothelial transmigration. Adhesion to HBMECs was quantified by immunofluorescence microscopy and qPCR, transmigration by qPCR and barrier integrity post-infection was assessed by measuring paracellular permeability with fluorescently labelled dextrans (70 kDa).

Results: Infection assays demonstrated strain- and species-specific differences in adhesion and traversal of the BBB model. CSF-derived clinical isolates showed significantly higher adhesion and translocation capacities than skin-derived isolates. Among the tested species, *B. garinii* and *B. bavariensis* exhibited the highest EC-invasion rates, whereas *B. afzelii* and *B. burgdorferi* sensu stricto displayed lower BBB interaction. In addition, infection with *B. garinii* was associated with increased BBB permeability, indicating disruption of barrier integrity.

Conclusion: Our findings demonstrate that *Borrelia* adhesion and translocation across the brain endothelium are strain-specific and may correlate with their clinically relevant neuroinvasive potential.

PS02.47

Within-host diversity and clonality in patients with *Enterococcus faecium* bloodstream infections: From colonization to infection

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Background: *Enterococcus faecium* is a frequent causative pathogen for bloodstream infections, particularly in immunocompromised patients. Most infections are endogenous infections and originate from the intestinal tract. Clinical risk factors for the development of endogenous bloodstream infections (BSI) with *E. faecium* are well studied, however knowledge is lacking concerning a strain specific virulence for the development of endogenous *E. faecium*-BSI. Especially given the epidemiological dominance of multilocus sequence typing (MLST) sequence types (ST) ST80 and ST117 in *E. faecium* infections in parts of Germany in recent years, the question arises as to whether strains of these two sequence types are more likely to cause invasive infections than other strains. In this study, we analyse the within-host diversity and clonality of intestinal and invasive *E. faecium* isolates in patients with *E. faecium* BSI.

Methods: Patients with *E. faecium* bloodstream infections were recruited between January 2023 and May 2024 at the University Hospital Münster. A pre incubated blood sample and an anal swab or stool sample from each patient were plated on Columbia CAP agar and checked for enterococcal grows. Five colonies that showed a typical morphology for *E. faecium* were randomly picked from the stool or anal culture. One colony was picked from the blood culture sample. All isolates were subjected to whole genome sequencing. Clonality was analysed using the core genome multilocus sequence typing (cgMLST). Isolates with an allele distance of ≤ 4 were considered as one single clone.

Results: In total, 138 *E. faecium* isolates (24 blood isolates, 114 anal/stool isolates) of 24 patients were included into the analysis. Clinically, 16 patients had an underlying (hematological-)oncological condition, four had liver cirrhosis, three had acute cholecystitis and one had cardiac surgery. ST117 (n=18, 75%) and ST80 (n=6, 25%) were the only MLST ST that were found in the blood samples. In approximately half of the patients (n=11, 46%) only one single *E. faecium* clone was detected in the blood culture isolate and all five intestinal isolates. Seven (29%) patients exhibited a polyclonal colonization of the intestine in which the blood culture isolate represented a clone of at least one intestinal isolate. Among these patients, a median of three out of five (mean=2.6) intestinal isolates were clonally related to the blood culture isolate. The blood culture isolates in those seven patients were genetically diverse, with four different core genome MLST cluster types (ST117 CT929, ST80 CT1470, ST117 CT2505,

ST80 CT2680). No clonality between the blood culture isolate and the intestinal isolates were found in six (25%) patients.

Conclusion: Endogenous infection from the intestine was the dominant route of infection in most patients and were caused exclusively by the ST80 and ST117 strains. No selection of specific *E. faecium* stains was observed in polyclonal intestinal colonization.

PS02.48

Influence of *Streptococcus suis* suilysin and D-alanine-D-alanyl carrier ligase on neutrophil effector functions under innate immune conditions

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Streptococcus suis (*S. suis*) is one of the most important pathogens in modern swine industry. While the bacterium is detected frequently in the upper respiratory tract of clinically healthy animals, it can also cause severe disease including septicemia and meningitis. Many of its virulence factors have been investigated, but the full picture of pathogenesis remains poorly understood. Among those factors known to modulate the host immune response are the toxin suilysin (SLY) and the D-alanylation of lipoteichoic acids in the bacterial cell wall, implemented by the enzyme D-alanine-D-alanyl carrier ligase (DltA).

We investigated the influence of both virulence factors on different neutrophil effector functions under innate immune conditions. For this we infected isolated porcine blood polymorphonuclear cells (PMN) with *S. suis* strain 10 and respective deletion mutants in the presence of colostrum deprived serum. Degranulation, oxidative burst and release of neutrophil extracellular traps, as important neutrophil effector functions against bacterial infections, were chosen as read-out functions.

Our results show that porcine PMN do not release myeloperoxidase from intracellular granules in response to infection with *S. suis* under innate immune conditions, independent of the presence of DltA or SLY. Similarly, no extracellular release of reactive oxygen species (ROS) could be observed. For intracellular ROS we found no significant production in response to wild-type *S. suis* or SLY-deficient mutants, while DltA-deficient mutants stimulated increased production. This result indicates a role for DltA, but not SLY, in modulating ROS production by PMN under innate immune conditions.

PS02.49

Invasion and intracellular development of UPEC in bladder epithelial cells is linked to glucose availability during *in vitro* infection

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Introduction: Uropathogenic *Escherichia coli* (UPEC) account for the majority of urinary tract infections (UTI). When UPEC infect the human bladder, bacteria cannot only replicate in the bladder lumen, but also invade superficial bladder cells. Intracellular UPEC are able to replicate intracellularly and to develop into intracellular bacterial communities (IBCs). The development of IBCs protects the bacteria from the immune system, treatment by antimicrobial agents and is one reason for recurrent UTIs. In order to replicate intracellularly bacteria have to scavenge nutrients from the host cell and escape detrimental effects of phagolysosomes. If UPEC survive and replicate intracellularly they might exit the host cells and start a second infection cycle.

Goals: Our aims are to (1) identify conditions influencing the invasion efficacy of UPEC, (2) linking the energy status of the host cell with the uptake frequency of UPEC and subsequent intracellular replication of bacteria.

Materials and Methods: We use an *in vitro* bladder epithelial model employing RT-112 bladder epithelial cells. After infection of RT-112 cells with UPEC, the invasion, intracellular survival and the escape of the bacteria from the cells were quantified. We influenced the infection by the glucose concentration in the cell culture medium during and after infection as well as by inhibition of glucose uptake by the host cells. On the host cell side, we determined ATP levels as a marker of the cells´ energy status. Also, the influence of UPEC on the mitochondrial metabolism was analyzed.

Results: We studied the influence of different glucose concentrations on the invasion of bladder cells by UPEC and the subsequent development of IBCs. We infected bladder epithelial cells with two different UPEC model isolates. Our results show that invasion of host cells by UPEC is drastically reduced in glucose-deficient medium. By employing inhibitors of glucose transport, we showed that UPEC invasion efficacy corresponds to glucose uptake. Our results also show that infection of bladder cells by UPEC negatively influenced the energy status and glycogen levels of host cells. We could also show a UPEC strain-specific influence on the mitochondrial metabolism of infected cells.

Discussion: Our results show that the invasion and intracellular development of UPEC is affected by the availability of glucose as the carbon source . Interestingly, the two model UPEC strains differ in their impact on the mitochondrial metabolism of the host cells. The mechanism and consequences how glucose availability affects the uptake of UPEC and their intracellular live cycle remains to be elucidated.

PS02.50

Characterization of *Staphylococcus aureus*-induced remodeling of the host ubiquitination machinery in periprosthetic joint infections

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Staphylococcus aureus is a leading cause of acute and chronic musculoskeletal infections, playing a central role in the pathogenesis of periprosthetic joint infections (PJI). A key factor in the chronicity of these infections is the ability of *S. aureus* to invade host cells and persist intracellularly, allowing the bacterium to evade both the host immune response and systemic antimicrobial therapy. The elimination of intracellular pathogens relies on specialized host defense mechanisms, among which the ubiquitination machinery is critical for the recognition and degradation of intracellular bacteria. However, the specific ubiquitinating enzymes involved in the response to *S. aureus* within bone and adjacent soft tissue infections remain poorly characterized.

In this study, we analyze the expression of selected ubiquitinating enzymes in soft tissue samples from patients with periprosthetic joint infections and *S. aureus*-infected osteoblast cell lines. Using qPCR and immunoblotting, we assess these enzymes at both the mRNA and protein levels. First results reveal significant alterations in the expression levels of ubiquitinating enzymes in clinical samples, suggesting their involvement in the host response to bone and joint infections and pointing to a role of the complex bone microenvironment in the regulation of ubiquitination and associated cellular pathways.

Collectively, these findings will provide a foundation for a deeper mechanistic understanding of how host-response ubiquitination signatures influence *S. aureus* clearance or persistence and suggest that targeting these enzymes may offer novel therapeutic strategies for the eradication of chronic bone infections and foreign body infections.

PS02.51

Exploring host deubiquitinases during *S. aureus* infection

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Introduction: Intracellular survival represents a key immune evasion strategy of *S. aureus*, shielding the pathogen from conventional antibiotics within host cells. Clearance of intracellular bacteria relies on ubiquitin-dependent host defence mechanisms, including E3 ligase-mediated ubiquitination of the bacterial surface and subsequent selective autophagy. This cascade is counterbalanced by deubiquitinating enzymes (DUBs) that remove ubiquitin modifications and thereby critically regulate the intracellular antibacterial immune response. Recent evidence suggests that *S. aureus* serine protease-like proteins (Spls) can proteolytically cleave host DUBs (1), yet the full scope of infection-driven DUB dysregulation and its functional consequences remain poorly understood.

Methods: Proteomic analysis of macrophage-like THP-1 cells infected with *S. aureus* USA300 was performed via LC-MS. In parallel, changes in DUB abundance were assessed by immunoblotting during infection. To evaluate the effect of *S. aureus* Spls on DUB abundance, two USA300 strains were used: wild-type USA300 and a USA300 strain lacking all Spls. DUB abundance changes were compared across both infection conditions to validate Spl-mediated cleavage of candidates including USP7, OTUD7B, and UCHL3.

Results: Macrophage-like THP-1 cells infected with *S. aureus* USA300 showed widespread changes in DUB abundance, with the majority of DUBs being downregulated, compared to uninfected cells. In addition to the previously validated Spl cleavage substrates (USP7, OTUD7B, and UCHL3), immunoblotting analysis identified four novel candidates USP13, VCPIP1, OTUB1 and USP10 whose abundance was reduced during infection. Whether the downregulation of these candidates is due to direct Spl-mediated cleavage or other infection-related mechanisms remains to be determined.

Summary: The obtained results demonstrate that specific DUBs are critical components of the host response to *S. aureus* in THP-1 macrophages. The observation that *S. aureus* proteases influence DUB stability indicates a targeted bacterial strategy to neutralize the host's ubiquitination machinery. By uncovering this mechanism of possible immune evasion, our study suggests an intrinsic role for the ubiquitination system in the defence against intracellular *S. aureus* and contributes to the broader understanding of host-pathogen interactions.

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PS02.52

Establishment of a cell culture model to study *Neisseria gonorrhoeae*-immune cell interaction

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Neisseria gonorrhoeae (GO), a Gram-negative, aerobic diplococcal bacterium, is the causative agent of the sexually transmitted infection gonorrhea. Many infections remain asymptomatic and resolve spontaneously; however, if left untreated, severe disease courses may occur, including bacterial dissemination and subsequent infertility. Worldwide, gonorrhea is the third most common sexually transmitted infection (STI). Although gonorrhea is still generally treatable with antibiotics, the number of reported GO infections exhibiting reduced susceptibility to the commonly used antibiotics ceftriaxone, cefixime, or azithromycin has steadily

increased since mandatory reporting was introduced in 2020, rising from 397 cases in 2021 to 1,111 cases in 2024 (Selb et al., Epid Bull 2023;45:3-20, DOI 10.25646/11749; Selb et al., Epid Bull 2025;38:3-23, DOI 10.25646/13419.2).

Despite extensive genomic characterization of clinical isolates, phenotypic analyses regarding pathogenicity-associated traits remain insufficiently addressed, not least because the corresponding *in vitro* assays are often technically demanding. The aim of this project is therefore to establish cell culture–based assays that enable the comparative analysis of different clinical GO isolates with manageable experimental effort.

To achieve a standardized and reproducible comparison between different GO isolates, the two human neutrophil-like cell lines (NLCs) PLB-985 and HL-60 were selected to establish a model for the investigation of GO phagocytosis and intracellular survival. The use of cell lines circumvents donor-dependent variability associated with primary neutrophils. As inoculum, a GO suspension grown in GCLB medium was used, which ensured reproducible bacterial fitness upon reaching a defined OD600. The corresponding CFU of the inoculum was determined by plating on PVX agar plates. Following synchronized infection by centrifugation at 10°C, NLCs were incubated with GO for 30 minutes at 37°C, after which extracellular bacteria were eliminated by gentamicin treatment. The number of intracellularly surviving GO was subsequently determined by plating on PVX plates.

Initially, different differentiation protocols for the NLCs were evaluated to induce a phagocytic capacity which enables following comparative experiments. Stimulation of HL-60 cells for five days with DMSO, Nutridoma, and ATRA induced clearly measurable phagocytosis of GO, whereas this could not be achieved in PLB-985 cells. Treatment with cytochalasin D, an inhibitor of phagocytosis, reduced the recovery of viable intracellular bacteria to nearly zero. Clinical isolates exhibited distinct recovery rates compared to the ATCC laboratory strain as well as among each other. Further investigations are required to determine whether these differences are attributable to evasion of phagocytosis or to increased susceptibility to intracellular killing mechanisms.

PS02.53
Discovery of compounds with previously undescribed host-dependent intracellular antimicrobial activity using an automated in vitro cystitis screening platform

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Background: Intracellular infection stages contribute to acute disease, persistence, recurrence and treatment failure in many infectious diseases[1]. Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and represent a clinically relevant disease with well-established intracellular bacterial infection stages[2-6]. However, most antimicrobial discovery approaches rely on extracellular screening conditions and therefore fail to identify host-dependent antimicrobial activities that emerge only in the context of infection. We established an automated infection-context screening platform to identify compounds with previously undescribed intracellular antimicrobial activity.

Methods: Human bladder epithelial cells (HTB-9) were infected with a luminescent *Klebsiella pneumoniae* strain (RB120). Following bacterial uptake, extracellular bacteria were eliminated using gentamicin, allowing intracellular reservoirs to establish. A total of 10,400 compounds from a structurally diverse synthetic small-molecule library were screened using an automated high-throughput infection assay. Intracellular bacterial survival was quantified by luminescence following host-cell lysis and bacterial outgrowth. Hits were validated using CFU-based secondary assays, including dose–response analyses, time-kill experiments, cytotoxicity testing, evaluation of activity against multidrug-resistant strains, and assessment of extracellular antibacterial activity under axenic growth conditions.

Results: The screening platform demonstrated excellent robustness, with *Z′*-factor values consistently exceeding 0.7. Screening of 10,400 compounds identified three compounds that were confirmed in secondary assays as exhibiting previously undescribed intracellular antimicrobial activity. These compounds showed potent dose-dependent activity without detectable host-cell toxicity and retained activity against carbapenem-resistant *K. pneumoniae* strains. Notably, none of the compounds displayed antibacterial activity against extracellular bacteria under axenic conditions, suggesting a host-dependent mode of action distinct from that of classical antibiotics. Conclusion: Infection-context screening enables the identification of antimicrobial activities that remain undetected in conventional extracellular assays. Using an intracellular UTI model, we identified three compounds with previously undescribed host-dependent intracellular antimicrobial activity. These findings highlight intracellular infection stages as promising targets for antimicrobial intervention and support infection-based screening approaches as a strategy to expand antimicrobial discovery beyond conventional antibiotics.

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PS02.54
Construction of *L. pneumophila* multi-phospholipase knockdown strains

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Introduction: *Legionella pneumophila*, an intracellularly replicating lung pathogen, expresses 19 phospholipases (PLs), some of which are known or predicted virulence factors. One example is VipD, a Dot/Icm-translocated patatin-like phospholipase activated by Rab5/Rab22. VipD targets endosomal membranes, removes PI(3)P, and blocks endosome fusion with the Legionella-containing vacuole (LCV), thereby helping *Legionella* escape degradation. Other PLs require activation by cofactor binding or proteolytic processing, including PlcABC, PlaA, and PlaC (1). Although some *Legionella* PLs have been linked to infection, individual PL

deletions do not strongly attenuate virulence in host-cell models, likely due to functional redundancy (2). Therefore, simultaneous depletion of the entire phospholipasome or selected PL groups is a promising strategy. To address this, we considered using a recently described multiplexed CRISPR interference system for *Legionella* (3).

Aim: CRISPRi enables precise, reversible, and programmable repression of bacterial genes without altering the DNA sequence, making it well suited for simultaneous multi-gene control with low toxicity. We generated three *L. pneumophila* CRISPRi knockdown strains targeting different phospholipase sets: 19, eight, or 15 PL genes. After validating knockdown efficiency by enzyme activity assays and RNA-seq, we performed an initial phenotypic analysis of intracellular growth in the environmental host *Acanthamoeba castellanii*.

Methods and Results: We successfully constructed three *L. pneumophila* knockdown strains targeting 19, 8, or 15 phospholipases. Transcriptome profiling showed efficient knockdown of 12, 7, or 10 target genes, corresponding to 60%, 88%, and 67% efficiency. This included efficient depletion of all characterized PLAs and PLCs contributing to the major catalytic activities of these groups. Fatty acid release assays using palmitoylphosphatidyl-glycerol and thin-layer chromatography confirmed nearly absent cell-associated PLA and PLC activities in the respective strains.

All strains grew normally in broth and proliferated intracellularly in *A. castellanii*. However, real-time monitoring of amoeba infections using a luciferase system (3) revealed delayed host-cell exit when 12 phospholipase genes were simultaneously depleted. Thus, simultaneous depletion of most *Legionella* phospholipases does not impair extracellular growth but suggests a role of the phospholipasome in host-cell exit. Future work will further investigate its involvement in this process.

Summary: We demonstrate CRISPRi-mediated simultaneous knockdown of up to 12 *L. pneumophila* phospholipases. Our data show that these PLs are dispensable for in vitro growth but suggest a role in host-cell exit.

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PS02.55

An NAD(H)-depletion-activated phospholipase promotes fluoroquinolone-mediated bacterial killing and autolysis

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Introduction: We recently discovered an unusual mechanism that controls the activity of the *Legionella pneumophila* phospholipase and virulence factor, PlaB (1). Physiological concentrations of NAD(H) stabilize inactive PlaB tetramers; however, low NAD(H) levels trigger tetramer deoligomerization into highly active protein dimers. These dimers pose a threat to *Legionella* when not localized at the surface of the bacterium because the cell membranes are no longer protected from PlaB's phospholipase activity. Bacterial NAD(H) can be targeted and degraded by NAD-hydrolyzing enzymes, such as NADases. Recent studies have shown that genotoxic stress induced by the fluoroquinolone antibiotic ciprofloxacin activates the GndRX NADase toxin-antitoxin module in *L. pneumophila*, which efficiently depletes cytosolic NAD(H) (2). Based on these findings, we hypothesized that the efficient killing and lysis of *L. pneumophila* by fluoroquinolones depends on PlaB. This makes the phospholipase a potential drug target.

Aim: Does the presence and activation of PlaB improve killing of *L. pneumophila* in cultures and infected host cells upon fluoroquinolone exposure?

Methods and Results: Consistent with the idea that NAD(H) is central to the regulation of PlaB's activation status, our gene co-occurrence studies revealed the conservation of *plaB* with genes encoding NAD(H) consumers, suggesting a functional link. Indeed, the co-expression of an NADase toxin and PlaB in *E. coli* and *L. pneumophila* resulted in depletion of cytosolic NAD(H) and accelerated bacterial cell death. Lipid analysis of *E. coli* expression cultures demonstrates that activation of cytosolic PlaB activity results in membrane phospholipid digestion, suggesting that toxin-driven NAD(H) depletion can kill by inducing self-digestion when PlaB is present. Gene expression analysis in *L. pneumophila* revealed the co-regulation of *plaB*, toxin, and antitoxin genes. All these genes are induced by fluoroquinolone exposure (2) and prolonged stress. Our results support a model in which PlaB is functionally linked with an NADase effector module as part of its stress response. Taking together, our findings suggest that PlaB activation could be exploited to sensitize *L. pneumophila* to better control it with antibiotic regimens in the future (Fig. 1).

Summary: PlaB can act as a cell death effector and autolysin when cytosolic NAD(H) levels are low, making it a promising future drug target candidate. PlaB co-occurs with NADase genes in *Legionella pneumophila*, among other organisms.

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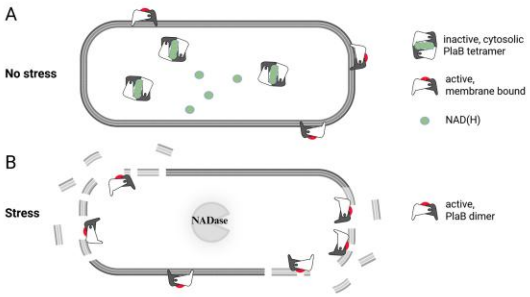
Fig. 1 Proposed model of active surface-associated and inactive cytosolic PlaB when (A) no stress, such as fluoroquinolone treatment, is present and (B) PlaB-driven autolysis under fluoroquinolone stress.

Fig. 1



PS02.56

A zoonotic *Streptococcus equi* subsp. *zooepidemicus* strain survives in activated blood neutrophils of pigs through SzM expression

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Introduction: *Streptococcus equi* subsp. *zooepidemicus* (SEZ) causes diseases in various animals and humans. In pigs, it is an emerging pathogen that causes septicemia and other severe pathologies. SEZ expresses the M-like protein SzM, which recruits host proteins such as fibrinogen to the bacterial surface.

The objective of this study was to investigate the survival mechanisms of a zoonotic SEZ strain in porcine blood. The zoonotic SEZ C33 strain proliferated in porcine blood of 10-week-old piglets *in vitro* despite comparably high levels of IgM binding to the bacterial surface.

Methods and Results: Comparison with the isogenic *szm* deletion mutant showed that SzM_C33 expression is crucial for porcine blood survival. Flow cytometry analysis revealed that SEZ C33 was associated with porcine blood granulocytes, exhibiting a prominent generation of reactive oxygen species. Cytochalasin D treatment significantly inhibited this association. Furthermore, we found viable intracellular SEZ C33 in a penicillin/streptomycin protection assay in porcine blood and purified porcine granulocytes, in agreement with the microscopic detection of streptococci in neutrophils. Experimental infection with SEZ C33 confirmed the virulence of this strain in pigs, including one case of fibrino-suppurative meningoencephalitis and arthritis of the tarsal joint.

Summary: In conclusion, SEZ C33, originally isolated from a diseased human, demonstrated SzM-mediated intracellular survival in porcine blood granulocytes *in vitro* and is most likely involved in the pathogenesis of diseases such as meningitis and arthritis *in vivo*.

PS02.57

Killing of *Rodentibacter heylii* in blood and reduced biofilm formation in tracheonasal washes after immunization with the RTX-protein RhiA depends on the application route and adjuvant

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Introduction: *Rodentibacter heylii (R. heylii)* is a frequently detected pathogen causing pneumonia, abscesses and mastitis in laboratory mice. In a recent infection experiment, we observed an unexpected high virulence of a strain harboring the new RTX-protein RhiA. Efficient colonization of the upper respiratory tract was detected 28 days after experimental infection. Immunohistology investigations indicated expression of RhiA in biofilms on the respiratory epithelium.

Objectives: This study investigates the working hypothesis that RhiA-based vaccinations with different adjuvants and application routes lead to distinct immune response and differences in protection against biofilm formation.

Materials and Methods: Different domains of RhiA were recombinantly expressed followed by verifying the immunogenicity by immunoblot analysis using convalescent sera. Biofilm assays with rRhiA_B specific IgY were conducted to investigate the role of rRhiA in biofilm formation. Furthermore, mice were vaccinated with rRhiA_B and different adjuvants and application routes: AdDP (intra nasal.), QS-21 (subcutan) or Alhydrogel (subcutan). Read out parameters included analysis of rRhiA_B specific IgG subtypes and IgA as well as induction of cytokines in splenocytes after stimulation with rRhiA_B. Bactericidal assays and challenge experiments are in progress to read out protective immunity.

Results: RhiA_B (aa 1460-2077) was immunogenic, as IgG from reconvalescent sera bound only on this protein in the immunoblot. Preincubation of bacteria with anti rRhiA_B IgY antibodies reduced biofilm formation as measured in the crystal violet assay and CFU quantification. Mice intranasally immunized with AdDP as adjuvant showed increased specific IgA levels in their tracheonasal washes, while subcutan applications led to high IgG levels. The RhiA vaccine with QS-21 induced high specific IgG2b and IgG2c levels, whereas no specific IgG2b or IgG2c could be detected when Alumn was used as adjuvant. Additionally, IgG3 levels were much higher after QS-21 immunization than after Alumn. Stimulation of splenocytes with rRhiA_B led to IFNgamma production in all RhiA vaccinated groups, whereas TNFalpha was significantly higher in Alumn and QS-21 groups than in the intranasal group, independent of the use of rRhiA_B. In vitro biofilm formation was found to be reduced in tracheonasal washes of intranasally vaccinated mice with high specific IgA. Efficient killing of *R. heylii* in blood was only observed in the Alumn (high specific IgG1) but not in the QS-21 (high specific IgG2b and IgG2c levels) group.

Summary: Vaccination with rRhiA_B led to different antibody profiles, depending on the used adjuvant and application route. Though anti-RhiA IgY antibodies inhibited biofilm formation in vitro, mucosal immunization with RhiA did not prevent efficient colonization of the respiratory tract. Induction of high IgG1 and IgG3 levels but not IgG2b and IgG2c was associated with systemic bactericidal immunity.

PS02.58

Metagenome and metatranscriptome analysis of wound debris in infected chronic wounds

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Aim: Wound biofilms are detected in more than 75% of chronic wounds and represent a major challenge in wound healing. These microbial "fortresses", often composed of different bacterial species, are highly tolerant to antimicrobial treatment. When various microorganisms enter a wound, they not only interact with human tissue but also with each other through synergistic or competitive mechanisms. In clinical routine, wound swabs and antibiograms are commonly used; however, it is known that approximately 99.8% of microorganisms cannot be routinely cultivated. Metagenome and metatranscriptome analyses therefore provide deeper and more comprehensive insights into the wound microbiome.

Method: Ten clinical wound debris collected during sharp debridement are being analyzed in detail at the molecular biological level. All participants were found to have a wound biofilm containing at least one of the most common bacterial genera associated with chronic wounds: Pseudomonas or Staphylococcus.

Results/ Discussion: Although a large proportion of the wound debris consisted of human genetic material, metagenomic analysis identified between 8 and 40 bacterial species per patient from approximately 30 genera. This provided more detailed information about the bacterial consortium than conventional microbiological swabs. Metatranscriptome analysis detected over 500 different genera, significantly exceeding the diversity revealed by metagenomics. In addition, these analyses offered valuable insights into pathogen activity and behavior and allows not only the pathogen but also the human immune response to observe.

Conclusion: Modern molecular techniques such as metagenome and metatranscriptome analyses enable a far more comprehensive characterization of microbial diversity in chronic wounds than traditional culture-based methods. While routine swabs detect only a small proportion of present microorganisms, molecular approaches reveal a highly complex and dynamic microbial community, including information on active gene expression. These deeper insights enhance our understanding of biofilm formation and pathogenic behavior and may significantly influence future diagnostic and therapeutic strategies for chronic wounds.

PS02.59

Microbial community structure and source contribution to the early neovaginal microbiota following penile inversion vaginoplasty

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Introduction: The neovagina is a surgically created new environment and is known to be colonized by microorganisms. The process by which this new environment is colonized is largely unknown but may impact women"s health. We characterized the neovaginal microbiome, along with the microbiomes of the tissues that formed the neovagina, as well as the gut, which may also be a source of colonization.

Methods: Thirty male-to-female transgender women were included. Using *16S rRNA* sequencing, we analysed the penile, scrotal, urethral and rectal microbiomes before surgery, and the neovaginal microbiome in the period of 2 to 13 months after vaginoplasty in 30 individuals.

Results: Each of the sites was characterized by a specific microbiota. The neovagina was characterized by the second highest bacterial richness and diversity after the gut. The neovaginal microbiota was dominated by anaerobic bacteria of the phyla Firmicutes and Bacteroidota. The most abundant genera were *Prevotella*, *Peptoniphilus*, *Anaerococcus* and *Porphyromonas*. These genera, together with *Dialister*, *Finegoldia* and *Actinomyces* formed a neovaginal core microbiota, representing genera present in more than 50% of the samples, each with a minimum relative abundance of 1%. More than half of the neovaginal bacteria were not shared with the tissue sites that formed the neovagina. The site with the highest percentage of shared neovaginal Amplicon Sequence Variants (ASVs) was the gut, followed by the urethra. Complementary aerobic culturing showed that on average 38.7% of neovaginal bacterial species were shared with at least one other site.

Conclusions: The early neovaginal microbiota is highly diverse, dominated by anaerobic bacteria and is distinct from the microbiota of the tissue sites that form the neovagina. As most neovaginal ASVs are unshared, this indicates that bacteria from other environments may colonize the neovagina in this early period after creation of this new environment. These findings suggest implications for neovaginal health, potentially informing studies on probiotic therapies to support the colonization of the neovagina with beneficial bacteria.

PS02.60

Temporal dynamics of the healthy human gut and oral microbiome and resistome: A 12-week longitudinal study

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Introduction: Although the human microbiome is highly individualized, most studies characterize it using only a single time-point sample. Whether this snapshot reliably represents the stability of an individual"s gut or oral microbial community

over time remains unclear, with important implications for microbiome-based diagnostics and antimicrobial resistance (AMR) surveillance.

Goals: We investigated how much variation in the gut and oral microbiome, including the associated resistome, occurs within versus between healthy individuals over a 12-week period, and how accurately a single sample reflects each individual"s average microbial state.

Materials and Methods: Eleven healthy adults (six female, five male; aged 27-61 years) each provided three consecutive stool and three consecutive saliva samples six weeks apart (weeks 0, 6, 12), resulting in 198 samples total. Libraries underwent Illumina metagenomic sequencing (median 36.8 million reads per sample after quality filtering) and a standard short-read pipeline (host depletion, MetaPhlAn 4, HUMAnN 3, RGI/CARD, StrainPhlAn 4) for microbiome analysis. Variance was partitioned by PERMANOVA on Aitchison distances and by linear mixed models with random intercepts for subject and subject-by-timepoint.

Results: Subject identity explained 54.4 % of compositional variance in stool and 57.6 % in saliva samples (PERMANOVA, both $P<0.001$), while timepoint contributed only 2.3 % and 1.3 %. Functional pathways exhibited reduced inter-individual partitioning compared to taxonomic profiles (subject R^2=40.7 % stool, 46.7 % saliva; both $P=0.001$), consistent with functional redundancy across phylogenetically distinct communities. The resistome displayed similarly pronounced subject-specific structuring: on Aitchison distances, subject identity explained 44.3 % of resistome variance in stool and 55.4 % in saliva,approaching the taxonomic level (both $P=0.001$), far exceeding any temporal contribution. Strain phylogenies were monophyletic within a subject for 55-91 % of individuals across the six tracked species. The practical impact of within-host variability differed by habitat: in saliva, a single randomly selected sample deviated from the individual mean by 8.9 % on average, with 72.7 % of samples falling within ±10 % of this mean; in stool, corresponding deviations were 17.7 %, with only39.5 % within ±10 %.

Summary: Across 12 weeks of habitual daily life, the gut and oral microbiomes of healthy adults exhibit stable, highly individualized compositional and resistome signatures that exceed temporal variability. A single saliva sample provides a reasonable approximation of an individual´s mean microbial state, whereas a single stool sample does not achieve comparable representativeness. Accordingly, robust individual-level characterization of the gut microbiome and resistome necessitates repeated sampling to account for within-host variability.

PS02.61
Ultrastructural morphology of granular bodies in erythocytes and possible mechanisms of their proliferation

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Introduction: Heinz bodies are granular intracellular inclusions found in erythrocytes. The leading hypothesis suggests that they form under conditions of oxidative stress and structural damage of hemoglobin. Recent evidence indicates that, in addition to chemical factors, members of the blood microbiota may also participate in their formation.

Objectives: The aim of this pilot study was to track the dynamics of Heinz body formation in erythrocytes under induced oxidative stress and to analyze the associated microbial profile.

Materials and Methods: High temperature and elevated concentrations of salts known to induce Heinz body formation were used as stress factors. Fresh blood samples from three healthy volunteers were incubated at 43 °C in the presence of menadione sodium bisulfite (vitamin K) and aminophenol. Morphological changes were monitored in real time using light microscopy, a BioFlux system, and scanning electron microscopy. Targeted 16S rDNA sequencing was performed to assess the microbial composition before and after incubation.

Results: Morphological analysis demonstrated the appearance of granular inclusions within the first minutes of treatment, followed by a marked increase in the percentage of erythrocytes containing Heinz bodies after 90–150 minutes. Microbiome analysis of DNA from untreated erythrocytes and isolated Heinz bodies showed predominant representation of the phyla Proteobacteria, Firmicutes, and Actinobacteria. No loss of microbial diversity was observed as a result of treatment or DNA isolation procedures. In stress-exposed samples containing Heinz bodies, we detected up to a thirty-fold increase in the number of reads for certain bacterial genera relative to untreated erythrocyte controls. In lysed erythrocyte preparations, we identified cultivable microbial structures measuring 170–180 nm with morphology similar to that of Heinz bodies. Vitamin K and aminophenol acted as strong chemical stressors promoting Heinz body formation and likely exhibited specific biochemical interactions with the cellular structures of different microbial taxa.

Summary: Our findings suggest a possible link between oxidative-stress-induced granulation in erythrocytes and quantitative and qualitative changes in the blood microbiota, highlighting the need for further investigation into the potential role of microbial factors in Heinz body formation.

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PS02.63
Pathogen detection in implant failure after spinal fusion by microbiological examination of pedicle screws

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Background: Implant failure following spinal fusion remains a major challenge in spine surgery, with septic loosening representing one of the most important differential diagnoses. As surgical revision options are often limited, targeted antimicrobial therapy is essential for successful treatment. However, reliable pathogen identification requires not only microbial detection but also recovery of viable bacteria for

phenotypic susceptibility testing. The aim of this prospective pilot study was to evaluate a simple and low-cost adjunctive diagnostic approach to improve microbiological detection rates.

Methods: Pedicle screw implants from patients with suspected septic loosening were collected in sterile containers filled with 150 mL sodium chloride solution. Following vigorous shaking, 10 mL of the fluid were inoculated into aerobic and anaerobic blood culture bottles and incubated for up to 14 days. Standard microbiological diagnostics included peri-implant swabs and/or tissue samples cultured on solid media and in enrichment broth.

Results: Between April 2021 and March 2026, 53 patients were enrolled, of whom 32 yielded interpretable microbiological results. In five patients, bacterial pathogens were detected by both the novel and the standard diagnostic approach. In six cases, examination of the pedicle screws showed bacterial growth despite negative standard cultures or identified additional pathogens not detected by standard diagnostics alone. In three cases, pathogens were detected exclusively by standard microbiological procedures. Concordant negative results were obtained in 17 cases. Time to positivity ranged from 8 to 76 hours; prolonged incubation beyond this interval up to 14 days did not further increase diagnostic yield in our cohort.

Conclusions: In this prospective pilot study, additional microbiological examination of pedicle screws increased diagnostic sensitivity in patients with suspected septic implant loosening. The proposed method can be readily implemented in routine microbiological laboratories at low cost and with minimal additional hands-on time, an important consideration in the context of increasing economic pressure and workforce shortages. Further studies are required to determine the diagnostic sensitivity and specificity of this approach, particularly in patients with suspected aseptic loosening.

PS02.64

Combining broad host range and anaerobic phage susceptibility routine testing – Are we moving closer to targeted therapy for *Cutibacterium acnes* infections?

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Background: Infections caused by *Cutibacterium acnes*, particularly foreign body-associated and other invasive infections, remain therapeutically challenging due to biofilm formation and limited antimicrobial susceptibility. Bacteriophages represent a promising complementary therapeutic approach; however, data on routine-compatible anaerobic phage susceptibility testing and host range are still limited.

Methods: The lytic *Cutibacterium* phage UMR_Nox was isolated and propagated under anaerobic conditions. A total of 68 clinical *C. acnes* isolates, including 44 invasive isolates from deep clinical specimens and 24 control isolates from superficial skin samples, were analyzed for phage susceptibility using spot test and an adapted routine-compatible anaerobic phage susceptibility testing workflow (anaPST). Lytic activity was initially assessed by visible lysis zones and subsequently characterized by efficiency of plating (EOP) analysis.

Results: The isolated phage demonstrated lytic activity against all tested *C. acnes* isolates, indicating a broad host range across invasive and commensal-associated strains. AnaPST proved feasible within routine laboratory workflows and enabled standardized phage susceptibility testing. Based on these findings, a multicenter German interlaboratory comparison for anaerobic phage susceptibility testing is currently being established with participation from multiple diagnostic laboratories.

Conclusion: The combination of broad lytic activity and feasible routine anaerobic susceptibility testing highlights the translational potential of *Cutibacterium acnes* phage UMR_Nox for future applications in *C. acnes*-associated infections, particularly implant-associated infections. Furthermore, standardized anaerobic phage diagnostics may support broader implementation of phage-based therapeutic strategies. Additional studies evaluating stability and biofilm-associated activity are ongoing.

PS02.65

Expanded mycological diagnostics from bacteria-positive blood culture bottles identifies masked fungal infections

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Background: High mortality rates make fungemia a critical systemic infection, yet its diagnosis remains problematic. Blood cultures (BCs) show a low sensitivity of only 50%, presumably because fungi circulate at low concentrations and grow less efficiently in BC bottles than bacteria. Hence there is a risk that bacteria overgrow fungal pathogens, causing missed co-infections. This prospective study aims to determine the frequency of false-negative findings for underlying fungemia in bacterial bloodstream infections and the usability of expanded mycological BC diagnostics.

Methods: All bacteria-positive BC bottles underwent expanded mycological diagnostics: BCs were incubated for another week and visually controlled for growth, i.e., formation of hyphal balls. Subsequently,, BCs were sub-cultured on Sabouraud agar, which was again incubated for ≥ 4 days. All recovered isolates were identified via MALDI-TOF MS.

Results: Over two years, all consecutive bacteria-positive BC bottles were screened for fungi. Out of 2,067 bacterial BCs, two (0.1%) grew *Candida glabrata*. Both cases occurred in ICU patients with enterococcal bloodstream infections. Notably, one case of masked fungemia was only detected through the expanded culture. Furthermore, among 88 bacteria-positive BC bottles used for cerebrospinal fluid cultures, one patient (1%) presented with a *Cryptococcus deneoformans* infection. This case would have been missed due to bacterial overgrowth by *Staphylococcus haemolyticus*.

Conclusion: These findings demonstrate that bacterial growth can mask underlying fungal infections in BC-based diagnostics. Expanded mycological testing proved to be able to increase diagnostic sensitivity and should be considered for

bacteria-positive BCs from patients at high risk for fungal infections.

PS02.66
Evaluation of gradient tests for the detection of azole-resistant *Aspergillus fumigatus*
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Introduction: Invasive infections with *Aspergillus fumigatus* are challenging in management and treatment. Due to the emergence of azole-resistant strains, current guidelines recommend susceptibility testing of clinically relevant isolates. Therefore, reliable methods for susceptibility testing which are easier and less elaborate than the EUCAST standard method are needed in routine microbiology diagnostic labs.

Objective: We evaluated gradient tests of two manufacturers for susceptibility testing of *Aspergillus fumigatus* with regard to comparability to standard EUCAST method broth microdilution.

Material and Methods: We selected 53 *Aspergillus fumigatus* isolates from the collection of the German National Reference Center for Invasive Fungal Infections (NRZMyk) that represented azole susceptible strains (n=18) and azole resistant strains (n=35), including 30 isolates representing all CYP51A mutations detected so far by the NRZMyk. All isolates were tested by standard EUCAST method broth microdilution. For all strains gradient tests of two manufacturers (ETEST® by bioMérieux and MIC Test Strip by Liofilchem®, bestbion^{dx}) were performed for itraconazole and voriconazole according to manufacturers' protocol and read at 48 hours. Minimum inhibitory concentrations (MIC) were determined independently by five laboratory staff members and interpreted according to the method-dependent epidemiological cutoff values proposed by Espinel-Ingroff *et al.* 2019.

Results: Categorical agreement with EUCAST MICs was found with gradient testing MICs in 83,0%/84,9% (ETEST®/MIC Test Strip) of isolates for voriconazole and 81,1%/79,2% of isolates for itraconazole. Using gradient testing, azole resistance was detected in 28/33 (ETEST®/MIC Test Strip) isolates. Major errors and very major errors were observed for both systems. Error rates could be lowered by using combination of gradient testing for voriconazole and itraconazole to 0,0%/1,9% (ETEST®/MIC Test Strip) for major errors and 13,2%/5,7% for very major errors. Most isolates carrying mutations were successfully identified by gradient testing (25 isolates with ETEST® and 28 isolates with MIC Test Strip).

Conclusion: Gradient testing methods are a suitable tool for the detection of azole resistance in *Aspergillus fumigatus*. If gradient test indicates azole resistance, we recommend to confirm azole resistance by EUCAST microdilution and CYP51A sequencing. However, further analyses are required to assess the reliability of routine gradient tests for *Aspergillus fumigatus* susceptibility testing.

PS02.67
Capture of viable bacteria from liquid samples to enhance sensitivity of classical microbiological methods
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Culturing of bacteria and fungi is a cost-efficent and well-established method for environmental monitoring, food & water safety testing, as well as clinical diagnostics. However, the methodology is limited by the sample volume that can be applied to the growth medium. This bottleneck is currently addressed by various concentration methods such as filtration and centrifugation protocols. In food testing it is circumvented by pre-culturing in liquid growth media. Each of these methods come with certain disadvantages.

The goal of this project is to develop a fast and user-friendly method to concentrate live bacteria from large volumes. Our aim is to capture bacteria with high cell release and survival rate without the use of filtration or centrifugation.

We implemented a workflow utilizing buffer-optimized magnetic separation. The performance of this workflow was tested using both Gram-positive and Gram-negative bacteria and capture efficiency was determined by comparing colony counts of plated bacterial populations before and after the capture process.

Preliminary results indicate a near-complete recovery of Gram-positive bacteria, showing strong potential for high-efficiency live bacterial capture. Ongoing work focuses on optimization of Gram-negative bacterial capture and maintaining viability across all bacterial types using common buffer conditions.

These findings demonstrate the feasibility of our approach to recover live bacteria from large-volume samples and even if present in very low concentration. With further optimization, this method holds significant potential for broad applications in rapid and accessible pathogen detection, microbiome studies, and various microbiological analyses where maintaining bacterial viability is essential and where classical microbiology"s sensitivity is currently hampered by insufficient sensitivity.

PS02.68
From target selection to molecular test development: A reproducible bioinformatic pipeline for oligo design and assay quality control
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Molecular assays for genetically diverse pathogens require oligonucleotide sets combining broad inclusivity across target variants with high specificity against related organisms, host

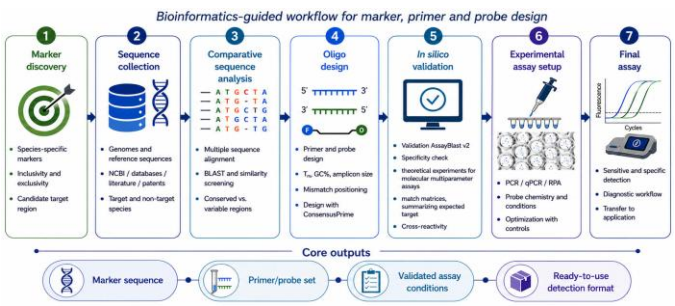
DNA and matrix background. We present a fully local workflow linking target selection, consensus oligo design and in silico quality control for qPCR, dPCR, multiplex PCR, isothermal amplification, point-of-care tests and DNA microarray assays.

Target sequences are curated as multi-FASTA input and processed with ConsensusPrime. The pipeline aligns sequences, removes duplicate, divergent or interfering entries, calculates consensus scores and identifies conserved regions for primer and probe design. User-defined Primer3 parameters generate candidate oligonucleotides; HTML summaries and final alignments document filtering, consensus regions and predicted oligos. Candidate sets are transferred to AssayBLAST for post-design quality control. Custom BLAST databases combine intended targets with off-target organisms, related taxa, human sequences and sample-specific background genomes. AssayBLAST evaluates binding on both strands, applies mismatch thresholds, reports critical mismatch positions and classifies interactions by strand orientation and spatial proximity.

The workflow supports the path from target definition to assay-ready primer/probe candidates. ConsensusPrime increases inclusivity by designing oligos from conserved regions of representative alignments rather than single references. AssayBLAST detects unintended binding, reverse-complement duplicates, missing or wrongly oriented primers, and predicted constant, linear or exponential amplification patterns. It also enables theoretical experiments for molecular multiparameter assays from NGS datasets, including raw sequencing data. These in silico experiments are represented as match matrices summarizing expected target–oligonucleotide interactions across large sample or genome collections. Such matrices provide a rational basis for practical assay optimization, which would otherwise require extensive wet-lab testing, and can support post-market surveillance by assessing new sequencing data against existing assay designs. These outputs support qPCR/dPCR assays requiring robust amplification, multiplex PCR panels requiring compatible primer combinations, and microarray assays requiring large oligonucleotide sets with minimal cross-reactivity. AssayBLAST adds automatic BLAST parameter optimization, modular BLAST interpretation and detailed TSV reports.

By combining ConsensusPrime and AssayBLAST, we establish a reproducible, data-sovereign workflow for molecular multiparameter assays. The approach reduces manual interpretation, supports scalable screening of oligonucleotide panels and provides an auditable in silico benchmark before synthesis and wet-lab validation. It can accelerate assay development for clinical microbiology, outbreak surveillance, antimicrobial-resistance diagnostics and pathogen detection.

Fig. 1



PS02.70

Development and validation of a protocol for microbial cell-free DNA Isolation from human plasma

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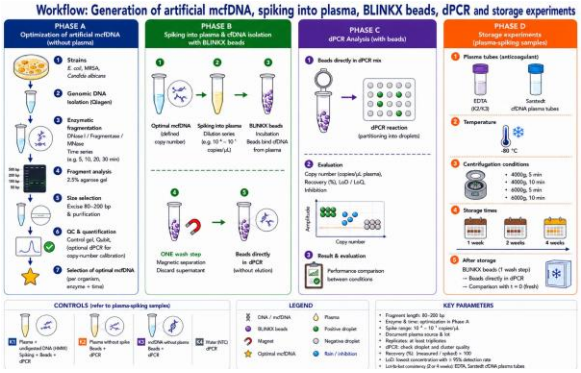
Microbial cell-free DNA (cfDNA) isolated from human blood plasma represents a promising biomarker for the non-invasive detection of bloodstream infections and invasive microbial diseases. However, the low abundance and fragmented nature of microbial cfDNA in plasma present major analytical challenges, requiring highly optimized extraction workflows. This study focuses on the development and validation of a standardized protocol for the efficient isolation of microbial cfDNA from human plasma samples.

Artificial microbial cfDNA fragments were generated in vitro from genomic DNA of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* using different enzymatic fragmentation approaches, including Fragmentase, DNase I, and micrococcal nuclease (MNase). Fragmentase showed the best overall performance among the tested enzymes, producing reproducible DNA fragment sizes between 20 and 500 bp after 30 min incubation at 30°C for all three investigated microbial species. The generated cfDNA fragments were purified using either a gel extraction-based method (Agarose Gel Extraction Kit, JenaBioScience, Germany) or a magnetic bead-based purification kit (DNA Clean & Concentrator MagBead Kit, ZymoResearch, Germany). The resulting fragment profiles were then used to establish defined spike-in plasma models mimicking clinically relevant microbial cfDNA conditions.

To improve pre-analytical sample processing, various centrifugation conditions differing in speed and duration were tested for efficient plasma recovery from whole blood while reducing residual cellular DNA contamination. Human plasma samples were then spiked with known concentrations of artificial microbial cfDNA to evaluate and optimize extraction efficiency. Following cfDNA isolation, nanoreactor-beads developed by BLINK AG (Jena) will be applied in a downstream quantitative PCR (qPCR) workflow regarding cfDNA recovery yield, fragment retention, and reproducibility. Furthermore, the impact of pre-analytical storage conditions was investigated by analyzing plasma samples collected in K2-EDTA tubes and stored at −80 °C for varying durations (1, 2, 3, and 4 weeks) prior to cfDNA isolation.

The developed workflow aims to improve the sensitivity and reliability of microbial cfDNA recovery from plasma to provide a robust methodological foundation for future diagnostic applications in infectious disease detection and monitoring.

Fig. 1



PS02.71

Association of Borrelia outer surface protein C antibodies with antibiotic-refractory course in paediatric Lyme arthritis

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Introduction: Lyme Borreliosis caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex is the most prevalent tick-borne disease in North America and Europe. Lyme arthritis (LA) represents a late manifestation of infection and patients usually respond well to antibiotic treatment. However, a subset of patients develops antibiotic-refractory Lyme arthritis (ARLA), characterized by persistent inflammatory symptoms despite adequate antibiotic therapy. Reliable biomarkers for early identification of patients at risk for ARLA are currently lacking.

Objectives: This study aimed to characterize serological immune signatures in paediatric LA patients and to identify Borrelia-specific antibody responses associated with the development of ARLA.

Material and Methods : We retrospectively analysed a German paediatric LA cohort, treated at the University Hospital Wuerzburg between 2015 and 2025. Clinical data, diagnostic parameters, and archived serum samples were available for all patients. In total, 62 patients with LA were included and stratified into ARLA (n =25) and antibiotic-responsive LA (n =37) groups according to clinical disease course. Borrelia-specific IgG immune signatures were assessed by recombinant line immunoblot analysis using eight recombinant Borrelia antigens. Antibody reactivity patterns were compared between both patient groups.

Results: Patients who developed ARLA were generally older at disease onset and more frequently presented with monoarticular disease. Independent of disease course, most patients exhibited IgG reactivity against several recombinant Borrelia antigens. Notably, antibodies directed against Borrelia outer surface protein C (OspC) were detected at a markedly higher frequency in the ARLA cohort compared to patients with antibiotic-responsive LA. These findings indicate a distinct serological profile associated with refractory disease progression.

Summary: Our data suggest that IgG immune response against OspC, may serve as potential early biomarker for identifying paediatric patients at increased risk of developing ARLA. Early recognition of such patients at the time of diagnosis could support risk assessment and facilitate more individualised therapeutic management. Further prospective studies are required to validate the predictive values of OspC-specific immune responses and to better understand the immunopathological mechanisms underlying ARLA.

PS02.72

Development of sample preparation from clinical swabs using ionic liquids with downstream multiplex detection and quantification of microbial pathogens

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Efficient and rapid sample preparation is essential for reliable molecular analysis of clinical specimens such as nasopharyngeal, wound, and rectal swabs. A major challenge is the efficient lysis of Gram-positive bacteria, whose thick peptidoglycan cell wall increases resistance to conventional chemical and physical lysis methods.

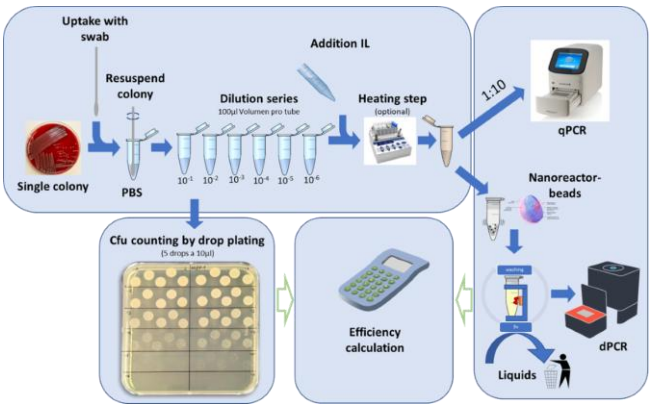
In this study, bacterial DNA extraction from clinical swab samples was optimized using comparative lysis protocols to maximize nucleic acid recovery and compatibility with downstream applications including qPCR and digital PCR. Classical polyester swabs and FLOQ® flocked nylon swabs (Copan Italia, Brescia, Italy) were evaluated using *Escherichia coli* and *Klebsiella pneumoniae* as Gram-negative model organisms and *Bacillus atrophaeus* and *Staphylococcus aureus* as Gram-positive representatives. Lysis conditions included phosphate-buffered saline (PBS) as control and ionic liquid (IL)-based lysis combined with heat treatment. DNA recovery and PCR compatibility were assessed directly and after magnetic bead–based purification using Nanoreactorbeads (BLINK AG, Jena, Germany).

Lysis efficiency depended on bacterial species and swab type. Gram-negative bacteria showed high DNA recovery under IL-based conditions, whereas Gram-positive organisms demonstrated lower but substantially improved lysis efficiencies compared to PBS controls. Compared to conventional buffers, ILs significantly improved bacterial lysis efficiency, achieving nearly 100% lysis for Gram-negative bacteria and approximately 70–80% for Gram-positive strains. However, residual ILs inhibited downstream PCR applications unless samples were diluted or purified before analysis. FLOQ® swabs showed slightly improved bacterial uptake and transfer efficiency, while Classical swabs enabled more effective DNA release. DNA-associated IL residues were efficiently removed using Nanoreactorbeads, enabling direct digital PCR with only minor performance losses.

The BLINK Nanoreactorbeads additionally enabled direct digital PCR–based detection and absolute quantification within the same workflow. Bead functionalization and encoding allow multiplex detection of genetic markers, including species-specific and antimicrobial resistance targets. All four model organisms were successfully quantified in parallel, and the platform currently supports simultaneous detection of up to 16 targets in a single assay.

Overall, the study demonstrates that ionic liquid–based lysis combined with magnetic Nanoreactorbead purification provides a robust and scalable workflow for nucleic acid extraction from clinical swab samples. Standardized sample handling, including agitation and resuspension procedures, proved critical for reproducibility and optimal DNA yield. The workflow represents a promising basis for integrated sample-to-result diagnostic pipelines and multiplex molecular diagnostics with high point-of-care potential.

Fig. 1



PS02.73
Advancing gastrointestinal diagnostics: a high-throughput multiplex PCR for comprehensive pathogen detection
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Background: Gastrointestinal infections remain a major global health burden, requiring rapid and accurate diagnostics to guide treatment and prevent complications. The LiquidArray® Gastrointestinal assay is designed as a syndromic multiplex PCR assay to detect and differentiate a broad spectrum of bacterial, viral, and parasitic pathogens in stool specimens. The new VER 2.0 update expands the assay"s diagnostic scope by adding *Aeromonas* spp. and enhancing differentiation capabilities, including separate detection of *stx1* and *stx2* and discrimination of *Yersinia enterocolitica* genes *ail* and *ystB*.

Materials and Methods: The assay combines real-time RT-PCR with LiquidArray technology, enabling simultaneous detection of 27 targets, including key virulence factors and toxin genes. Performance was assessed using a diverse set of clinical stool samples and external quality panels. Nucleic acids were extracted using GenoXtract platforms (Bruker) and analyzed on FluoroCycler XT (Bruker) with automated interpretation via FluoroSoftware XT-IVD. Comparator methods included established multiplex PCR panels and reference assays to determine agreement and analytical performance.

Results: LiquidArray® Gastrointestinal VER 2.0 showed high sensitivity and specificity across all major pathogen groups and consistently identified clinically relevant bacterial, viral, and parasitic targets. The assay provided accurate differentiation of clinically relevant targets, such as *Clostridioides difficile* toxins A and B, Shiga toxin genes (*stx1* vs. *stx2*), and *Yersinia enterocolitica* genes (*ail* vs. *ystB*), demonstrating strong concordance with comparator methods. Analytical validation confirmed low limits of detection, broad inclusivity, and absence of cross-reactivity. Workflow precision was stable across automated and manual workflows, supporting reproducibility in laboratory settings.

Conclusion: LiquidArray® Gastrointestinal VER 2.0 provides a comprehensive and differentiated approach to gastrointestinal pathogen detection. The expanded target panel and improved discriminatory capabilities offer added clinical value, while the semi-automated workflow and integrated software interpretation support efficient implementation in routine diagnostics, supporting timely and accurate clinical decision-making.

PS02.74
NGS based cultur-independent pathogen identification in respiratory secretions of ICU patients: A comparison between Illumina short read and Nanopore long read sequencing vs. conventional microbiology
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Rapid and accurate identification of respiratory pathogens in critically ill patients remains a significant challenge in intensive care units (ICUs). Conventional culture-based microbiological (CBM) diagnostics are often limited by prolonged turnaround times and reduced sensitivity. In this study, six ICU patients with suspected lower respiratory tract infections were investigated, comparing standard CBM methods with culture-independent next-generation sequencing (NGS) for pathogen identification in respiratory samples.

Tracheal secretions were collected by bronchoscopy from 6 ventilated ICU patients with suspected lower respiratory tract infection. They were analyzed under sterile conditions using conventional microbiological techniques, including culture and phenotypic identification. In parallel, DNA was extracted from the same specimens without cultivation or enrichment and subjected to NGS to enable comprehensive detection of bacterial DNA. Two sequencing strategies were applied. Short-read whole-metagenome sequencing (WMS) was performed on an Illumina MiSeq instrument with LiveGene real-time data retrieval, enabling rapid identification of pathogens and antimicrobial resistance determinants during the sequencing run. Additionally, full-length 16S rRNA gene amplicons were sequenced using Oxford Nanopore Technologies long-read sequencing to achieve species-level taxonomic resolution.

Concordance between short read WGS and long read 16S full-length sequencing was very high, but concordance between conventional microbiological diagnostics and NGS was observed in only one of the five cases, where both methods identified the same pathogen. In the remaining four cases, classical culture yielded either no growth or detected only a limited number of bacterial species. In contrast, NGS identified a substantially broader spectrum of bacteria in all samples, including multiple organisms that were not detected by conventional methods. In 4 from 6 cases, potentially clinical relevant bacterial findings were exclusively identified by NGS.

These results demonstrate the increased sensitivity of NGS in detecting microbial diversity in tracheal secretions from ICU patients. However, the clinical relevance of additional bacterial species detected solely by NGS remains uncertain, as the method cannot reliably distinguish between colonization, contamination, and true infection.

In conclusion, NGS provides a more comprehensive characterization of the respiratory microbiome compared to classical microbiological diagnostics in ICU patients. While it shows promise as a complementary diagnostic approach, further studies are needed to establish standardized interpretation criteria and to clarify its role in guiding clinical decision-making and antimicrobial therapy.

Attachment: Table 1: Results in NGS and CBM with abundancy and clinical relevance

Fig. 1

Table 1: Results in NGS and CBM with abundancy and clinical relevance

Patient/Sample	NGS Result	abundance	Clinical relevance	CBM Result	abundance	Clinical relevance
1	Streptococcus sp.	low	high	Staphylococcus aureus	high	high
	Escherichia coli	low	High	Candida species	low	low
	Veillonella sp.	low	high			
	Lautropia mirabilis	low	low			
	Capnocytophaga sputigena	low	low			
2	Parvimonas micra	low	low	Serratia marcescens	moderate	high
	Prevotella oris	low	moderate	Candida dubliniensis	low	low
	Fusobacterium gonidiaformans	low	low			
	Streptococcus constellatus	low	moderate			
	Dialister pneumosintes	low	low			
3	Staphylococcus aureus plus identified resistance ErmC	moderate	High	Staphylococcus aureus	high	high
	Neisseria sp.			Candida species	low	low
	Capnocytophaga gingivalis / Capnocytophaga sputigena	low	high			
	Actinomyces sp.	low	low			
4	negative	n.a.	Low	Herpes simplex (cultural)	high	high
5	Pseudomonas sp.	low	High	No Result		
	Alternaria alternata	low	low			
	Cutibacterium acnes	low	low			
	Klebsiella varicola	Moderate	High			
	Metamycoplasma sp.	low	low			
6	Cutibacterium acnes	low	low	Candida species	high	low

PS02.75
Diagnostic algorithm for same-day evaluation of penicillin susceptibility in *Staphylococcus aureus*

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Bloodstream infections caused by *Staphylococcus aureus* are associated with high mortality rates and require rapid and targeted antibiotic therapy. Preliminary results from the *S. aureus* Network Adaptive Platform (SNAP) Trial suggest that penicillin G may be a beneficial treatment option. The proportion of penicillin-sensitive *S. aureus* isolates (PSSA) ranges up to 35% depending on the study. Penicillin resistance is primarily mediated by the production of a penicillinase, which is encoded by *blaZ* in S. aureus. The aim of this study was to develop a diagnostic algorithm that enables a reliable and rapid determination of penicillin susceptibility.

A total of 240 *S. aureus* isolates from bloodstream infections were included. The diagnostic performance parameters of various phenotypic and molecular biological methods were compared (Table 1). A *blaZ* PCR was used as the gold standard.

Of the included isolates, 32.1% (n = 77/240) were *blaZ*-negative. Agar diffusion with evaluation of the inhibition zone according to EUCAST achieved a sensitivity of 100% and a specificity of 99%. MIC determination using VITEK 2 showed a sensitivity of 83% and a specificity of 100%, with a false-positive rate of 17% (n = 28/240). The nitrocefin-based rapid test (NT) using four-hour-old cultures showed the lowest sensitivity at 70%, but achieved a positive predictive value of 100% with a specificity of 100%. Real-time PCR (rtPCR) attained a sensitivity of 98% and a specificity of 100% at a Ct cutoff of 19.68 cycles (derived from the Youden index). The combination of NT and rtPCR achieved comparable diagnostic performance parameters to agar diffusion with zone measurement, with a shorter turnaround time (approximately 6 h vs. 16–24 h).

The use of VITEK 2 alone for the determination of penicillin MIC does not permit the reliable exclusion of penicillin resistance in *S. aureus*. Agar diffusion and zone measurement represent a highly reliable method for assessing penicillin susceptibility, but results are not available until the following day. For the early assessment of penicillin susceptibility in routine diagnostics, we propose a stepwise diagnostic algorithm: The NT is suitable as a "rule-in" test for the detection of penicillinase. If the test result is negative, a confirmatory test using rtPCR is necessary.

Fig. 1

Method	Interpretation	*blaZ*-neg. (n = 77)	*blaZ*-pos. (n = 163)	Sensitivity	Specificity	PPV	NPV	VME	ME
Disk diffusion (1 U)[1]	Sensitive	76	0	100%	99%	99%	100%	0%	1%
	Resistant	1	163						
MIC VITEK[1]	≤0.125 mg/L = S	77	28	83%	100%	100%	73%	17%	0%
	>0.125 mg/L = R	0	135						
MIC epsilometer test[1]	≤0.125 mg/L = S	76	9	94%	99%	99%	89%	6%	1%
	>0.125 mg/L = R	1	154						
Nitrocefin-based (NT) test	Negative	77	49	70%	100%	100%	61%	30%	0%
	Positive	0	114						
Real-time (rt) PCR	Negative	77	3	98%	100%	100%	96%	2%	0%
	Positive	0	160						
NT + rtPCR	Negative	77	0	100%	100%	100%	100%	0%	0%
	Positive	0	163						

[1]EUCAST interpretation; PPV: positive predictive value; NPV: negative predictive value; VME: very major error; ME: major error

PS02.76
Development of a quantifiable high-throughput coagulase assay for identification of coagulase-positive staphylococci

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Staphylococcus aureus is a major pathogen affecting both humans and animals and, including methicillin-resistant lineages, remains a global concern due to its virulence and increasing multidrug resistance. Coagulase and clumping factor are important virulence determinants that mediate fibrinogen binding and plasma clot formation and have long served as classical phenotypic markers for the identification of *S. aureus* and other coagulase-positive staphylococci. These organisms cause a broad range of clinical entities, from mild skin and soft tissue infections to severe invasive diseases. Conventional species identification is based on clot formation in plasma using glass tubes, requiring relatively large reagent volumes and subjective visual interpretation. These limitations hinder standardization, high-throughput, and quantitative analysis.

We established a standardized microplate-based assay enabling quantitative detection of coagulase activity in a 96-well format with minimal hands-on time and reduced reagent consumption. Overnight cultures were mixed with rabbit plasma in microplates, and optical density at 620 nm was monitored continuously using a plate reader at variable temperatures. Initial optical density values were subtracted from subsequent measurements to account for baseline differences. Colonies from agar plates were tested using the conventional glass tube assay for validation of results.

Coagulase-positive *S. aureus* reference strains showed a reproducible increase in optical density, whereas *S. epidermidis* used as a coagulase-negative control, showed no OD increase and no visible clot formation. Several *S. aureus* isolates and coagulase-positive *S. pseudintermedius* strains demonstrated characteristic increases in OD, often with sigmoidal kinetics, and selected isolates produced already detectable signals within 15 min after assay initiation. The microplate assay results were fully concordant with those from conventional tube-based testing while offering continuous and quantitative readout, lower plasma volume requirements, and substantially higher throughput. The assay is scalable, automation compatible and facilitates earlier termination once a plateau is reached, thereby substantially reducing the turnaround time. These features make it a promising platform for screening large numbers of strains and isolates and for comparative studies of coagulase serotypes and enzyme activity across host species.

PS02.77
Bridging the sim-to-real gap in influenza CPE diagnostics: Performance of EfficientNet-B0 trained on WHO reference strains versus clinical isolates

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Introduction: Manual cytopathic effect (CPE) assessment in routine influenza virus culture is labour-intensive and subject to inter-observer variability. Deep learning-based image analysis offers automation potential; however, models trained on well-characterized reference material frequently underperform against morphologically variable clinical isolates — a "sim-to-real gap." Quantifying this gap is a prerequisite for translating AI-assisted CPE readout into routine diagnostics.

Objectives: To compare EfficientNet-B0 classification performance trained on WHO-characterized influenza reference strains versus clinical isolates under identical architectural and preprocessing conditions, and to assess the diagnostic utility and domain-shift limitations of each approach.

Materials and Methods: Two EfficientNet-B0 models were fine-tuned independently using a 70/15/15 stratified split (seed=42) and identical preprocessing (centre crop to 224×224, ImageNet normalisation). Model A was trained on 858 images from WHO reference strains (Negative=224, Flu A=386, Flu B=248). Model B was trained on 4,697 images from sentinel surveillance isolates — influenza-positive nasopharyngeal swabs collected from patients with acute respiratory illness during the 2024/25 season and cultured at the National Reference Centre for influenza (Negative=2,694, Flu A=1,297, Flu B=706). Both used ImageNet-pretrained weights with early stopping on validation macro-F1 (patience=3). Performance was evaluated on held-out test sets as precision, recall, and F1-score per class.

Results: Model A (reference strains, test n=184) achieved macro-F1=0.97 with near-perfect classification (Negative: F1=0.95; Flu A: F1=0.97; Flu B: F1=1.00). Model B (sentinel surveillance isolates, test n=1,007) reached 88% accuracy, with strong negative screening (F1=0.99) but reduced subtype discrimination (Flu A: F1=0.81; Flu B: F1=0.57). The gap was most pronounced for Flu B (precision: 1.00→0.68; recall: 1.00→0.49), reflecting morphological variability and subclade heterogeneity of circulating clinical strains.

Summary: Identical conditions produced markedly different outcomes by training data origin, directly demonstrating the sim-to-real gap in CPE-based AI diagnostics. AI-assisted negative screening showed immediate utility (F1=0.99), offering a reliable tool to reduce manual microscopy workload. Subclade-level classification of sentinel surveillance isolates remains challenging, driven by morphological similarity among Influenza A subtypes and limited Flu B diversity. Future work will explore multi-modal integration of CPE imaging with NGS-derived surface protein profiles and phylogenetic metadata to improve subtype resolution and support robust clinical deployment.

Reference:

Tan M, Le QV. EfficientNet: Rethinking Model Scaling for Convolutional Neural Networks. arXiv:1905.11946, 2020.

Figure 1. Confusion Matrices: WHO reference strains (A) vs. sentinel surveillance isolates, 2024/2025 (B)

Fig. 1

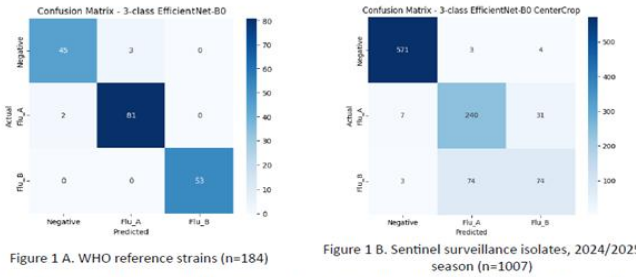

Figure 1 A. WHO reference strains (n=184)

Figure 1 B. Sentinel surveillance isolates, 2024/2025 season (n=1007)

Figure 1. Confusion Matrices: WHO reference strains (A) vs. sentinel surveillance isolates, 2024/2025 (B)

PS02.78
Evaluation of the phenotypic rapid antimicrobial QuickMIC system in clinical routine compared the the VITEK-2 system

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One in three deaths in Germany in 2021 is linked to sepsis. Between 1990 and 2021, the number of deaths has increased from 148 to 247 per 100,000 inhabitants. (1) Early, targeted antibiotic therapy is crucial and requires rapid resistance testing. The QuickMIC system (QM) (Gradientech AB) is a phenotypic rapid antimicrobial susceptibility testing system based on microfluidics. It generates a linear antibiotic gradient in a 3D agarose hydrogel. It is validated for Gram-negative bacteria, which are introduced directly from the positive blood culture and automatically monitored via real-time imaging. The minimum inhibitory concentration (MIC) is determined for up to 12 antibiotics. (3) The aim of this study is to evaluate the reliability of QM in routine clinical use. It is compared with the VITEK-2 system (V2) (bioMérieux).

Following a positive, automatically generated blood culture signal, the presence of Gram-negative rods was confirmed by Gram staining. In parallel with the QM measurement, the blood culture was streaked onto agar plates (McConkey, bioMérieux). After a short incubation period (8 h), identification was performed using the direct transfer method via the MALDI-TOF Sirius (Bruker), and an antibiogram was generated using V2.

A total of 49 QM measurements were performed (October 2025 to April 2026). Of these, one was completely terminated. In 27 runs, at least one antibiotic measurement ended with an error message. Of a potential 600 measurable antibiotic-isolate combinations, 6.00 % were abandoned. The discontinuation rate for the comparative measurements using V2 is 0.64 %. The Essential Agreement (EA) was calculated from the MIC values. It indicates how often the MICs from QM and V2 are identical. The overall EA is 88.7 %. The Categorical Agreement (CA) was determined based on the EUCAST 16 interpretations. The overall CA is 93.98 %. The bias is intended to illustrate how much the MICs differ from one another and whether they represent an underestimation or an overestimation. The overall bias is very low at 0.15 titer units. The highest bias was observed for gentamicin (2.00), where an average of two titer steps higher was measured. The mean measurement time for QM is 4 h 17 min, which is clearly shorter than that for V2 at 10 h 43 min.

One of the biggest advantages of QM over V2 is its short measuring time. QM and V2 produce consistent MIC values and interpretations. The failure rate of the QM is many times higher than that of the V2. If this is reduced, the QM can provide rapid, reliable resistance testing. This enables timely de-escalation or adjustment of the prescribed antibiotic therapy.

(1) Romeike, Heike (2025): Weltweiter Sepsis-Bericht: Immenser Handlungsbedarf in Deutschland – Sterblichkeit stagniert auf hohem Niveau Stand, [am 27.05.2026]

(2) García-Rivera, Celia et al. (2024) Evaluation of the quickmic system in the rapid diagnosis of Gram-negative bacilli bacteremia, in Microbiology Spectrum, Bd. 12, Nr. 10, S.1-8

PS02.79
Development of a dual-target qPCR assay for rapid detection of *Candidozyma auris*

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Introduction: *Candidozyma auris* is an emerging multidrug-resistant yeast classified as a priority fungal pathogen by the World Health Organization (WHO). Owing to its ability to persist on environmental surfaces for prolonged periods, *C. auris* is associated with healthcare-associated outbreaks and poses major challenges for infection prevention and control.

Objectives: This study aimed to develop and evaluate an in-house fully automated real-time PCR (qPCR) assay for the rapid and specific detection of *Candidozyma auris*. A dual-target design was implemented to increase assay robustness against genetic variability among established and newly emerging *C. auris* clades.

Materials and Methods: The assay targets the *C. auris* ITS1 region (channel 4), ITS2 region (channel 2), and an internal control target (channel 5). Primers and probes were partially adapted from previously published assays (ITS2: Freitas et al., *mSphere*, 2020; ITS1: Feng et al., *Analytical Chemistry*, 2024) and subsequently optimized to improve clade inclusivity, reduce primer–probe interactions, and ensure compatibility with the integrated full-process control system. The assay was validated in accordance with IVDR requirements for laboratory-developed tests (LDTs) on the fully automated high-throughput Cobas 5800/6800/8800 platforms (Roche). Analytical sensitivity, linearity, and precision were assessed using standards quantified by digital PCR. Analytical specificity was evaluated using a panel of 56 yeast and mold isolates. The reference strain used for analytical performance characterization (LOD and linearity) was *C. auris* CDC B11903 (clade IV).

Results: The assay demonstrated a limit of detection of 111 CFU/mL (95% CI: 79.5–216) for ITS1 and 66.8 CFU/mL (95% CI: 47.4–127) for ITS2. Linearity was confirmed over seven log10 dilution steps (Ct 9.7–31.2 for ITS1 and Ct 11.7–35.5 for ITS2) for both targets. Intra- and inter-assay variability were below 1ct for both targets. The dual-target *C. auris* assay achieved 100% analytical inclusivity and 100% analytical specificity using the isolate collection, including seven clinical *C. auris* strains representing clades I–IV, as well as closely related species including members of the *Candida haemulonii* complex. In an initial clinical evaluation using remnant diagnostic eSwab samples (n = 33), the assay demonstrated 100% positive and negative agreement (24/24 true negatives;

9/9 true positives) compared with culture and MALDI-TOF MS as the composite reference standard.

Conclusion: The developed dual-target qPCR assay enables rapid, highly sensitive, and specific detection of *Candidozyma auris* with robust performance on fully automated Cobas workflows. The assay may support rapid screening and early detection of *C. auris*, thereby facilitating timely implementation of infection prevention and control measures.

PS02.80

A new selective medium for isolating the emerging pathogen *Ochrobactrum* allows its improved monitoring in complex sample matrices

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Ochrobactrum spp. are aerobic, environmentally widespread Gram-negative bacteria that are increasingly recognized as opportunistic pathogens in both humans and animals. Especially *O. anthropic* and *O. intermedium* have been associated with nosocomial infections, antimicrobial resistance, and misidentification with closely related members of the Brucellaceae, highlighting the need for reliable and safe cultivation methods. Despite their growing relevance in clinical and environmental microbiology, selective isolation of *Ochrobactrum* from complex sample matrices remains challenging.

So far, the isolation of *Ochrobactrum* from environmental samples has mainly relied on selective media originally designed for classical *Brucella* species or on non-specific media allowing the growth of multiple bacterial genera simultaneously. The former approach bears the risk of unintentionally promoting the growth of risk group 3 organisms, thereby limiting its applicability under standard BSL2 laboratory conditions.

Here, we report the development of an *Ochrobactrum*-selective medium based on MacConkey agar supplemented with cell wall biosynthesis inhibitors, exploiting the genus" intrinsic resistance to most ß-lactam antibiotics. This modified MacConkey-*Ochrobactrum* (mMCO) selective medium efficiently inhibits the growth of bacterial and fungal contaminants commonly present in environmental and complex biological samples, while simultaneously interfering with the proliferation of classical risk group 3 *Brucella* species. Consequently, the medium can be handled safely under BSL2 conditions.

A total of eight *Ochrobactrum* species (*O. anthropi, O. intermedium, O. tritici, O. pseudogrignonensis, O. oryzae, O. soli, O. gallinifaecis, and O. teleogrylli*) represented by 32 different strains were evaluated for growth on mMCO. Seven species (27 strains) showed successful replication and colony formation. Importantly, none of the 11 tested contaminating bacteria, including *Acinetobacter* spp., *Escherichia coli*, *Pseudomonas* spp., and *Klebsiella* spp., were able to grow on the medium.

Finally, the new mMCO selective medium was applied for the isolation of *Ochrobactrum* from fecal samples of zoo animals, previously identified as *Ochrobactrum*-positive. The former isolation success could be reproduced, while substantially reducing the growth of background microbiota.

In conclusion, mMCO represents a novel selective medium for the targeted isolation of *Ochrobactrum* spp. from complex environmental and biological samples. Its strong selectivity, combined with the inhibition of classical *Brucella* species enables safer handling under BSL2 conditions and facilitates routine screening approaches. The medium therefore provides a valuable tool for future ecological, veterinary, and clinical investigations of *Ochrobactrum* spp.

PS02.81

Validation of a fully automated laboratory-developed qpcr panel for the detection of atypical pneumonia pathogens

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Background: Diagnosing atypical pneumonia remains challenging due to limitations in routine microbiological diagnostics.

Aim: To enable timely detection and optimal pathogen-directed management, we aimed to establish a fully automated, in-house developed PCR panel for the detection of the most common pathogens causing atypical pneumonia.

Materials and Methods: The PCR panel targets *Pneumocystis jirovecii* (channel 1), *Legionella pneumophila* (channel 2), *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Chlamydia psittaci* (channel 3), *Legionella* species (channel 4), and an internal control (channel 5) in a single reaction. The assay was validated in compliance with IVDR requirements for laboratory-developed tests (LDTs) on a fully automated, high-throughput platform (Cobas 5800/6800/8800, Roche). Analytical sensitivity and specificity were assessed using clinical isolates quantified by digital PCR. The limit of detection (LoD) was determined by Probit analysis using 2-fold dilution series, 21 replicates, and 8 dilution steps. Linearity was evaluated using 10-fold dilution series. For clinical validation and assessment of specificity and sensitivity, 79–80 select samples per target were analyzed and compared with CE-IVD PCR assays. In addition, clinical specimens tested in routine diagnostics between January 2024 and May 2026 were retrospectively analyzed.

Results: Diagnostic performance: The LoD was 50.5 copies/ml (95% CI: 36.9–84.0) for *P. jirovecii*, 396 copies/ml (95% CI: 326–617) for *L. pneumophila*, 215 copies/ml (95% CI: 153–350) for *Legionella species*, 19.3 copies/ml (95% CI: 14.5–35.6) for *C. pneumoniae*, 209 copies/ml (95% CI: 159–322) for *C. psittaci* and 129 copies/ml (95% CI: 92.4–211) for *M. pneumoniae*. Linearity was confirmed for all targets over 6–8 log steps (slopes: −3.36 to −3.56, $r^2 > 0.995$). No cross-reactivity was observed when testing a panel of 31 viral and bacterial pathogens. Specificity ranged from 95.5% (95% CI: 87.6%–98.5%) to 97.3% (95% CI: 90.7%–99.3%), while sensitivity ranged from 91.7% (95% CI: 64.6%–98.5%) to 100% (95% CI: 61%–100%) across all targets. Clinical performance: In total, n= 7427 clinical samples were analyzed (all for the bacterial targets, n=3237 additionally for *P. jirovecii*).

Positivity rates were 0.3% for *L. pneumophila*, 2.3% for *M. pneumoniae*, 0.1% for *C. pneumoniae* and 0.05% for *C. psittaci*, and 7.6% for *P. jirovecii* (median Ct 34,9, range 19,6–38,8). Of 168 positive samples for *M. pneumoniae*, 129 were detected in 2024, consistent with the reported increase in *M. pneumoniae* pneumonia cases in Germany. Conclusion: The assay demonstrated excellent analytical and clinical performance for the detection of five of the most common pathogens causing atypical pneumonia.

PS02.82

Long-term evaluation of preanalytical and analytical factors influencing blood culture pathogen recovery

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Question: Blood cultures remain the cornerstone for diagnosing bloodstream infections, yet their diagnostic performance is strongly influenced by numerous analytical and preanalytical factors. We present the analysis of more than a decade of routine microbiological data assessing the effects of (a) medium, (b) presumptive antibiotic exposure, and (c) transport time on pathogen growth from blood cultures.

Methods: We analysed microbiological data from a hospital trust consisting of three hospitals in Berlin, Germany, between 2012 and 2023. Secondary data use was approved by the ethics committee of the Brandenburg State Medical Association (ref. 2025-133-BO-ff). The dataset included >150,000 blood culture requests and approx. 820,000 associated time-stamped metadata attributes. Blood culture bottles by Waters (formerly BD) (types: "standard" aerobic/anaerobic, "plus" aerobic/anaerobic, "lytic", and "pedibact") were incubated using Bactec devices. Urinary inhibition tests submitted within a time window 1 hour preceding to 48 hours following the blood culture request were used as surrogate markers for presumptive antibiotic exposure. Microbial diversity was quantified with the Shannon index. Statistical significance was evaluated using bootstrapping for diversity, chi-squared tests for proportions, and Wilcoxon rank-sum tests for incubation times.

Results: Among more than 150,000 requests, 16.2% were positive, yielding approximately 27,400 microbial isolates. In inhibition-negative requests, both positivity proportions and Shannon diversities were significantly higher in 5 of 6 bottle types (all $p < 0.01$, except pedibact: inverse trend). Following the introduction of a satellite laboratory adjacent to one of the hospitals in April 2020, median transport time at that site plummeted from 15h to 2.6h, correlating with significantly increased microbial diversities in both "lytic" and "aerobic plus" bottles ($p < 0.01$). Presumptive antibiotic exposure significantly prolonged incubation times in standard as well as aerobic and anaerobic plus media ($p < 0.05$), with standard media consistently displaying the shortest times to positivity.

Conclusions: Presumptive antimicrobial exposure and pre-analytical logistics substantially impact blood culture efficacy. While presumptive antibiotic use is associated with reduced pathogen recovery and delayed detection, incubation on site significantly broadens the detectable microbial diversity, likely by fostering the survival of fastidious organisms. Counterintuitively, standard media displayed the shortest times to detection. These findings underscore the critical need for optimized transport pathways and tailored media selection in clinical routine. Further results will be presented at the conference.

Fig. 1: microbial diversities in inhibition positive and negative bottles by medium type

Fig. 2: incubation times by inhibition status and blood culture bottle combination

Fig. 1

medium type	diversity (inhibition negative)	diversity (inhibition positive)	p-value
aerobic standard	1.780	1.440	<0.01
anaerobic standard	1.711	1.494	<0.01
aerobic plus	1.597	1.094	<0.01
anaerobic plus	1.530	1.145	<0.01
lytic	1.647	0.930	<0.01
pedibact	0.282	0.297	<0.01

Fig. 2

	lytic pair			plus pair			standard pair		
Characteristic	FALSE N = 581[1]	TRUE N = 116[1]	p-value[2]	FALSE N = 446[1]	TRUE N = 342[1]	p-value[2]	FALSE N = 516[1]	TRUE N = 102[1]	p-value[2]
aerobic incubation time	19 (12, 54)	22 (14, 65)	0.087	19 (11, 59)	24 (13, 95)	0.014	13 (7, 41)	18 (10, 89)	0.046
anaerobic incubation time	22 (11, 120)	120 (21, 120)	<0.001	24 (13, 68)	31 (17, 120)	<0.001	18 (7, 78)	29 (10, 120)	0.006

[1] Median (Q1, Q3)
[2] Wilcoxon rank sum test

PS02.83

Analytical and clinical validation of a lab-developed carbapenemase 9-plex assay using a novel, temperature-dependent qPCR probe system

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Background: Carbapenemase-producing Gram-negative bacteria are major cost drivers in healthcare and are associated with adverse patient outcomes. Conventional qPCR multiplexing is constrained by the number of optical channels. This study aimed to develop and validate a lab-developed (LDT) 9-plex qPCR assay that combines a novel temperature-dependent reporter system (Target-mediated Branched Overlap Extension, TBOE) with TaqMan probes to detect and differentiate eight carbapenemase genes plus a pan-16S loading control in a single well.

Methods: Assays were designed using recent sequence information to ensure compatibility and broad inclusivity. The targets blaVIM, blaOXA-48-like, blaKPC and blaOXA-40/24-like are detected via the TBOE reporter system and blaIMP, blaNDM, blaGES and blaOXA-23-like via TaqMan probes, using five optical channels and two temperature categories. Reactions employed standard commercial master mix and qPCR thermocyclers with amplification- and melt-curve analysis. Analytical validation was performed with dPCR-quantified bacterial stocks to generate dilution series. Clinical validation compared clinical isolates against conventional qPCR.

Results: Analytical sensitivity (LoD) ranged between 5,970 copies/ml and 32,000 copies/ml for TaqMan assays and

between 4,260 copies/ml and 97,900 copies/ml for TBOE assays. Accordingly, the pan-16S extraction control was calibrated for a validity threshold of 100,000 copies/ml. Linear range was assessed over a Ct range of at least 19.7 to 31.5 (blaVIM); repeatability ranged from 0.116 Ct (blaOXA-48) to 0.372 Ct (blaVIM). Precision assessed by variance component analysis showed a within-lab imprecision ranging from 0.0115 Ct (blaKPC) to 0.421 Ct (blaNDM). Exclusivity in 17 different Gram-negative species (60 samples) showed no cross-reactivity. Clinical validation (n=66 gram-negative carbapenemase positive isolates) demonstrated 100% positive and 100% negative agreement: blaKPC (7/7), blaOXA-48-like (13/13), blaNDM (5/5), blaVIM (32/32), blaIMP (4/4), blaGES (3/3), blaOXA-23-like (1/1), blaOXA-40/24-like (1/1).

Discussion and Conclusion: The presented 9-plexTaqman/TBOE LDT assay demonstrated robust analytical performance and perfect agreement in the clinical isolate comparison set. Temperature-dependent multiplexing strategies allow expansion of the potential number of qPCR targets on existing diagnostic hardware. Consolidating more targets with this approach has the potential to significantly increase efficiency and throughput for molecular targets such as antimicrobial resistance genes.

PS02.85

Development of a rapid test for the detection of antibiotic resistant *Neisseria gonorrhoeae*

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Introduction: *Neisseria gonorrhoeae* (NG) is the second most prevalent sexually transmitted bacterial pathogen worldwide and an increasing public health concern due to rising antimicrobial resistance (AMR). Current diagnostic methods mainly rely on nucleic acid amplification tests requiring specialized laboratory infrastructure. Rapid point-of-care assays capable of simultaneously identifying NG and resistance determinants are still lacking.

Objectives: Aim of the project is the development of a rapid lateral flow assay (LFA) for NG detection combined with the identification of antibiotic resistance-associated RNA targets.

Materials and Methods: Comparative genome analysis and transcriptional profiling were used to identify conserved and highly expressed NG targets. Five antigens were selected and recombinantly expressed in *E. coli* to immunize mice for monoclonal antibodies (mAbs) generation by hybridoma technology. Two antigens, NG0101 and NG0177, were further characterized due to confirmed binding of antibody clones to endogenous NG proteins in lysates. Mabs against both antigens were conjugated to gold nanoparticles and evaluated by competition analysis and LFA half-strip testing.

For RNA-based AMR detection, a heteroduplex LFA approach was developed. Resistance in NG is linked to point mutations in e.g. *gyrA* (ciprofloxacin) or *penA* (penicillin). Based on a heteroduplex LFA principle, biotinylated DNA probes were immobilized via streptavidin plates to capture complementary RNA targets. DNA-RNA hybrids were detected using the monoclonal antibody S9.6 recognizing RNA/DNA heteroduplexes. S9.6 was purified from hybridoma culture supernatant and characterized by SDS-PAGE and binding ELISA. For LFA application, S9.6 was conjugated to gold nanoparticles to enable visible signal generation. Initial proof-of-concept experiments were performed using synthetic 16S rRNA and *rplP* RNA fragments and corresponding DNA-probes. Heteroduplex formation was additionally analyzed by agarose gel electrophoresis.

Results: Competition analysis identified several non-overlapping monoclonal antibody pairs against NG0101 and NG0177 suitable for sandwich-LFA applications. Preliminary half-strip experiments demonstrated target-specific signal generation with selected antibody combinations. Ongoing analyses include testing with clinical NG isolates under near real-life conditions.

S9.6 purification yielded functional antibody and specific RNA/DNA heteroduplex formation was demonstrated by agarose gel mobility shift assays. Streptavidin-based immobilization enabled efficient biotinyl. DNA-probe/RNA capture on ELISA plates and on nitrocellulose, producing detectable signals upon gold-labelled S9.6 binding.

Conclusion: This project demonstrates substantial progress towards a combined antigen- and RNA-based LFA for rapid NG diagnostics and AMR detection. This integrated approach may enable rapid point-of-care diagnostics for resistant gonococcal infections.

PS02.86

Imported case of Vibrio cholerae O1 El Tor in Bulgaria: Genomic and epidemiologic characterization

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Introduction: Cholera is an acute diarrheal disease caused by Vibrio cholerae, transmitted primarily through ingestion of contaminated food or water. It remains a global public health concern, with endemic areas in Africa, South Asia, and South America.

We report an imported case of cholera in Bulgaria including phylogenomic characterization of the isolated Vibrio cholerae strain. This is the first confirmed imported cholera case in Bulgaria in over a century. The patient was an Indian citizen, 23-year-old university student in medicine in Bulgaria, who had traveled to New Delhi, India, between August 13 and September 2, 2024. On September 3, he developed acute watery diarrhea without additional symptoms. The case was laboratory-confirmed, as Vibrio cholerae at the National Reference Laboratory for Special Bacterial Pathogens, at the National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria.

Stool samples were cultured for the presence of vibrios. The isolated strain was examined for oxidase activity and identified by MALDI-TOF MS, slide agglutination tests, and multiplex PCR. To determine genetic lineage and potential virulence/resistance markers whole-genome shotgun (WGS)

sequencing was also performed. The assembled genome consisted of 94 contigs, with an estimated total length of 4,024,112 nucleotides. The average G+C content was 47.50%. The Contig N50 (the shortest sequence length at 50% of the genome) was 215,351 nucleotides, with an L50 (the smallest number of contigs whose length sum produces N50) of 7 contigs.

Summary: The last recorded case of cholera in Bulgaria was in 1921, when it was imported again. This case illustrates the continued vulnerability of non-endemic countries to imported cholera through international travel and underscores the need for sustained laboratory capacity, molecular surveillance, and public health preparedness.

PS02.87

Specific PET-imaging of bacterial infections using novel carbohydrate-based imaging probes

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Life-threatening bacterial infections often present with non-specific symptoms. Distinguishing infectious from non-infectious conditions and localizing bacteria are therefore critical for patient management. Although 2-deoxy-2-[^{18}F]fluoro-D-glucose positron emission tomography ([^{18}F]FDG-PET) is widely used for infection imaging, it visualizes the host immune response rather than bacteria directly. We therefore aimed to develop bacteria-specific ^{18}F-labeled carbohydrate-based PET tracers for direct bacterial detection.

We analyzed the *in vitro* uptake of two novel probes, [^{18}F]NT155 (sakebiose-based) and [^{18}F]LGO314 (maltotriose-based), both carrying an ^{18}F label at the C2 position. Tracer uptake was quantified using a gamma counter, and stability was assessed in human and mouse serum. In addition, [^{18}F]LGO314 was evaluated *in vivo* in PET biodistribution studies and a subcutaneous infection model. Bacteria were injected into the shoulder region of mice, followed 3 h later by tracer administration via the tail vein. Tracer accumulation was analyzed by PET imaging.

[^{18}F]LGO314 was taken up *in vitro* by *Staphylococcus aureus* (Sa), *Escherichia coli* (Ec), and *Klebsiella pneumoniae* (Kp), but not by *Pseudomonas aeruginosa* (Pa). Sa and Ec infections could be detected by PET imaging using [^{18}F]LGO314. However, the imaging signal was low and may not provide sufficient sensitivity for detecting low bacterial burdens.

Our results highlight several challenges. First, tracer uptake is relatively slow, with the highest gamma-counter signal observed after 1 h of incubation with Sa, whereas [^{18}F]LGO314 exhibits a short blood circulation time, resulting in suboptimal bacterial uptake *in vivo*. Additionally, the tracer is gradually washed out of bacteria, preventing sufficient intracellular accumulation for reliable diagnosis. A possible explanation is the metabolic cleavage of the glycosidic bonds, followed by further catabolism of the released [^{18}F]FDG. This contrasts with mammalian cells, where [^{18}F]FDG accumulates over time through metabolic trapping.

To overcome limited tracer uptake, we developed [^{18}F]NT155, a tracer based on the disaccharide sakebiose. Disaccharides are transported into bacteria via the phosphotransferase system, which generally enables faster uptake than the ATP-binding cassette transport systems responsible for maltotriose uptake. Initial *in vitro* experiments with [^{18}F]NT155 demonstrated significantly faster and higher uptake than [^{18}F]LGO314, particularly in Sa. The tracer was also taken up by Kp, but not by Ec. First *in vivo* studies are currently under investigation.

In summary, we present two novel PET tracers, [^{18}F]LGO314 and [^{18}F]NT155, that show species-dependent uptake patterns in bacteria. Ongoing studies will determine their suitability for sensitive *in vivo* imaging of bacterial infections and whether a sakebiose-based tracer offers advantages regarding metabolic trapping and bacterial retention.

PS02.88

Performance assessment of a new multiplex molecular panel for acute gastroenteritis: Single-center prospective analysis

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Introduction: Acute gastroenteritis is a frequent cause of outpatient consultation and early hospitalization, with diagnostic workflows often relying on heterogeneous and time-consuming methods. Multiplex molecular panels may enhance pathogen detection and standardize diagnostic approaches.

Objectives: To prospectively evaluate the performance of the RUO Xpert GI Panel (Cepheid) compared with the BioFire Gastrointestinal Panel (bioMérieux) for gastrointestinal pathogen detection, and to assess agreement and time to result in a real-world setting.

Materials and Methods: This prospective single-center study was conducted in Essen, Germany. Residual de-identified stool specimens collected in Cary-Blair medium for routine diagnostics were tested using the Xpert GI Panel (RUO) and compared with BioFire. The head-to-head comparison focused on 11 targets: *Salmonella* spp., *Shigella*/EIEC, Shiga toxin 1/2, *Campylobacter* spp., *Yersinia enterocolitica*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Giardia intestinalis*, *Cryptosporidium* spp., and norovirus. Discrepant results were resolved by a third method to establish a consensus reference for sensitivity and specificity estimation. Time to result was prospectively recorded.

Results: A total of 118 samples were included (positivity rate 15%). The cohort comprised 49% women and 51% men; 97% were older than 5 years. Patients were mainly inpatients hospitalized <3 days (78%), followed by outpatients (18.6%) and emergency department patients (3.4%). Eighteen positive detections were observed, including co-detections. Concordant positives included *Salmonella* spp. (n=2), *Campylobacter* spp. (n=4), norovirus (n=9), and Shiga toxin 1/2 (n=2). Co-detections were identified in 2 samples by BioFire alone, 2 by

Xpert alone, and 1 by both methods. Nine samples were discordant. Most discrepancies were attributable to BioFire, including five false-positive norovirus results and two false-positive *Campylobacter* spp., as well as one false-negative *Yersinia enterocolitica*. One false-positive norovirus result was observed with Xpert. After discrepancy resolution, Xpert demonstrated 100% sensitivity and >99% specificity across all detected pathogens. Median time to result was 78 minutes for Xpert and 74 minutes for BioFire.

Summary: In this single-center study, the Xpert GI Panel (RUO) demonstrated excellent diagnostic performance with 100% sensitivity and >99% specificity after discrepancy resolution. Discordances were limited and mainly attributable to the comparator. Comparable turnaround times support the use of multiplex molecular testing for routine gastroenteritis diagnostics; assessment of clinical impact warrants further study.

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