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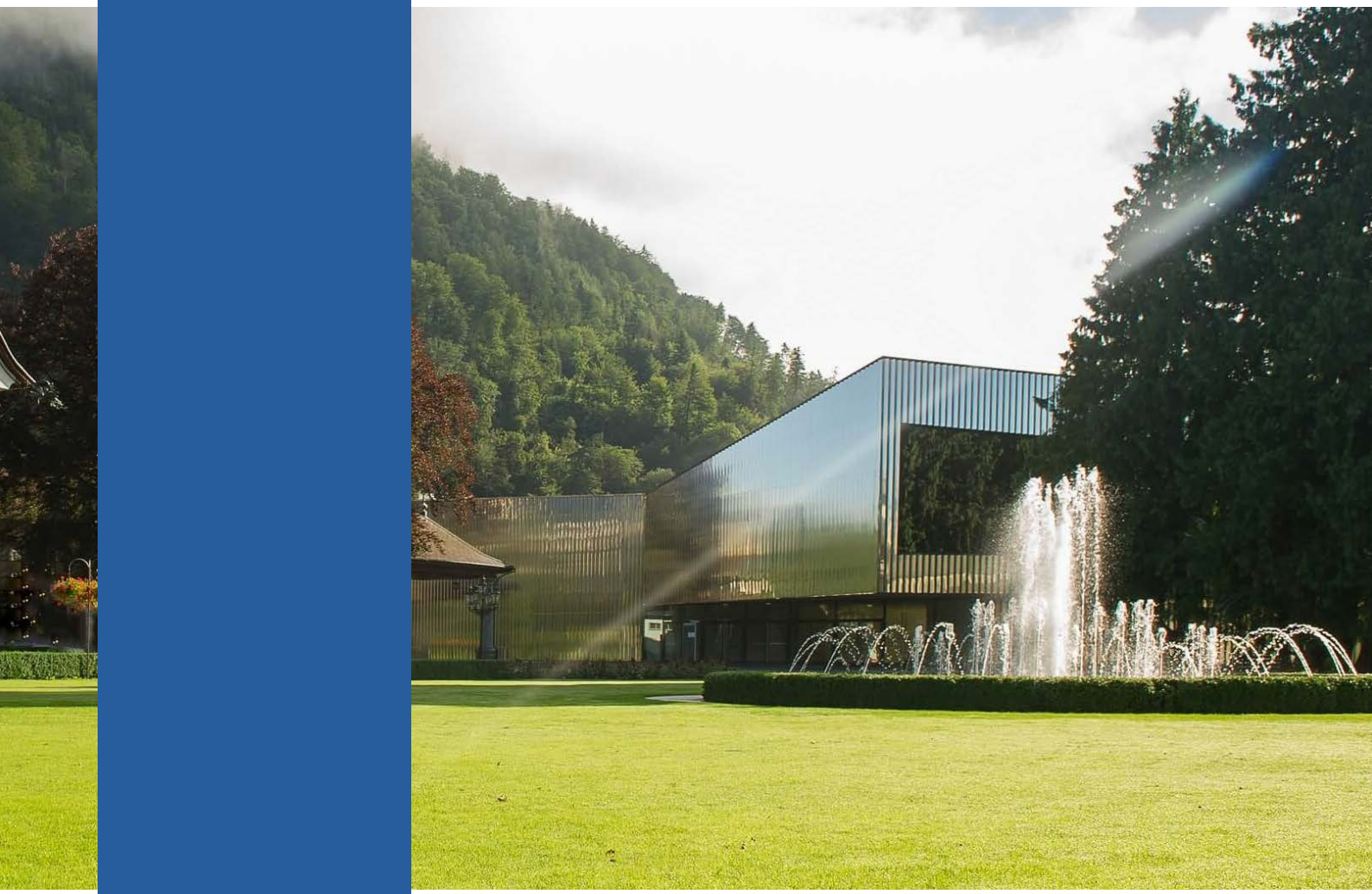

ABSTRACT BOOK

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KEYNOTE TALK

K-01 Gut microbiota-engineering for improved child growth

Pascale Vonaesch & team

University of Lausanne, Lausanne, Switzerland

Chronic undernutrition in childhood can lead to stunted child growth, a condition with far-reaching consequences, including cognitive delays and weakened immune responses. This issue affects approximately 100 million children under the age of 5 worldwide, with the highest prevalence found in low- and middle-income countries. In our recent research, we focused on investigating changes in the gastrointestinal microbiome composition in these children and could elucidate two bacterial signatures: an overabundance of oral bacteria within the small intestine, termed small intestinal oral bacterial overgrowth (SIOBO) as well as a reduction in butyrate producers and bile converters in the fecal microbiome. SIOBO is especially characterized by an overgrowth of oral taxa, such as *Streptococcus salivarius* and has been associated with impaired nutrient uptake as well as gut barrier dysfunction. In my talk, I will share some recent advances on how host-derived metabolites such as bile acids interact with and shape the microbiome and will also share recent results on how we can engineer the microbiome using (pro)phages and especially lysins isolated thereof. Combined with complementary nutritional interventions, the use of phage lysins and other microbiota-modulating approaches may offer a precision medicine strategy to remodel dysbiotic microbiota and improve growth outcomes in children.

KEYNOTE TALK

K-02 Exploring the interplay between bacteria & MGEs

Rafael Pinilla-Redondo

University of Copenhagen, Copenhagen, Denmark

K-03 Value-based laboratory medicine

Mario Plebani

University of Paadova-Italy, Italy

Healthcare systems are under intense pressure to increase value while ensuring sustainability. Following the outbreak of the Coronavirus (Covid-19) pandemic, there has been growing interest in the concepts of value-based medicine (VBM) and value-based laboratory medicine (VBLM) as a means of improving the quality, sustainability and safety of care. The specialty of laboratory medicine, particularly clinical microbiology, is a good example of health care resource shared across all levels of health care delivery, virtually all medical specialties, and patient care at all ages. Laboratory medicine is well positioned to support the transition to value-based healthcare as it helps to improve clinical outcomes and healthcare sustainability by reducing the time to diagnosis, improving diagnostic accuracy, providing effective guidance for tailored therapies and monitoring, and supporting screening and wellness care. However, the perception of the value of laboratory medicine is still limited, often lacking visibility to patients and the public. In addition, in recent decades, clinical laboratories have sought to improve the ratio between outcomes and costs by increasing efficiency and reducing the cost per test rather than improving clinical outcomes. Clinical laboratories have been pioneers in introducing process measures (internal quality control, external quality assurance and the model of quality indicators) to improve the accuracy and reliability of laboratory results and should now play a key role in promoting value-based laboratory medicine to ensure more effective, safe and patient-centered clinical diagnostic and therapeutic pathways. In recent discourse, a set of ten commandments has been proposed as a framework for the implementation of this process in clinical practice addressing the necessity of ensuring the quality of the entire testing process, the pivotal role of standardization and harmonization, and the emphasis on the measurement and enhancement of clinical outcomes.

KEYNOTE TALK

K-04 Drivers of phage transport in soil

Lukas Wick

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K-05 MxB-mediated inhibition of herpesvirus infection

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MxA and MxB are antiviral members of the dynamin family of GTPases with MxA restricting several RNA viruses and MxB restricting lentiviruses and herpesviruses. MxB is localized at the nuclear pores at basal expression, but also accumulates in cytosolic biomolecular condensates upon IFN induction. The BioID MxB interactome includes many nucleoporins and proteins of cytoplasmic ribonucleoprotein granules (Moschonas 2024, *Cell Host Microbe).* Cell extracts containing active MxB and GTP disassemble HSV-1, HSV-2, and VZV capsids, while MxA or GTPase hydrolysis-deficient MxB or truncated MxB(26-715) do not (Serrero 2022, *eLife).*
Using epithelial cell lines with inducible expression of MxA, MxB, MxB mutants, or MxA/B chimeric proteins, we show that MxB restriction requires its N-terminal domain and oligomerization. While the MxB GTPase activity contributed to restriction, the MxB(T151A) mutant, which cannot hydrolyze GTP but binds to capsids, also perturbed HSV-1 infection. Experiments with HSV-1 inocula with CLICKable genomes show that MxB also disassembles capsids in cells, leading to premature genome uncoating and fewer nuclear incoming genomes.
Since MxB restricts α-, β-, and γ-herpesviruses, we investigated whether MxB could target the evolutionarily conserved portal cap that consists of two pentamers of a minor capsid protein (HSV1-pUL25, KSHV-pORF19). Pentameric, but not monomeric, KSHV-pORF19 inhibited MxB-induced capsid disassembly. Surface Plasmon Resonance (SPR) showed that MxB N-terminal domain binds directly to pentameric or monomeric KSHV-pORF19 with similar affinities.
Our data indicate that upon IFN induction, MxB and NPC proteins form cytosolic biomolecular condensates, which bind incoming capsids and impair their nuclear targeting efficiency. MxB restricts HSV-1 through a GTPase-independent mode, where capsid binding may block host interactions or stabilize capsids, and a GTP-hydrolysis-dependent mechanism, where conformational changes promote capsid disassembly. The resulting premature release of HSV-1 genomes into the cytosol might activate DNA sensors, such as cGAS, IFI16, or AIM2, and enhance host innate immunity defenses.

MAIN TALKS – SHORT TALKS

M-01 Diagnostic and Clinical Applications of Viral Metagenomic Sequencing

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Metagenomic next-generation sequencing (mNGS) is emerging as a transformative diagnostic tool in clinical virology, enabling comprehensive, unbiased detection of viral pathogens. It is particularly valuable in diagnostically challenging infectious syndromes, such as encephalitis and lower respiratory tract infections, where conventional diagnostics fail to identify a causative virus in up to 60% of cases. Our group evaluated a targeted hybrid-capture mNGS approach using the Twist Bioscience Comprehensive Viral Panel, covering over 3,000 RNA and DNA viruses. This approach offers a 10–100-fold increase in sensitivity compared to untargeted metagenomics, significantly enhancing viral detection in samples with low viral loads or high DNA background. Realizing this diagnostic potential in clinical practice demands rigorous quality control. We incorporated internal controls for qualitative validation and quantitative normalization, along with positive and negative controls to ensure assay reliability and monitor for contamination. We also systematically assessed key wet-lab parameters, including nucleic acid extraction and cDNA synthesis protocols, demonstrating their substantial impact on diagnostic yield. Despite these advancements in wet-lab processes, bioinformatic analysis remains a critical challenge. Stringent human-read depletion, while essential to reduce host background, can inadvertently remove virus-derived reads, especially from integrated or low-abundance viruses. Thus, tailored bioinformatic pipelines and careful thresholding are essential to maintain diagnostic sensitivity.

We currently apply this workflow across diverse patient cohorts, including transplant recipients with lower respiratory tract infections, patients with neuroinflammatory syndromes, and oncology patients with suspected viral etiologies (e.g., HPV-negative tumors). To ensure accurate interpretation and clinical relevance of mNGS findings, we strongly advocate for interdisciplinary review of mNGS results through dedicated «diagnostic microbiology boards». Our experience highlights the growing value of viral metagenomics in routine diagnostics for pathogen detection in complex or unresolved clinical cases, and supports its transition from a specialized research method to a reliable, clinically actionable diagnostic platform.

S-01 Cure of a difficult-to-treat Pseudomonas aeruginosa canine chronic otitis externa (OE) with the PYO bacteriophage cocktail followed by antibiotics

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A Labrador presented with severe chronic otitis externa. The ear canal was thickened and covered with foul-smelling purulent secretions, the walls showing extensive erythema with ulcerations. *Pseudomonas aeruginosa* was identified and standard of care (SoC, gentamycin + polymyxin) treatment had failed. Phage therapy, which uses specific bacterial viruses called bacteriophages, was proposed. PYO bacteriophage cocktail (Eliava biopreparation, Georgia) was applied locally into the ear canal after soft cleaning over three consecutive days (0.5mL per day). *P. aeruginosa* and phage loads were monitored from ear swabs. Antibiotic and phage susceptibility was assessed on recovered clones. Resistance mechanisms were investigated through comparative genomics. Before onset of treatment, *P. aeruginosa* isolates were fully susceptible to PYO *in vitro*. After a 2-$\log_{10}$ *P. aeruginosa* load reduction by D2 post-treatment, levels returned to baseline. Interestingly, rapid and significant clinical improvement was observed, with reduced secretions and pain. We found that already at D1, the pathogen population changed with the emergence of phage-resistant clones, without modification of antibiograms. These clones harboured large genomic deletions or mutations impacting genes involved in O-antigen and type IV pilus biosynthesis known as phage receptors. Still, phages persisted in the ear canal for three weeks, with evidence of phage-susceptible *P. aeruginosa* at D65. Finally, instillations of SoC at D65 and D90 cleared the infection after >2 years of ineffectiveness. Phage therapy alone significantly improved clinical symptoms and modified the *P. aeruginosa* ear community towards mixed-resistance profiles. Although antibiograms remained similar, we believe that this pathogen community switch could explain the observed successful therapeutic outcome. Decrease in virulence or capacity to form biofilm of the selected phage-resistant clones might be involved and are currently investigated.

S-02 Engineered phage therapy against catheter-associated urinary tract infections (CAUTI)

Dr. Fatih Abudla [1], Dr. Enea Maffei [2], Dr. Marion Bichet [3], Ms. Berta Puig Collderram [1], Dr. Sonja Milek 1, Mr. Robert Miarecki [1], Dr. Jiemin Du [2], Ms. Ellen-Aleksandra Svorjova [4], Prof. Martin J. Loessner [4], Mr. Matthieu Labriet [3], Mr. Valentin Borgeat [3], Prof. John McKinney [3], Mr. Ashim Dubey [5], Prof. Adrian Egli [5], Mr. Benjamin Sellner [6], Prof. Christoph Dehio [6], Prof. Thomas Kessler [1], Dr. Lorenz Leitner [1],Dr. Shawna McCallin [7]

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Catheter-associated urinary tract infections (CAUTI) are the most frequent nosocomial bacterial infections and leading cause of secondary bloodstream infections. Widespread antibiotic use in CAUTI management plays a critical role in the emergence and spread of antimicrobial resistance (AMR). Bacteriophages offer a potential alternative to antibiotics to combat resistant bacteria and to treat UTIs. Here, we present preclinical data for a two-phage cocktail, uroCOLE7-01, targeting uropathogenic Escherichia coli, the leading cause of UTIs and a WHO priority pathogen.

The two phages, E2 and 41S, were engineered using homologous recombination and CRISPR-Cas counterselection to incorporate the endonuclease colicin E7. Spot assays and turbidity reduction assays (TRAs) were used to determine the host range and lytic activity in different media. A urothelial transwell cell culture model and an ascending UTI mouse model were used to evaluate uroCOLE7-01's localisation, pharmacokinetics and pharmacodynamics.

The engineered phages E2 and 41S infected 58% and 37% of strains, respectively, for a combined host range of 68% on a panel of 260 clinical E. coli isolates. The engineered cocktail suppressed bacterial growth more than wild-type phages in TRAs and decreased the development of phage resistance compared to the wild type (30% versus 55%, respectively). uroCOLE7-01 significantly reduced bacterial loads in cell culture, as well as in the bladder, kidney, and urine of phage-treated mice (>1log). Phages were rapidly cleared from the bloodstream after intravenous administration and were present in target tissues of the bladder and kidney within 15 min.

The preclinical results highlight the possibility of efficient development of phage-based therapeutics, enabled by advanced gene editing techniques. The phages are currently being produced under good manufacturing practices, with the prospect of a phase I trial by the end of 2025. This work addresses the urgent need for alternative interventions, especially in high-burden, low-resource settings where AMR impact is greatest.

M-02 From the physiology of methanogens to a robust biological methanation process

Wenyu Gu

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Area: Applied and Environmental Microbiology

Methanogenesis is a form of anaerobic respiration that generates methane as the final product of metabolism. It is carried out by a group of archaeal microorganisms called methanogens. Methanogens play a crucial role in the global carbon cycle by participating in the anaerobic breakdown of organic matter into CH4 and CO_2. They are also of biotechnological significance, being central to the development of renewable energy technologies such as power-to-methane and anaerobic digestion. Understanding methanogens' physiology is essential for optimizing their performance in engineered systems.
Hydrogenotrophic methanogens are of particular interest. Taking the example of power-to-methane, methane can be generated from hydrogen produced via water electrolysis powered by renewable energy. This hydrogen is combined with captured waste CO_2 and converted into synthetic natural gas through methanogenesis. This renewable, carbon-neutral fuel can be stored and distributed using existing natural gas infrastructure, offering a viable solution for buffering the variability of renewable electricity generation.
We will show that, by systematically characterizing the physiology of a model hydrogenotrophic methanogen—including cellular resource allocation strategies, responses to fluctuations in substrate availability, and exploration of co-cultures—we can develop a robust understanding of microbial methanation under application-relevant conditions. These insights will inform process designs to support the reliable deployment of the biological methanation process. More broadly, this work will provide fundamental insights into microbial physiology in fluctuating natural and engineered ecosystems.

S-03 Methylphosphonate driven methane production in oxic marine environments

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Methylphosphonate is an organic phosphorus compound characterised by a stable carbon-phosphorus bond. Together with other phosphonates it constitutes a substantial fraction of the organic phosphorus pool found in the marine environment. Phosphorus is an essential growth-limiting nutrient and the use of organic phosphorus compounds is prevalent in phosphate-limited surface waters. However, microbial usage of methylphosphonate by the carbon-phosphorus lyase pathway results in the formation of methane, a potent greenhouse gas. Methylphosphonate utilisation in oxygenated surface waters therefore directly contributes to marine methane emissions and ongoing climate perturbations. To date, the extent and distribution of this process in marine waters remains underexplored.

We combined stable isotope incubation experiments with metagenomic and metatranscriptomic analyses to quantify rates of aerobic methane formation and to characterise the microbial community responsible for methylphosphonate utilisation in the phosphate-limited western tropical North Atlantic. Over 24 hours, methylphosphonate was transformed to methane throughout the upper 200 metres of the water column, with the highest median rates (0.4 nmol methane L^{-1} d^{-1}) measured in surface waters. With increasing depth, methane formation rates were repressed but not inhibited to median rates of ~0.06 nmol methane L^{-1} d^{-1} in the presence of phosphate. Interestingly, the phosphorus liberated from methylphosphonate transformation could sustain a substantial amount of carbon dioxide fixation (median 11%) in the investigated stations emphasising the ecological importance of this pathway. The utilisation of methylphosphonate, by the carbon-phosphorus lyase pathway, was both encoded and transcribed by key hetero- and phototrophic microbial taxa, such as *Pelagibacterales*, *SAR116* and *Trichodesmium*. While the large cyanobacteria were dominant in the surface waters, heterotrophic taxa became more prevalent with increasing depth. Our more recent data indicate that some of these key taxa have a global distribution and may contribute to methylphosphonate utilisation also in other oligotrophic environments, such as the Mediterranean Sea.

S-04 Growth in low carbon conditions reveals amino-acid-coupled iron uptake

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Bacteria in nature encounter substrates at widely varying concentrations, yet studies of bacterial physiology have focused more on nutrient type than concentration, partly due to challenges in maintaining low concentrations. We developed a Millifluidic Continuous Culture Device (MCCD) to culture bacteria under precisely controlled nutrient conditions, including very low concentrations, in a manner suitable for proteomic analysis. Using the MCCD, we cultured *Escherichia coli* with a mixture of amino acids as the sole carbon source at three concentrations supporting growth rates spanning a fivefold range. Surprisingly, at the lowest concentration, cells exhibited proteomic signatures of iron shortage despite equal iron levels across conditions. We observed the uptake of labeled iron-histidine and iron-cysteine complexes, indicating that amino acids facilitated iron acquisition, and that amino-acid-bound iron is bioavailable to *E. coli*. These findings reveal a previously unknown mechanism of bacterial iron acquisition that emerged under the flow imposed by the MCCD, which likely diluted the siderophore pool and reduced their efficacy. This work highlights the importance of studying bacterial physiology under low nutrient concentrations and demonstrates how physical conditions, such as flow, shape microbial nutrient acquisition strategies.

M-03 Unique relationships between phages and spore-forming bacteria. Lessons from Bacillus subtilis

Anna Dragos

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S-05 Functional dissection of nucleoside diphosphate kinase reveals its central role in Chlamydia trachomatis development

Dr. Giti Ghazi-Soltani [1], Dr. Simone Adams [1], Dr. Carole Kebbi Beghdadi [1], Prof. Scot Ouellette [2], Prof. Gilbert Greub [1]

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C. trachomatis is an obligate intracellular pathogen that alternates between the infectious elementary bodies (EBs) and the replicative reticulate bodies (RBs). Despite its medical significance, little is known about the genetic regulation of its developmental cycle. Nucleoside diphosphate kinase (Ndk) is a multifunctional protein involved in cellular nucleotide maintenance, cell development, gene regulation, and evasion of host defenses across diverse organisms. To investigate the role of *ndk* in *C. trachomatis* (*ctndk*), the study used CRISPR interference (CRISPRi) to knockdown *ctndk*. Knockdown resulted in smaller inclusions, impaired RB-to-EB conversion, reduced EB infectivity and failure to lyse host cells. *ctndk* knockdown also resulted in abnormal glycogen accumulation, suggesting a previously unrecognized role in glycogen catabolism. These defects were fully rescued by complementation with wild-type CtNdk. However, point mutations in key catalytic residues, H115A (ATP-binding/autophosphorylation-deficient) and H52A/R85A (phosphotransfer-deficient) did not rescue the phenotype. These results suggest that CtNdk promotes *C. trachomatis* development through ATP-binding and subsequent phosphotransferase activity, possibly phosphorylating unknown protein substrates. In conclusion, our findings establish CtNdk as a crucial regulator of *C. trachomatis* development and suggest that it plays multifaceted roles across multiple stages of the developmental cycle.

S-06*/P-81* Influence of conserved Cys-residues in a transcription factor on ICEclc activation in Pseudomonas putida

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Horizontal gene transfer (HGT) is major mechanism for producing bacterial genome variation. Different HGT mechanisms are known in great detail, but less is known about the underlying regulatory system(s) and their response to environmental signals.
Here, we use the Integrative and Conjugative Element ICE*clc* of *Pseudomonas putida* as a model system. ICE*clc* encodes genes allowing the degradation of 3-chlorobenzoate (3CBA). Its activation is tightly regulated, involving a combination of classical regulators—MfsR and TciR (a TetR- and LysR-type regulator, respectively)—as well as the recently discovered BisR and BisDC families [1]. Activation and excision of ICE*clc* is constrained to a small subset of cells, named 'transfer competent' (tc) cells, and is highest when cells have been grown to stationary phase with 3CBA as unique carbon source (2-5% of cells) [2]. Recent work also showed that 3CBA metabolism induces a strong oxidative stress, suggesting that cells experiencing above-average oxidative stress are more likely to activate the tc program.
To investigate the potential link of oxidative stress to ICE*clc* activation, we focused on the TciR transcription activator. TciR has weak homology to OxyR, the oxidative stress regulator of *Escherichia coli*. In silico analysis identified four cysteines in TciR, including one (C199) perfectly aligned with one of OxyR reactive cysteines [3]. Based on this, we constructed four mutants in *P. putida* ICE*clc tciR* carrying an individual cysteine-to-serine substitution, and monitored their effects on ICE*clc* activation using a fluorescent transcriptional reporter and on ICE conjugation. Replacement of 3 out of 4 Cys-residues by Ser decreased ICE*clc* activation by more than tenfold, whereas the fourth (C203S) reduced activation by two-fold. This supports the hypothesis of implication of oxidative stress in ICE*clc* activation, possibly involving intra-molecular disulfide bond formation, modulating TciR oligomerization that affect its activation of the *bisR* promoter.

M-04 **Fungal models of Target Of Rapamycin signaling**

Robbie LOEWITH

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Rapamycin is a bacterial macrolide discovered by virtue of its antifungal activity. The Target Of Rapamycin, TOR, is an atypical Ser/Thr protein kinase broadly conserved across eukaryotic species. Rapamycin is a clinically approved drug used in immunosuppression, oncology as well as other indications including off-label use as an agent to increase longevity. These clinical ramifications drive strong interest in understanding TOR signaling at both the molecular and atomic levels, e.g. for drug design purposes.

We use budding yeast *(Saccharomyces cerevisiae)* as a model system to study TOR signaling. We found that TOR functions in two distinct multiprotein complexes, TORC1 and TORC2. TORC1 primarily resides on the vacuole membrane and functions in a rapamycin-sensitive signaling pathway that couples nutrient adequacy and inadequacy to cell growth and autophagy respectively. Thus, TORC1 regulates cell mass/volume homeostasis. TORC2 primarily resides at the plasma membrane (PM) and functions in a rapamycin-insensitive pathway that couples membrane mechanical stress to downstream targets that affect biophysical properties of the PM. Thus, TORC2 regulates cell surface homeostasis.

I will present our recent efforts using cryoEM to reveal at near atomic resolution how TORC1 and TORC2 operate in these separate signaling pathways to coordinately control cell growth. I will end with a brief outlook on our recent incorporation of *Magnaporthe oryzae* as a new fungal model to study TOR signaling, highlighting how this line of investigation may lead to new approaches to tackle rice blast disease.

S-07 Nuclear basket proteins regulate the distribution and mobility of nuclear pore complexes in budding yeast

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Nuclear pore complexes (NPCs) mediate all traffic between the nucleus and the cytoplasm. The nuclear basket is a prominent feature of the nuclear pore complex (NPC) and plays important roles in mRNA export and chromatin organization. In budding yeast, basket formation is the last step of NPC assembly and baskets are associated only with a subpopulation of NPCs. We use recombination induced tag exchange and expansion microscopy to visualize yeast nuclear pore complexes at single pore resolution to determine the architecture, assembly mechanism and dynamics of the nuclear basket and its interaction with the nucleolus. Our data suggest that the distribution of NPCs on the nucleus is governed by multiple interaction of nuclear basket proteins with the nuclear interior.
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S-08 Colloid osmotic pressure dictates nuclear size and efficient gene silencing

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In many cell types, the nuclear-to-cell volume ratio (N/C ratio) remains constant during growth. Increased nuclear size during growth coincides with altered transcriptome and impaired genome homeostasis. In yeast, the N/C ratio is approximately 8%. How this ratio is maintained and how changes in nuclear size affect nuclear function remain poorly understood. Previous work suggested the N/C ratio is set by osmotic equilibrium. However, as small osmolytes can diffuse freely through the nuclear pores, it was unclear how such an osmotic equilibrium could be established. Here we investigated the hypothesis that the N/C ratio is determined by the osmotic pressure formed by soluble proteins in the cytoplasm and the nucleus (colloid osmotic pressure).
To this end, we overexpressed proteins in the nucleus and in the cytoplasm and examined the effects on the N/C ratio and on the transcriptome. We used the high-copy yeast 2µ plasmid, engineered to express *LEU2* weakly under a truncated *LEU2* promoter. This allowed for selection of yeast cells harboring higher plasmid copy numbers (~40 to ~200 copies) in leucine-deficient medium. We inserted a construct expressing three tandem fluorescent proteins (3xFP) under an estradiol-inducible promoter. By adding a nuclear localization signal (NLS) to 3xFP (yielding 3xFP-NLS), we successfully accumulated large amounts of fluorescent protein in the nucleus. Cells expressing 3xFP-NLS showed an increased N/C ratio from 8% to ~17%, while those expressing cytoplasmic 3xFP had a decreased ratio of ~6%. Transcriptome analysis by mRNA-seq revealed that cells with elevated N/C ratio (3xFP-NLS) exhibited increased expression of typically repressed genomic regions such as retrotransposons and subtelomeric genes. In contrast, cells with reduced N/C ratio (3xFP) showed the opposite trend. These findings demonstrate that the N/C ratio is determined by a colloid osmotic pressure and that changes in the N/C ratio can have reciprocal and genome-wide effects on transcription.

M-05 NGS applications at the Swiss veterinary virological reference laboratory

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The Institute of Virology and Immunology (IVI) functions as the Swiss national reference laboratory (NRL) for the majority of reportable viral diseases in veterinary medicine. Notably, it is the sole institution permitted to handle highly contagious animal viruses, such as foot-and-mouth disease virus.
In the last decade, climate change and increased mobility have led to an unprecedented threat from epizootic diseases in Europe. Modern sequencing tools have become an important help in addressing the growing challenges in animal disease control. At IVI, there are two principal areas of application for NGS in the diagnostic department: firstly, full genome sequencing of viruses for fast and precise typing, and secondly, virome analysis, which allows the diversity of viruses in a sample to be investigated.
An important application for i) is full-genome sequencing of avian influenza viruses (AIV) for which the IVI functions as NRL. H5N1 viruses in particular have killed billions of birds worldwide in recent years and are increasingly spreading to mammals. Amplicon resequencing using Oxford nanopore technology and subsequent genotyping encompassing all 8 genomic segments is pivotal to molecular tracing of outbreaks and the detection of reassortments.
For virome analysis, non-specific/metagenomic short-read sequencing and subsequent combination of diverse mapping approaches are utilised to determine viral nucleic acids in diverse sample materials. The method is employed with the objective of detecting viruses in undiagnosed clinical cases or outbreaks, for early detection of “novel” or emerging viruses in life-stock, wild-life or potential vectors such as ticks, and to monitor “commensal” viruses in SPF animals.
In both areas, the utilisation of contemporary sequencing repositories is imperative for the interpretation of results to be meaningful. Adopting a One Health and open science approach, we are sharing our sequences on international and national databases such as the Swiss Pathogen Surveillance Platform (SPSP).

S-09* Exploring Nanomotion Technology for Phage Therapy: A Novel Diagnostic Tool to Combat Antimicrobial Resistance

Mr. Anthony Vocat [1], Dr. Amanda Luraschi-Eggemann [1], Dr. Grzegorz Józwiak [2], Ms. Julia Dolezel [3], Ms. Zahra Azizi [3], Mr. Shreyas Vasantham [3], Dr. Salomé Gutiérrez [3], Dr. Danuta Cichocka [4], Dr. Alexander Sturm [4], Dr. Grégory Resch [1]

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Background

Antimicrobial resistance (AMR) is an escalating global health threat, responsible for hundreds of thousands of deaths annually, with projections reaching 10 million fatalities per year by 2050 without effective interventions. Phage therapy, utilizing bacteriophages to target drug-resistant bacteria, presents a promising complementary approach to antibiotic treatments. This study explores the application of nanomotion technology for rapid, growth-independent in vitro phage susceptibility testing (PST).

Methods

We developed Phenotech PST, an innovative technology that simplifies empirical drop test assays (DTA) by measuring the real-time nanomotions (vibrations) of living bacterial cells. These vibrations are detected via micromechanical sensors (cantilevers). Clinical isolates of *Pseudomonas aeruginosa* (n=47) were exposed to five distinct bacteriophages across 171 experiments. Machine learning algorithms were applied to analyze the resulting nanomotion data. Additionally, PST was performed directly on patient sputum samples, circumventing traditional bacterial isolation steps.

Results

Phenotech PST significantly reduced the time-to-result (TTR) from approximately 16 hours for standard DTA to just 6 hours. By analyzing variance slopes of nanomotion signals, the technology accurately classified phage activity, correlating with observed lysis patterns in DTA. While no significant signal differences were found between inactive phages and control samples ($p > 0.99$), Phenotech PST achieved 91% accuracy in distinguishing clear and turbid lysis outcomes, with 97.5% sensitivity and 83% specificity ($p < 0.001$). Preliminary sputum testing results were promising, though further optimization is needed for standardization.

Conclusions

Nanomotion technology demonstrates significant potential for enhancing PST and guiding phage therapy decisions. By providing a rapid, reliable, and growth-independent readout, this method addresses key limitations of traditional assays. Future work will focus on refining sputum testing protocols, incorporating temperature-controlled conditions at 37°C, and optimizing TTR. Our findings contribute to the advancement of phage-based diagnostics, offering a valuable tool for combating AMR.

S-10

Enhanced molecular detection of tick-borne encephalitis virus in CSF using dual-target PCR assays: A paradigm shift in tick-borne encephalitis diagnosis?

Dr. Onya Opota [1], Dr. Florian TAGINI [2], Mr. Valentin Loup [2], Ms. Zahera Dance [2], Dr. Alix T. Coste [3], Prof. Gilbert Greub [1]

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Backgrounds
Tick-borne encephalitis virus (TBEV) is a neurotropic flavivirus causing TBE. While serology is the diagnostic standard, it has limitations in early infection and in immunocompromised patients. Although PCR in cerebrospinal fluid (CSF) has historically been considered low yield, it may offer unexpected diagnostic value in selected cases.

Methods
We implemented and compare the analytical and clinical performance of two real-time PCR assays targeting the envelope (E) gene and the 3' untranslated region (3'UTR) of TBEV on our automated molecular diagnostic platform. Validation included assessment of reproducibility, limit of detection using plasmid dilutions, specificity against 41 pathogens and 30 TBEV-negative CSF samples, and inclusivity using clinical specimens and infected cell cultures.

Results
Both PCR assays showed high analytical sensitivity, with detection rates of 100% for 5 copies and 40% and 44% for 1 copy per reaction for PCR U and PCR E, respectively. Performed in triplicates, their practical sensitivity approached 1 copy per reaction. Reproducibility met diagnostic standards, and both assays demonstrated 100% specificity with no cross-reactivity. The dual-target PCR assay was introduced into routine diagnostics in June 2024. Among the 47 clinical CSF samples tested from June to December 2024, five cases with clinically and/or serologically confirmed TBEV infection were PCR-positive in CSF. Two were positive with both assays, while three were positive only with PCR U, suggesting higher clinical sensitivity for the 3'UTR-targeted assay.

Conclusions
Contrary to previous assumptions that TBEV RNA is rarely detectable in CSF, our findings demonstrate that molecular detection using dual-target PCR assays can significantly contribute to diagnosis in selected cases. This suggests a paradigm shift, emphasizing the role of early PCR testing as a complement to serology, particularly in immunocompromised patients and in the early phases of infection.

M-06 The Importance of Soil Microbial Processes as Drivers of Atmospheric Greenhouse Gas Concentrations

Prof. Dr. Klaus Butterbach-Bahl

Danish Pioneer Center Land-CRAFT, University of Aarhus, Aarhus, Denmark

Climate change is closely linked to changes in the atmospheric concentrations of relevant trace gases, specifically CO_2, CH_4, and N_2O. CO_2 is estimated to contribute about two-thirds of anthropogenic climate warming, while CH_4 and N_2O contribute about 20-30% and 6%, respectively. The importance of microbial processes in controlling the atmospheric concentrations of these gases is often overlooked. For instance, heterotrophic respiration is the primary source of atmospheric CO_2. Approximately two-thirds of all methane emission sources are linked to microbial processes, particularly methanogenesis. The same is true for N_2O, with approximately 90% of emission sources linked to processes such as nitrification and denitrification. However, microbial processes act as both a source and a sink for atmospheric trace gases. Examples include photosynthesis (plant and microbial), methane oxidation, and denitrification. Thus, observed biosphere-atmosphere fluxes result from the simultaneous production and consumption of these gases. Why is this important? Human activities greatly affect the exchange of climate-relevant trace gases between the biosphere and the atmosphere. Agriculture is a main driver of climate change. It is estimated that approximately one-third of all anthropogenic greenhouse gas emissions are linked to food and feed production. This is due to changes in land use, the mineralization of organic matter by microbes, the volatilization of soil carbon in the form of CO_2, and methane (CH_4) and nitrous oxide (N_2O) emissions from livestock, rice production, and the increased use of fertilizers, which disrupt the global nitrogen cycle. Therefore, limiting climate change requires developing mitigation strategies to reduce GHG emissions associated with agriculture. In my talk, I will present a few potential options to limit GHG emissions from agricultural sources linked to microbial activities.

S-11* Eis2: a Swiss knife turning a resistance determinant into a weapon against Mycobacterium abscessus

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Mycobacterium abscessus (Mab) is an emerging pathogen and one of the most drug-resistant bacteria, having major therapeutic challenges. In this study, we characterize a chloramphenicol resistance determinant and strategically repurpose a known aminoglycoside resistance determinant for prodrug activation of phenicol derivatives.

We identify WhiB7-dependent O-acetyltransferase *cat* (MAB_2989) as the primary determinant of intrinsic chloramphenicol resistance in Mab. We show that florfenicol, a fluorinated analog of chloramphenicol, overcomes this resistance by substituting the 3-hydroxyl group with a fluorine atom, thereby avoiding O-acetylation by Cat.

WhiB7-dependent promiscuous aminoglycoside N-acetyltransferase MAB_4532c (Eis2) is conferring resistance to amikacin, a cornerstone of Mab treatment, as well as other aminoglycosides such as kanamycin B, hygromycin B, and the cyclic peptide capreomycin. Here, we exploit the broad substrate spectrum of Eis2 as a prodrug activator. Eis2 efficiently N-acetylates florfenicol amine (FF-NH_2), a major florfenicol metabolite lacking translational inhibition, into the active ribosome-targeting compound FF-acetyl (FF-Ac). Converted FF-NH_2, which shows narrow-spectrum activity against the *M. abscessus chelonae* clade, induces *eis2* expression, triggering a feed-forward loop that enhances its own activation and potency by balancing WhiB7-dependent intrinsic Mab resistance.

By bypassing one resistance mechanism (Cat) and repurposing another for prodrug activation (Eis2), FF-NH_2 represents a promising therapeutic approach. It avoids toxicity linked to mitochondrial ribosome inhibition, enhances susceptibility to other ribosome-targeting antibiotics through collateral sensitivity, and demonstrates in vivo efficacy in a pre-clinical GM-CSF knockout mouse model of *M. abscessus* infection.

These findings underscore *eis2* as a molecular "Swiss knife" in Mab, playing key roles in transforming a resistance determinant into a therapeutic tool. This approach offers a new angle to disarm the opportunistic pathogen's intrinsic defenses. Ongoing development of next-generation phenicol derivatives aims to enhance antibacterial potency and improve pharmacokinetic properties.

S-12 Comparative analysis of cloacal microbiota in Chlamydiota-infected and uninfected snakes

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Members of the phylum *Chlamydiota* have been identified as emerging pathogens in snakes. A study by Rüegg *et al*. (2015) reported *Chlamydia pneumoniae* as the likely cause of fatal infection in a snake. This study aims to investigate how *Chlamydiota* infection in the cloaca influences the composition and diversity of the cloacal microbiota in snakes.

To achieve this, we compared the cloacal swabs samples from 14 snakes infected with *Chlamydiota* to 40 uninfected controls. Infection status was determined using the broad-range pan-*Chlamydiota* and pan-*Chlamydiaceae* PCR assays. Microbiota profiling was conducted through sequencing of the 16S rRNA (V3-V4) gene on an Illumina NextSeq 1000. Sequencing reads were processed using zAMP, an in-house bioinformatics pipeline, while visualization and downstream statistical analyses were performed using R.

Compared to the control group, non-rarified bacterial diversity, as measured by the Chao1 (richness) and the Shannon index (richness and evenness), was significantly higher in *Chlamydiota*-infected snakes ($p<0.0001$ and $p<0.001$, respectively). Microbial community composition differed significantly between infected and uninfected groups, as indicated by Jaccard ($p<0.001$) and Bray-Curtis ($p<0.0012$) distance metrics, with clear clustering patterns observed. Furthermore, differential abundance analysis using MaAsLin2 identified a significantly lower abundance of *Achromobacter* spp. in infected snakes compared to uninfected controls (coefficient=-5.66, $p<0.0001$).

These findings suggest that *Chlamydiota* infection in snakes could be associated with alterations in cloacal microbiota diversity and composition. The observed depletion of *Achromobacter* spp. in infected individuals may reflect microbial imbalances linked to chlamydial infection, suggesting further investigation into the ecological and functional implications of these shifts.

S-13 Tracking Microbial Evolution in Raclette du Valais AOP

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The history of cheese-making is deeply intertwined with the evolution of microbial communities, from spontaneous fermentation to modern, standardized practices. Despite centuries of refinement, the most profound shifts in cheese production occurred in the last century, driven by advances in microbiology and technology. These changes have shaped the microbes within cheese, yet the specific impacts on bacterial and viral communities remain underexplored.

In this study, we examine Raclette du Valais AOP, a traditional Swiss cheese, using preserved cheese wheels from 1875 to 2017 from the Val d'Anniviers. This unique collection of samples, spanning over 140 years, provides an opportunity to track microbial shifts through a period of rapid change in cheese-making technology. By extracting microbial DNA from these ancient cheeses, we were able to analyze how bacterial communities evolved in response to technological developments, including the rise of antibiotics in animal husbandry.

Our results reveal that significant changes in microbial diversity coincide with shifts in cheese production practices. Notably, we found that domestication of lactic acid bacteria (LAB) predates the studied period, and that bacteriophages commonly found in modern cheese making were already present in 1875. These findings highlight how microbial communities have adapted or not to changing production methods and how human intervention, through practices like antibiotic use, has influenced these ecosystems in remote alpine cheese making.

This research offers a glimpse into the microbial changes of a traditional fermented food, providing valuable insights into how cheese-making has shaped the microbial world.

S-14*/MT-08*/P-16*

dismantling walls to grow - understanding the effect of physiological and environmental influences on L-form conversion during β-lactam treatment

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The bacterial cell wall and essential in hypoosmotic conditions for bacterial cell integrity. However, in hyperosmotic conditions, a subpopulation can shed the cell wall and convert to an amorphous, often avirulent L-Form state. This conversion is induced by cell wall targeting antibiotics such as β-lactams or other cell wall antagonists such as phage-derived endolysins. The bacterial L-Form can slowly proliferate in the presence of the β-lactams, and a population of L-Forms can revert frequently to the virulent walled state after antibiotic treatment is ended. While increased mutagenesis enables more efficient L-Form growth, the conversion is completely independent of genetic alterations. Over the decades of L-Form research, there have been multiple reports of L-Forms isolated from human patients. We previously showed that bacterial L-Forms can be induced in human host cells as well as in human urine by treatment with cell wall antagonists. However, it is currently not known which role L-Forms play exactly in antibiotic treatment failure and the mechanism of L-Form conversion remains elusive. Here we investigate the effects of cyclic di-AMP, the master regulator of cell wall homeostasis, on the ability of *Listeria monocytogenes* to convert to the L-Form state. We show that cyclic di-AMP is required for L-Form conversion and investigate the physiological role of cyclic di-AMP during β-lactam treatment in human host cells. We compare changes in L-Form conversion rates of *Listeria* populations to the general tolerance to β-lactam treatment and unravel the role of temperature in L-Form growth. L-Forms comprise an understudied field within antibiotic resilience and understanding the mechanisms of L-Form conversion will bring light to the role of the elusive L-Forms during antibiotic treatment failures in human patients and domestic animals.

S-15 Efficacy of dimercaptosuccinic acid in combination with imipenem in a murine model of experimental sepsis due to metallo-ß-lactamase-producing Pseudomonas aeruginosa

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Meso-dimercaptosuccinic acid (DMSA) is a heavy metal chelator used in the clinic in case of poisoning. *In vitro* studies have shown that DMSA inhibits the activity of metallo-carbapenemases (MBLs), which require zinc in their catalytic site. The aim was to evaluate the adjuvant efficacy of DMSA together with imipenem in a mouse model of sepsis caused by non- and MBL-producing strains of *P. aeruginosa*.

Pa01 reference strain was used together with its isogenic NDM-1, IMP-1, IMP-10, IMP-13 and VIM-2 producing mutant. For each strain, femalesmice were inoculated intraperitoneally (ip) with the minimum lethal dose previously characterized for each strain. Treatment lasted 24 hours and started two hours post-inoculation and animals were randomly grouped into: i) controls, untreated; ii) imipenem (100mg/kg/q2h/ip/24h); iii) DMSA (200mg/kg/q4h/ip/24h); iv) imipenem+DMSA. Immediately after death or slaughter, spleen and blood were aseptically removed for bacterial count assessment. Positive blood cultures were expressed as percentages. Bacterial spleen concentrations (log_{10} CFU/g) and bacterial blood concentrations (log_{10} CFU/mL) were expressed as means±SD. Differences in bacterial concentrations were compared and bloodstream infections (BSI) rates were compared. A p-value < 0.05 was considered significant. SPSS v26.0 was used.

- DMSA in monotherapy has no effect decreasing bacterial concentration.
- The combination of imipenem and DMSA (i) reduced spleen bacterial counts compared to imipenem monotherapy only in murine experimental sepsis caused by NDM-producing *P. aeruginosa* strain; and (ii) reduced blood bacterial counts and BSI compared to imipenem monotherapy in experimental sepsis caused by IMP-producing P. aeruginosa strain.
- DMSA restored imipenem activity for all the MBLs-producing strain tested.
- DMSA plus imipenem were more efficacious than monotherapies in diminishing spleen bacterial concentration in NDM-producer.
- DMSA plus imipenem were more efficacious than monotherapies in reducing blood bacterial concentration or BSI in IMP-producer.
- This finding may have important implications in the treatment of infections caused by these antibiotic-resistant bacteria.

S-16 ZAMP in-silico: evaluating impact of primer, database and classifiers choice on taxonomic classification accuracy

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Introduction

Amplicon sequencing is a widely used tool for studying microbial communities due to its precision, sensitivity, and low cost (Callahan et al., 2019). However, it suffers from biases introduced during DNA extraction, PCR, sequencing, and bioinformatics processing, which distort relative abundance estimates (Starke et al., 2021; McLaren et al., 2019). Primer choice can also skew amplification of certain taxa (Sipos et al., 2007). To assess classifier performance under controlled conditions, we present zAMP-insilico, a Snakemake pipeline for benchmarking 16S rRNA taxonomic classifiers across primers and databases.

Methods

Starting with 1296 bacterial pathogens, zAMP in-silico automates reference genome retrieval using assembly_finder (Chaabane et al., 2024), amplicon extraction and simulation with cutadapt (Martin, 2011) and in_silico_pcr (github.com/egonozer/in_silico_pcr), respectively. Dereplication was executed with vsearch (Rognes et al., 2016). Taxonomic classification was performed using sintax, rdp, dada2, and kraken2 with SILVA138.1, Greengenes2 (2024.09)[CB3] [FC4] , and EzBioCloud (2017). The pipeline supports database region extraction based on primers (e.g., V3-V4), enabling comparisons between trimmed and full-length databases. Classifier predictions are compared to NCBI taxonomy using precision, recall, and F1-score metrics.

Results

At genus level, Greengenes2 is the best performing database across classifiers (mean F1=0.85), followed by EzBiocloud (F1=0.81). At species level, trimmed [CB1] [FC2] databases overall underperformed compared to the default (median F1=0.53 vs. 0.57). However, sintax combined with Greengenes2 and SILVA showed a notable improvement with V3–V4 extraction (F1=0.57 to 0.72 for Greengenes2, and F1=0.5 to 0.68 for SILVA). For EzBiocloud, the rdp classifier scored the highest using V3-V4 extraction (F1=0.57 to 0.72).

Conclusion

Our results highlight the importance of selecting the appropriate classifier and database for specific applications. In fact, some database and classifier combination like Greengenes2 and sintax benefited from V3–V4 region extraction, showing improved pathogenic species-level accuracy.

M-07 Carbon-Fluorine Bond Cleavage Across Microbiomes

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Fluorinated compounds are used for agrochemical, pharmaceutical, and numerous industrial applications. In many of these compounds, fluorine is incorporated to enhance the half-life and improve bioavailability. The human gut microbiota is known for its xenobiotic biotransformation capabilities, but it was unknown whether gut microbial enzymes could break carbon-fluorine bonds. Here, through the development of a rapid, miniaturized assay to detect fluoride release during whole-cell screening, we discovered active defluorinases encoded by human gut bacteria. Through alanine scanning, molecular dynamics, and chimeric design, we found a disordered C-terminal segment crucial for defluorination. Swapping this domain enabled defluorination in a non-defluorinating enzyme, revealing a C-terminal signature that can guide improved predictions of enzyme-substrate specificity for fluorinated compounds directly from microbiome data. More broadly, this lays the foundation for predicting defluorination activity across human and environmental microbiomes to better understand fluorinated xenobiotic biotransformation and fate.

S-17*

Horizontal transfer of ICEclc-like elements in Pseudomonas aeruginosa clinical isolates

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Integrative and conjugative elements (ICEs) are widespread autonomous mobile DNA within bacterial chromosomes. ICEs contain the genes necessary for excision from the chromosome, conjugative transfer to a new recipient cell, and chromosomal reintegration. They can also carry accessory genes that, while not essential for transfer, confer adaptive phenotypes to the host, contributing to bacterial survival under stressful or changing conditions.

Here, we characterized the presence, distribution, and variation of ICEs related to ICE*clc* among *Pseudomonas aeruginosa* clinical isolates within a geographically restrained environment to understand the factors shaping their evolution. We examined 181 *P. aeruginosa* genomes from patient and hospital environment isolates, mostly obtained from a single hospital over 20 years. Nearly 90% of the isolates carried one or more ICE*clc*-like elements with varying degrees of conservation of ICE*clc* lifestyle and transfer genes. ICE clones largely matched host clonal phylogeny but not exclusively, indicating both clonal evolution and horizontal transfer in the hospital environment. Variable gene regions were notably enriched for heavy metal resistance genes, potential efflux systems, and multidrug resistance proteins.

We then studied the activation and transfer of a representative subset of ICE*clc*-type elements and found that ICE excision could be induced in *P. aeruginosa* by ectopic expression of BisDC, the known master regulator of ICE*clc* activation, pointing to a similar regulatory cascade. Some elements were successfully transferred to *P. putida*, where they conferred increased tolerance to specific heavy metals. We also assessed ICE excision and transfer dynamics in response to different classes of stressors using qPCR-based quantification and conjugation assays. This work aimed to explore whether clinical conditions or antimicrobial treatment could inadvertently promote ICE-mediated gene transfer, with implications for understanding the evolution of antibiotic resistance and virulence.

S-18 Exploration of AMR Enrichment Techniques for Monitoring Environmental Waters and Wastewater

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Antimicrobial resistance (AMR) is considered a pressing public health issue in Switzerland and globally. In clinical settings, AMR threatens effective treatment, leading to increased healthcare costs and higher mortality rates. The One Health approach recognizes the interconnectedness of human, animal, and environmental health and underpins the need for environmental research. AMR is comprised of thousands of ever-evolving antibiotic resistance genes (ARGs), each with varying antibiotic targets, hosts, mechanisms of action, mobilities, and potencies. These factors make studying AMR a particularly challenging task. These challenges are further complicated by limitations in available analytical techniques. Traditional approaches such as quantitative PCR (qPCR) and culture-based methods remain widely used but offer only a partial view of the broader AMR landscape. Recently, metagenomics has shown promise in addressing this gap by enabling deep sequencing and the simultaneous detection of thousands of ARGs. Metagenomics remains limited in its application for widespread environmental monitoring, in part, due to the cost of deep sequencing required to capture low abundant targets. However, recent advancements in enrichment focused library preparations show promise by more effectively utilizing sequencing depth on targets of interest, in the process eliminating the need for more costly, deeper sequencing. This study explores the implementation of enrichment-based library preparations and their suitability for applications in environmental waters. Specifically, four enrichment kits employing different enrichment mechanisms (i.e. hybridization capture vs amplification) and target gene selections (ranging from approximately 400 to over 2000 shared and unique ARGs) and direct deep sequencing were compared across a range of environmental samples with varying degrees of suspected AMR contamination (wastewater treatment influent and effluent, wastewater-impacted and non-impacted lake water). We provide a direct comparison of kit performance in terms of target retrieval, sensitivity, preservation of quantitative information and discuss their application for studying and monitoring AMR contamination in aquatic environments.

S-19 The cyanosphere microbiota affects the growth of toxic benthic cyanobacteria

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Toxin-producing cyanobacteria have become an increasing threat to ecosystems, with serious consequences for wildlife and domestic animals.
Cyanobacteria often coexist with other microorganism such as heterotrophic bacteria, and ecologically meaningful interactions such as cross-feeding and phylosymbiosis have been documented in planktonic cyanobacteria. However, much less is known about such interactions in benthic cyanobacteria. *Microcoleus anatoxicus*, a particularly problematic toxic benthic species, has a notably reduced genome compared to other non-toxic *Microcoleus* species. Genes involved in vitamin biosynthesis are missing in complete genomes, and it has been hypothesized that the toxic species depends on microbial interactions for acquiring essential nutrients. In this study, we investigate this hypothesis by evaluating the interactions between *M. anatoxicus* and heterotrophic bacteria isolated from mats dominated either by *M. anatoxicus* or by a non-toxic *Oscillatoriales* (genus *Potamolinea*). We co-cultured these heterotrophs with an axenic strain of *M. anatoxicus* to evaluate their effect on growth. Both stimulatory and inhibitory effects were observed, prompting further investigation into the underlying molecular mechanisms.
By uncovering biotic factors that influence the proliferation of toxic cyanobacteria, our findings offer valuable insights into the ecological dynamics of harmful algal blooms.

M-08 What phage-bacteria interactions teach us on innate immunity

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Across the tree of life, genetic conflicts between hosts and pathogens have fueled the diversification of intracellular defenses that collectively define innate immunity. While prokaryotic and eukaryotic immunity have long been considered very distinct, the recent discoveries of multiple defense systems protecting bacteria against phages have profoundly challenged this paradigm. In particular, some components of eukaryotic innate immunity were shown to have a deep evolutionary origin in bacterial defenses. Illustrated by ATP nucleosidases, caspases and TIR domains, the existence of ancient immune components now suggests that phage-bacteria interactions can illuminate the genetic conflicts between eukaryotic hosts and their pathogens.

S-20 Type IV-A3 CRISPR-Cas: A plasmid-encoded strategy in the arms race of mobile elements

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CRISPR-Cas systems play a crucial role in bacterial immunity. Type IV systems, however, are plasmid-encoded, and their CRISPR array contents suggest a role in mediating inter-plasmid conflicts rather than providing host defense. We previously demonstrated that the type IV-A CRISPR-Cas can robustly interfere with conjugative plasmids through transcriptional repression. Here, we aim to shed light on the molecular mechanism and ecological function underlying these systems.
Using the IV-A CRISPR-Cas system from a clinical *Klebsiella pneumoniae*, we conducted a high-throughput genetic screen targeting 31,000 chromosomal positions in *Escherichia coli*. Our results reveal an action beyond long-range transcriptional silencing. Mismatch analyses identified a crucial seed region for target interference and a remarkable tolerance for mismatches between CRISPR RNA and protospacer. Bioinformatic analyses pinpointed the plasmid families and genetic regions preferentially targeted by type IV-A systems, suggesting they specifically block the horizontal transfer of competing plasmids. To test this, we interrogated the population-level effects of IV-A3 interference with a naturally targeted multidrug resistance plasmid. Together, this work provides new insights into the molecular mechanisms and the role of type IV-A3 CRISPR-Cas in shaping the dynamics of clinically important plasmids.

S-21* Suit up – Engineering Bacteriophages For Enhanced Therapeutic Efficacy

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Urinary tract infections (UTIs) are the most common bacterial infection worldwide. The lifetime incidence in women reaches 50 % and often requires repeated courses of antibiotic treatment. Recently, the use of bacteriophages (phages) – the viruses of bacteria – to fight bacterial infections has regained interest as an alternative approach, called phage therapy. In personalized phage therapy, a phage's host range is assessed to match the patient's infecting bacterial strain prior to treatment. However, despite effectiveness against the bacteria *in vitro*, phage therapy still fails in 20–30% of cases. This is largely due to the development of phage resistance among other reasons. We previously demonstrated that Nanoluciferase can serve as a reporter payload to simplify phage host range assessment. Here we present coliphage 41S, a broad-spectrum lytic virus with a multivalent adsorption apparatus. We used CRISPR assisted engineering to equip 41S with different heterologous payloads that are produced during the regular lytic cycle of the phage. We determined the host range using Nanoluciferase against a panel of clinical uropathogenic *Escherichia coli* isolates and could show complementarity to previously tested phages. To enhance bactericidal efficacy and suppress resistance, we further engineered 41S to deliver toxic effector proteins Colicin 5 and Colicin E7. Finally, we validated the efficacy of our engineered phages in physiologically relevant conditions, like pooled human urine. In sum, we identify 41S as a strong candidate to expand the current phage therapy arsenal and show that arming phages with bactericidal payloads is a promising strategy to improve treatment outcomes.

S-22 Discrimination of exogenous DNA by mitotic heterochromatin in fission yeast

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Despite the necessity of immunity, our understanding of exogenous DNA discrimination from endogenous/chromosomal DNA within eukaryotic nuclei is limited. Such a discrimination mechanism is essential for eliminating exogenous DNA and safeguarding eukaryotic genomes. Since most eukaryotes are unicellular, investigating such cell-autonomous mechanisms is a compelling research area.

We explored exogenous DNA discrimination mechanisms in fission yeast *Schizosaccharomyces pombe* by using a replicative plasmid as a model for exogenous DNA. Visualising the plasmid using the *tetO*-TetR system revealed its asymmetric partition during mitosis, resulting in rapid elimination from the cell population. The plasmid tends to localise at the nuclear periphery, which serves as a scaffold for heterochromatin regions. Mutations that abrogated the formation and maintenance of heterochromatin domains randomised the plasmid partitioning patterns, slowing down their loss from the cell population. Chromatin immunoprecipitation assay revealed enrichment of the heterochromatin marker on the plasmid subregions. These heterochromatin marks were deposited on the plasmid in an RNA interference-dependent manner. Consistent with heterochromatin regions on chromosomes clustering together in the nucleus, the exogenous DNA clustered in the wild-type and was dispersed more in the mutant cells. Thus, clustering the exogenous DNA to one or a few units drives its asymmetric partition. We suggest that the absence of an active centromere on the plasmid protects it from Aurora B kinase-dependent dissociation of heterochromatin domains during mitosis. Accordingly, the plasmid was barely phosphorylated in mitotic cells, unlike in the heterochromatinised regions of the chromosomes. Furthermore, tethering Aurora B to the plasmid randomises its partition patterns. Together, our results suggest that the heterochromatic regions persisting during mitosis serve as a marker for nonself-DNA, as Aurora B recruitment occurs at the centromere, which identifies self-DNA and thus promotes the elimination of nonself-DNA through asymmetric partitioning.

S-23 Slipping Past the Slime: How Phages Crack E. coli's Colanic-Acid Armor

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Bacteria and bacteriophages are locked in a continuous evolutionary arms race. Many bacterial defenses—such as restriction-modification, CRISPR–Cas, or abortive infection—protect against specific phages but leave vulnerabilities to untargeted viruses. Conversely, the production of polysaccharide barriers like the widely conserved colanic acid capsule of *Escherichia coli* are thought to broadly impede phage infection by physically shielding the bacteria. However, it has remained unknown how effectively colanic acid protects *E. coli* against diverse phages which limits our ability to adequately consider the role of capsule barriers for phage therapy or ecology.

Here we show that the colanic acid capsule broadly inhibits numerous diverse *E. coli* phages except those carrying specialized tailspike proteins targeting this glycan. Dedicated isolation experiments readily identified diverse new phages that target colanic acid as host receptor. Genomic analyses identified tailspikes possibly recognizing colanic acid analogous to known tailspikes of other phages targeting different glycans. Replacing a lipopolysaccharide-targeting tailspike in another phage with one from our new phages indeed confirmed that these tailspikes are necessary and sufficient to target colanic acid as host receptor. Thus, colanic acid emerges as an underappreciated but important factor in *E. coli* phage biology which can both protect the bacteria as a capsule but also sensitize them to specialized viruses.

Because colanic acid overexpression often evolves under phage pressure, integrating colanic-acid-targeting phages into therapeutic cocktails could prevent capsule-based resistance and enhance treatments against multidrug-resistant pathogens. More broadly, our findings illustrate the importance of bacterial surface polysaccharides for phage-host ecology and the design of phage-based therapeutics.

M-09 Exploiting divergent biology of two fission yeasts to understand membrane function

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Biological membranes are semi-permeable lipid barriers delimiting cells and subcellular compartments. By recruiting and scaffolding specific proteins and protein complexes, membranes also serve as platforms for cellular communication, signalling and metabolism. The specific features of the membrane depend on its lipid composition. I will present our recent work aimed at understanding how lipid metabolism impacts on membrane function and cellular physiology using comparative and synthetic approaches in two related fission yeast species with different lifestyles. Briefly, we show that a popular model system Schizosaccharomyces pombe and its less known relative Schizosaccharomyces japonicus exhibit strikingly different membrane lipid composition and provide the mechanistic explanation for this divergence. I will further argue that these differences in lipid metabolism may be at root of the profound changes to cellular physiology that occurred in the evolution of the fission yeast clade.

S-24

Antagonistic pathways of lipid transfer proteins (LTPs) for de novo membrane assembly in fission yeast

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Fungal sporulation involves *de novo* synthesis of the spore plasma membrane, named the forespore membrane (FSM), which surrounds each meiotic nucleus. Biomembrane lipid composition is regulated by synthesis, degradation, and lipid transport. Lipid transfer proteins (LTPs) facilitate non-vesicular lipid transport. In *Schizosaccharomyces pombe*, Ltc1, from the LTP anchored at membrane contact sites (LAM) family, transports ergosterol from the plasma membrane (PM) to the endosomes, and Osh41, from the oxysterol-binding homology (Osh) protein family, conversely promotes PM ergosterol levels [1]. However, neither LTP is essential, suggesting redundancy with other transport mechanisms, and their role in sporulation remains unknown.
We have undertaken a systematic exploration of the function of LTPs in fission yeast. Our findings on Osh-family LTPs show that: (i) Concurrent Ltc1/Osh3 or Osh41/Osh2 deletions are synthetically lethal, indicating redundancy; (ii) Deletion of neither *osh41* nor *osh42* causes major effect on sporulation, yet *osh41Δosh42Δ* double mutant exhibits complete failure of spore formation; (iii) *osh3* deletion partially restores *osh41Δ* mutant PM-ergosterol levels and circumvents the sporulation failure of *osh41Δosh42Δ* cells, suggesting antagonistic functions between Osh41/Osh42 and Osh3. Our results highlight both functional redundancy and antagonistic functions among lipid transfer protein families.
[1] Marek, M., Vincenzetti, V. & Martin, S. G., J. Cell Biol. 219, e202001147 (2020).

S-25 The role of positively charged peptides in innate immune of yeast

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Exogenous DNA is abundant in the environment, and organisms must defend themselves against it. While bacterial systems like restriction-modification and CRISPR, or cGAS-STING in metazoans, are well-characterized, little is known about similar mechanisms in other eukaryotes. To address this point, we use *Saccharomyces cerevisiae* to investigate how unicellular eukaryotes restrict exogenous DNA.

When performing a transformation experiment, we observe that nearly all cells internalize exogenous DNA, yet stable genetic modification occurs in only one in a thousand. Since centromeric plasmids are used, homologous recombination is not required, suggesting a restriction mechanism acting post cellular entry but before nuclear integration.

Using a genome-wide transposon screen, we identified genes that prevent DNA transformation, including an interesting subset that encodes short, positively charged, uncharacterized polypeptides. These polypeptides are particularly intriguing due to their potential ability to bind DNA in the cytoplasm.

Our data confirm their role, as mutants exhibit increased sensitivity to exogenous DNA. Furthermore, these polypeptides bind DNA *in vitro* and undergo phase separation upon mixing with DNA. Our ongoing work focuses on two key aspects: characterizing the interaction between incoming exogenous DNA and these short polypeptides and identifying the downstream mechanism involved in eliminating it.

Together, our results suggest that *S. cerevisiae* is protected from incoming DNA by an ensemble of cytoplasmic proteins that recognize it through their DNA-binding properties. Notably, several of the identified genes are not conserved across *Saccharomyces*, suggesting that the adaptability of this defense system may stem from the de novo emergence of these genes.

S-26* Hypoosmotic stress leads to rapid formation of chitin-containing protective caps at the hyphal tips of the basidiomycete Coprinopsis cinerea

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The agaricomycete *Coprinopsis cinerea* is a well-established model organism for filamentous basidiomycetes. As a coprophilous fungus, *C. cinerea* hyphae are exposed to hypoosmotic stress e.g. upon exposure of the dung pellets to heavy rain or water floodings. Thanks to the fungal cell wall, the volume of the subapical hyphal compartments does not increase under these conditions, but the turgor pressure increases by the water uptake. This can become critical at the hyphal tips where the cell wall is thinner due to polar growth. Accordingly, hypoosmotic stress leads to bursting of these compartments in many filamentous fungi, including mucoromycetes and ascomycetes.

During our investigation of the response of the basidiomycete *C. cinerea* to hypoosmotic stress, we observed that exposure to distilled water induced a distinct dome-like structure at the hyphal tips. In light microscopy, this structure appeared as a dense, opaque cap and was consistently present at nearly all hyphal tips. Notably, once the cap was formed, hyphal tips ceased to grow, even after returning to isosmotic conditions. Instead, hyphal growth resumed by branching of subapical compartments. Staining with calcofluor white (CFW) resulted in strong fluorescence, suggesting that the cap is rich in chitin. Accordingly, treatment with Nikkomycin Z, a chitin synthase inhibitor, prevented formation of the caps and led to hyphal tip bursting as well as swelling of subapical compartments into balloon-like structures. These results indicate that *C.cinerea* responds to hypoosmotic stress by an unprecedented rapid fortification of the cell wall at the hyphal tip.

Ongoing experiments aim at the characterization of the biochemical composition of the cap and the identification of the signalling modules and enzymes involved in its biosynthesis. We are also investigating whether similar responses occur in other agaricomycetes. Understanding this response could provide new insights into fungal resistance mechanisms towards abiotic and biotic stresses.

S-27 Exploration of novel mechanisms of azole resistance in Candida auris

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Candida auris is a pathogenic yeast of particular concern because of its ability to cause nosocomial outbreaks of invasive candidiasis (IC) and to develop resistance to all current antifungal drug classes. Most *C. auris* clinical isolates are resistant to fluconazole, an azole drug that is used for the treatment of IC. Azole resistance may arise from diverse mechanisms, such as mutations of the target gene (*ERG11*) or upregulation of efflux pumps via gain of function mutations of the transcription factors *TAC1* and/or *MRR1*. To explore novel mechanisms of azole resistance in *C. auris*, we applied an *in vitro* evolutionary protocol to induce azole resistance in a *TAC1A*/*TAC1B*/*MRR1* triple deletion strain. Azole-resistant isolates without *ERG11* mutations were further analyzed. In addition to a whole chromosome aneuploidy of chromosome 5, amino-acid substitutions were recovered in the transcription factor Upc2 (N592S, L499F), the ubiquitin ligase complex consisting of Ubr2 (P708T, H1275P) and Mub1 (Y765*) and the mitochondrial protein Mrs7 (D293H). Genetic introduction of these mutations in an azole-susceptible wild-type *C. auris* isolate of clade IV resulted in significantly decreased azole susceptibility. Real-time reverse transcription PCR analyses allowed to assess the link between these mutated proteins and some known modulators of azole resistance, such as Erg11, the efflux pumps Cdr1 and Mdr1 or the transcription factor Rpn4. In conclusion, this work provides further insights in the complex and multiple pathways of azole resistance of *C. auris*. Further analyses would be warranted to assess their respective role in azole resistance of clinical isolates.

M-10 **Genomics at the human-animal interface: Insights into emerging viruses and virus-host dynamics in reservoirs**

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M-11 MHC class II as novel entry receptor for influenza A viruses

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While influenza A viruses (IAVs) typically initiate infection by binding to sialic acid (Sia), a sugar moiety on host cell glycans, via their hemagglutinin (HA), our findings reveal an alternative entry mechanism exploited by multiple IAV subtypes involving the major histocompatibility complex class II (MHC-II). The bat-derived IAV subtypes H17N10 and H18N11 were the first shown to lack binding to the canonical receptor Sia and instead utilize MHC-II–including human leukocyte antigen (HLA)-DR and its homologs from multiple species, such as bats, chickens, and pigs–as a proteinaceous entry receptor. This alternative receptor usage suggests that bat IAVs may have a broader vertebrate tropism, potentially extending beyond their original bat hosts. More recently, we identified a novel subtype in ducks, designated H19, with similar receptor binding properties. Like H17 and H18, H19 does not bind Sia but instead uses MHC-II in a species-specific manner. While MHC-II from ducks, swans, and bats supports H19 entry, MHC-II from humans, chickens, and pigs does not–suggesting a restricted host range and limited zoonotic potential. We mapped key residues in MHC-II that govern this species specificity. Strikingly, we also found that conventional IAVs of the H2N2 subtype, responsible for the 1957 Asian flu pandemic, exhibit dual receptor usage. H2N2 can utilize both Sia and MHC-II independently for viral entry. Using receptor-deficient cell lines, we demonstrated that H2N2 is capable of infecting cells via MHC-II alone, in a Sia-independent manner. Notably, MHC-II-dependent infection occurs not only in differentiated human bronchial epithelial cells, but also in professional antigen-presenting cells, such as macrophages–highlighting a potential role in zoonotic transmission and immune modulation. Our findings redefine IAV receptor biology, revealing MHC-II as an alternative entry receptor for multiple subtypes. This alternative pathway carries significant implications for viral evolution, host adaptation, and pandemic preparedness.

S-28*/P-79* Staphylococcal Phage Diversity in a Single Wastewater Treatment Ecosystem

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Antimicrobial resistance (AMR) is a global health threat, with *Staphylococcus aureus* recognized as a major contributor. Bacteriophages, potent bacterial predators, have re-emerged as promising alternatives to traditional antibiotics. However, some phages can also facilitate horizontal gene transfer of AMR genes via transduction. This duality underscores the need to reconcile their therapeutic potential with their role in bacterial evolution, and to develop informed strategies to address the AMR pandemic. In a previous study (Göller et al., 2021), we isolated and preliminarily characterized 94 Staphylococcal phages from a wastewater treatment plant in Zürich. Of these, 40 underwent whole-genome sequencing (WGS), and the morphology of 56 was analyzed by transmission electron microscopy (TEM). Here, we present a comprehensive characterization of this diverse phage collection. Comparative genomic analyses of the initial 40 phages against all Staphylococcal phages from the public domain (Virus-host database) support the assignment of two new viral families, three new genera, and 21 putative species to the International Committee on Taxonomy of Viruses. We achieved >90% (88/94) phage recovery and propagation of the >5-year-old phage stocks from 4°C and/or -80°C. Following optimization of phage propagation (solid lysis) and DNA extraction protocols, we performed WGS on an additional 49 phages using Illumina technology. Genomes were assembled with SPAdes and annotated using Pharokka, using Prodigal for gene prediction. Intriguingly, several genomes contain predicted introns truncating the large terminase subunit gene (terL), suggesting potential regulatory or splicing functions. Morphology for 35 phages was determined via TEM after polyethylene glycol precipitation and cesium chloride gradient purification. All phages showed icosahedral capsids and varied tail lengths, corresponding to myovirus- and siphovirus like morphotypes. Altogether, this work lays the foundation for further analyses on phage mediated bacterial evolution and for a rational selection of phage candidates for therapeutic and biocontrol purposes against a range of Staphylococcal species.

S-29*/ P-86* **A dual approach to discover and isolate staphylococcal temperate bacteriophages**

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Bacteriophages lead bacterial evolution and are re-emerging as promising tools to target resistant pathogens. Little is known about temperate phages in members of Staphylococcaceae family other than *Staphylococcus aureus*. These prophages may contribute to gene transfer, adaptation, and interactions with therapeutic phages—factors that need to be better understood to fully assess the risks and potential of phage-based interventions.

We developed a scoring system to identify optimal lysogens (strains carrying prophages) of the Staphylococcaceae family to explore their temperate phage landscape and to assess their bacterial adaptation properties. For this, an *in silico* pipeline was established to analyze 70 genomes from our institute. A score weight was defined for each trait of interest and manual curation was conducted. The ideal candidate harbors at least one intact prophage (Phastest), multiple plasmids (PlasmidFinder), virulence (VFDB) and resistance factors (CARD, ResFinder) or phage satellites (SatelliteFinder). 74% of the strains analyzed contain predicted intact prophages, carrying either one (34%), two (29%) or three (11%). Coagulase positive staphylococci (CoPS) were enriched in prophages (95%), followed by coagulase negative staphylococci (CoNS) (81%) and *Macrococcus* (72%). Overall, 21% of predicted lysogens additionally carry plasmid/s, 50% carry AMR gene/s and 13% carry virulence gene/s. Five lysogens (7.1% of all strains) co-carry plasmid/s, AMR/s and virulence gene/s. Concurrently, we established a robust UV induction protocol to induce CoNS prophages from selected strains. The protocol was successfully tested on selected diverse lysogens. Ongoing studies focus on the molecular characterization and taxonomic classification of these inducible CoNS phages along with their involvement in shaping host bacterial traits.

We highlight high predicted prophage content and additional adaptive elements among staphylococci. This dual approach combining bioinformatic screening and experimental induction offers a scalable strategy to discover and harness novel functional temperate staphylococcal phages, enabling to appraise their contribution to generate staphylococcal networks.

M-12 Diagnosis of Parasitic Infections: Challenges and Advances in Detection Methods

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Parasitic infections represent a significant public health concern, not only in tropical and subtropical regions but also in temperate countries such as Switzerland, and their spread is enhanced by climate change, international travel, migration and globalization. These infections can lead to a wide range of clinical manifestations, sometimes with severe consequences, and are often caused by organisms with complex and sometimes cryptic life cycles. The diagnosis of parasitic diseases remains a substantial challenge due to the ability of parasites to evade immune detection and persist in host tissues, often without overt clinical signs. Reliable diagnosis requires a tailored approach based on the suspected pathogen, including direct methods such as microscopy and molecular techniques (e.g., PCR), as well as indirect approaches such as serological assays. This presentation provides an overview of current diagnostic strategies, highlights their advantages and limitations, and discusses emerging technologies aimed at improving diagnostic accuracy. Enhanced diagnostic capabilities are essential for timely treatment, effective disease surveillance, and improved patient outcomes.

S-30*/MT-05*/P-13* Uncovering global antimicrobial resistance in Capnocytophaga: insights from the largest genomic dataset to date

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Background
Certain *Capnocytophaga* species, most notably *C.* canimorsus, are common members of the oral microbiota of dogs and cats and can cause severe, sometimes life-threatening, infections in humans, typically following animal bites. Due to their slow and fastidious growth, species-level identification and antibiotic susceptibility testing are often delayed, leading clinicians to rely on empirical β-lactam therapy. However, emerging antimicrobial resistance (AMR) threatens the efficacy of these first-line treatments, while global data on AMR prevalence in *Capnocytophaga* remain sparse. We aimed to investigate distribution of AMR genes in *Capnocytophaga* isolates from human infections and domestic animals across 20 countries and match these findings with phenotypic testing.

Methods
We established the largest globally representative collection of (zoonotic) *Capnocytophaga* isolates to date (n=900; 536 human-clinical, 364 animal-oral) through the Global *Capnocytophaga* Consortium. This cohort includes isolates from 20 countries across 4 continents. Whole-genome sequencing (Illumina) was performed on 477 isolates. AMR genes were detected using AMRFinder+, ABRicate or AbriTAMR. Long-read sequencing (Oxford Nanopore) was performed on a subset (n=15) of isolates representing diverse geographies and resistance profiles.

Results
Acquired resistance genes were identified in 65/477 isolates (13.6%), spanning 13/20 countries, from both human (43%) and animal (57%) sources. These genes are associated with resistance to multiple antibiotic classes including β-lactams, aminoglycosides, macrolides, and tetracyclines.
Long-read sequencing revealed chromosomal integration of all detected AMR genes, even though plasmids were present in 6/15 screened isolates.
β-lactam resistance was detected in 36/477 (7.5%) isolates, predominantly involving the *blaOXA-347* gene (n=27). Two isolates harbored putative novel class D β-lactamase gene. Functional characterization experiments (via cloning and recombinant expression) and phenotypic susceptibility testing are underway to establish genotype-phenotype correlations.

Conclusion
This unprecedented dataset provides a critical baseline for AMR monitoring in *Capnocytophaga* and reveals a novel *blaOXA* gene with potential clinical relevance.

S-31 Genomic analysis of ompA-genotype L4 Chlamydia trachomatis in Switzerland and across Europe

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Lymphogranuloma venereum (LGV) is a sexually transmitted disease caused by *Chlamydia trachomatis* (CT) serovars L1–L3. Since 2003, cases have increased in Europe, initially linked to the *ompA*-genotype L2b, followed by a rise in *ompA*-genotype L2 cases from 2010. In 2025, a new lineage of CT carrying *ompA*-genotype L4 was identified in Argentina. Although *ompA*-genotype L4 has not yet been confirmed in Europe through whole-genome sequencing, identical *ompA* sequences have been reported in France, Portugal, Spain, and the Netherlands. Given the challenges of obtaining complete genomes from clinical samples, this study investigates the presence of the L4 *ompA*-genotype in Europe.

Clinical samples diagnosed positive for CT with a high load (Ct < 30) were obtained from retrospective studies across Switzerland and from European collaborators. Target enrichment (SureSelect XT, Agilent) and Illumina NextSeq1000 (PE150) sequencing were used. Reads were mapped to reference genome L2b_AM884177, quality controlled for >95% genome coverage and read depth >5x, and alignments created using snippy v4.6.0. Recombination-adjusted phylogenetic analysis was performed using Gubbins v3.3.0.

Whole genome sequences were analysed in the context of all published LGV genomes. Phylogenetic analysis revealed samples from Switzerland and wider Europe clustering within the *ompA*-genotype L4 lineage, while others cluster with *ompA*-genotype L2b. Recombination analysis also suggests genetic exchange across the *omp*A locus between *ompA*-genotype L2b and *ompA*-genotype L4 lineages. Overall, the genomic data indicates additional SNP diversity among circulating LGV strains in Europe.

Using target enrichment methodology, the circulation of the novel *ompA*-genotype L4 lineage was demonstrated for the first time in Switzerland and across Europe. This analysis shows a wider diversity of *ompA*-genotype L4 genomes than previously known, including genome recombination with *ompA*-genotype L2b. Further work will track transmission links between Europe and South America, investigate the global movement of *ompA*-genotype L4 and date the ancestor of this lineage.

S-32 CASE REPORT: Disseminated Borrelia infection in severe immunocomprimised patient

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We report a disseminated *Borrelia afzelii* infection in a patient of 61 years with multiorgan involvement, immunosuppressed with recurrent IVIG infusions. Clinical symptoms included odynodysphagia, fatigue, appetite weight loss over the past 6 months, diarrhea and intermittent fever.
Laboratory results show increased inflammatory parameters, severe anemia and cholestasis. The thyroid function tests were unremarkable.
A liver biopsy showed severe acute cholangitis with ductopenia. Initially, a consensus concluded on auto-immune cholangitis and budesonide was started.
Nasopharyngeal swabs for Sars-Cov-2, Influenza A/B and RSV were negative. Stool analysis show no evidence for *Clostridium*, *Campylobacter*, *Salmonella*, *Shigella,* toxoplasma gondii, giardia lamblia, cryptosporidium hominis/parvum, Entamoeba histolytica or *Tropheryma Whipplei*. Serology for Borrelia, HIV, CMV, EBV, Parvovirus B19, Brucella, Coxiella brunetti, bartonella henselae and syphilis were negatived. Bronchoscopy showed no evidence for a recurrent herpetic infection, previously treated and cytology was unremarkable. A PET-CT showed diffuse uptake in the thyroid gland and the right colic angle suggesting a thyroiditis and colitis.
Finally, a panbaterial NGS was conducted on the liver biopsy and reveals positive for *Borrelia afzelii* confirming a Borrelia hepatitis. At this point, an antibiotherapy with ceftriaxone is initiated and the budesonide treatment is gradually tapered off. Following these results, Borrelia PCR in the blood reveals positive as well, raising suspicion for a disseminated borreliosis with confirmed hepatic and suspected cardiac, colic and thyroid involvement.
Lyme disease can present as a systemic disease with aspecific symptoms and unusual organ dysfunction. This case also shows the diagnostic challenges and the relevance of PCR and NGS sequencing in patients with hypogammaglobulinemia and severe immunosuppression. In this case, lyme disease explains the mutiple organ involvement and atypical clinical presentation of our patient.

S-33 First case of Brucella suis Biovar 1 infection in a dog in Switzerland

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Brucella (B.) species are zoonotic and constitute a public health threat with *B. canis*, *B. ovis* and *B. suis* Biovar 2 being less virulent for human than *B. suis* Biovar 1, *B. abortus* and *B. melitensis*. Canine brucellosis is caused mainly by *B. canis,* while *B. suis* infections have been reported mostly in Australian pig hunting dogs. Association of the *B. suis* infection with consumption of biologically appropriate raw food (BARF) has been shown in Netherlands and potential causality was discussed in a canine case from Germany. *B. canis* is sporadically diagnosed in Switzerland and *B. suis* Biovar-2 is present in wild boar and brown hare.
We report a case of brucellosis in a dog from Switzerland, neither having a history of hunting nor originating from a country, where *B. suis* in domestic animals is present. However, the dog was fed with BARF product of unknown composition. The intact male dog showed clinical signs (fever and epididymitis) consistent with brucellosis. Urine sample sent to our laboratory tested positive for *Brucella* spp. in Real-time PCR and culture. Serology was negative in the lateral flow test (rough *B.* antigen) and positive in tests based on smooth *B.* antigen (Rose-Bengal test, complement fixation test and iELISA. Culture and subsequent whole genome sequencing revealed the isolate as *B. suis* Biovar-1, ST-14. The most closely related strain was shown to be strain 21RB23181 isolated in 2021 from an infected dog in Germany.
Considering lack of any previous report and seronegativity in tests based on smooth *B.* antigen, the infection by *B. suis* in dogs has not been diagnosed previously in Switzerland. Cases of *B. suis* Biovar-1 in dogs in Europe are being published more frequently and dog owners, veterinarians and physicians need to keep an eye on this unexpected pathogen of highly zoonotic importance.

M-13 Microbially Mediated Soil GHG Fluxes in Alpine Ecosystems: Seasonal and Diurnal Patterns Under Current and Warmer Conditions

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Anthropogenic climate warming disproportionately affects alpine and Arctic environments, including European permafrost-affected ecosystems. The European Alps are experiencing accelerated warming and snow cover loss, resulting in the replacement of alpine tundra environments with boreal forest. Currently, research on soil greenhouse gas (GHG) fluxes and their relation to microbial dynamics in high-altitude ecosystems is limited, particularly regarding the effects of seasonality and the diurnal cycle. This knowledge gap makes it difficult to quantify annual GHG fluxes and predict how they will respond to warming.

To address this, we determined in-situ CO_2 and CH_4 fluxes across all four seasons and the diurnal cycle in high-altitude alpine systems. We related the direction (positive vs. negative flux) and magnitude of these GHG fluxes to physicochemical soil properties and microbial diversity, while also assessing the impact of future warming through experimental in-situ warming using passive warming chambers (1.5–2.5°C above ambient). Across all sites, we observed positive CO_2 fluxes and negative CH_4 fluxes, indicating these high-altitude soils act as CO_2 sources and CH_4 sinks. Both seasonality and diurnal cycles influenced the fluxes; the CO_2 flux increased with temperature and artificial warming, while the CH_4 flux showed no response to warming but was linked strongly with soil moisture and the abundance of methanotrophic microorganisms. Our findings suggest that future warming will potentially exasperate microbially mediated soil emissions of CO2, while CH4 uptake will likely remain unaffected in alpine soils.

S-34 Spatial structure, chemotaxis and quorum sensing shape bacterial biomass accumulation in complex porous media

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It is hard to assess how biotic and abiotic properties control the accumulation of bacterial biomass in spatially structured media. We explore flow-mediated interactions allowing E. coli to colonize a porous structure composed of dead-end pores (DEPs) and transmitting pores (TPs). Gradients of the autoinducer-2 promote bacterial chemotactic accumulation in DEPs. This results in hot-spots of resource consumption, which triggers the mechanical evasion of biomass from nutrients and oxygen depleted DEPs. These observations demonstrate how micro-scale physical structure and complex flow, coupled with quorum sensing and chemotaxis, control the accumulation of bacterial biomass in a spatially structured environments.

S-35*/MT-12*/P-20* **From seeding to post-harvesting: sugar kelp microbiome decoding for sustainable European aquaculture**

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After rapid initial growth, the European seaweed aquaculture sector has entered a stagnation phase, presenting the unique opportunity to resolve scientific gaps crucial to sustainable and profitable sector advancement. Sugar kelp (*Saccharina latissima*) is a widely applied seaweed in food, cosmetics, bioremediation and more. During cultivation, early-life sugar kelp stages are glued onto ropes and eventually attach firmly using their holdfasts. Attachment is challenged by many factors, causing a >90% loss of unattached seeded sugar kelp. Research hints towards microbes playing an important role in early-life sugar kelp development, potentially fostering seeding attachment. Mechanical detachment of sugar kelp during harvest or detaching post-harvest remains pose additional economic and ecological risks. To limit these risks, the Dutch SEASEEDS project aims to improve cultivated sugar kelp attachment using a multidisciplinary approach, including research on allied microbiota. First, we characterized sugar kelp-associated bacterial and fungal communities by 16S and 18S rRNA gene amplicon sequencing. We collected sugar kelp and seawater samples throughout the cultivation cycle at the nearshore small-scale Oosterschelde site, seeded in by Hortimare B.V. and operated by The Seaweed Company. In turn, the microbial key players at sugar kelp detachment-prone cultivation stages (i.e., seeding, harvesting and post-harvesting) can be identified and potentially used to improve sugar kelp attachment. Secondly, we compare that study with sugar kelp samples from the first Dutch large-scale offshore farm, North Sea Farm 1, operated by the North Sea Farmers. Thirdly, we analyze sugar kelp samples from distinct European cultivation sites. These spatial comparisons allow to assess the generality of the observed microbial communities associated with cultivated sugar kelp. The entire monitoring efforts provides essential microbial insights that support the European seaweed cultivation sector advancement, promoting both economic viability and ecological sustainability.

S-36 Environmental cryo-electron tomography of ultra-small microorganisms in a deep Alpine subsurface ecosystem

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The terrestrial subsurface environment is a reservoir for unique microbial communities, often characterized by a relatively high abundance of bacteria belonging to the Candidate Phyla Radiation (CPR) group. These microorganisms have small cell sizes and highly reduced genomes, lacking genes essential for many metabolic pathways. Therefore, CPR bacteria are thought to be obligate symbionts relying on nutrient supply from host cells. However, their symbiotic lifestyle remains poorly understood in the subsurface biosphere. Recently, the microbial community of the Swiss deep-subsurface environment became accessible via the BedrettoLab Deep Life Observatory (DELOS) in Ticino. We developed a workflow combining 16S amplicon and shotgun sequencing with cryo-electron tomography (cryo-ET), enabling us to perform both microbial metagenomics and high-resolution three-dimensional imaging of microbial cells from DELOS samples. Using a custom-made filtration unit, we concentrated cells from a water sample collected beneath one kilometer of granite rock to extract genomic DNA and prepare cells for cryo-ET. First, we found that CPR bacteria made up a significant fraction of the microbial community based on the results of DNA sequencing. Second, we observed an extremely low number of microbial cells on the EM grids using cryo-ET. However, CPR-like cells indeed represented a substantial portion of the total cell pool. Third, we found multiple examples of tubular cell-cell connections between semi-lysed CPR-like cells and the S-layer-like cell envelope of a filamentous microorganism. Finally, we developed an approach to DNA sequencing of microbial community preserved on EM grids to increase confidence in the correlation between metagenomic and cryo-ET results. In conclusion, we advanced our environmental cryo-ET approach and used it to gain insights into microbial diversity in the Alpine subsurface ecosystem.

S-37 Phylogenomics of anatoxin-a-producing Microcoleus species at a local and global geographic scale

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Microcoleus anatoxicus and closely related *Microcoleus* species *[Matx]* are ubiquitous benthic cyanobacteria capable of producing anatoxin-a derivatives. Their proliferation is commonly associated with the death of vertebrates, including domestic animals, at the shores of rivers and lakes. Despite recent sequencing efforts, we lack an overview of the genomic diversity and biogeography of this problematic *Microcoleus* clade. Here, we analyze a set of a dozen new complete *Matx* genomes and metagenome-assembled genomes (MAGs) collected, over the span of five years, from L'Areuse and other Swiss rivers and lakes. We compare the *Matx* sequences from *L'Areuse* with genomes from other regions of Switzerland and from around the world. We find that proliferations happening in Switzerland over the years are caused by one dominant cohesive lineage (ANI>=99.0%) which is closely related to, but distinct from, lineages causing similar problems on other continents. In addition, the anatoxin-a biosynthesis operon found in Swiss *Matx* populations is unique, confirming that this genomic region is prone to recombination and diversification. Overall, our results suggest that *Matx* populations present a quite strong geographic structure, in particular compared to planktonic cyanobacteria; this structure could be linked to dispersal barriers or, alternatively, to local adaptations which could be key to understand the ecological success of *M. anatoxicus,* both globally and locally.

Keywords: Microcoleus, anatoxin-a, phylogeny, genomics, biogeography

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M-14 A Bacterial Pirate Ship – Mechanism of Bacterial Predation via Ixotrophy

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Bacteria in aquatic environments must adapt to rapidly changing nutrient conditions. Some Bacteroidetes species have developed a predatory strategy known as ixotrophy. Despite its ecological relevance, the molecular mechanisms underlying ixotrophy remain poorly understood.

We applied a multiscale imaging approach—combining light microscopy, cryo–electron tomography, single-particle cryo–electron microscopy, and single-cell Raman microspectroscopy with functional assays—to elucidate how the model strain *Aureispira* captures and kills prey. Our findings reveal that *Aureispira* uses specialized extracellular structures, secreted via a type IX secretion system (T9SS) and resembling grappling hooks, to latch onto prey cells, particularly interacting with their flagella. Following capture, a type VI secretion system (T6SS) punctures the prey cell, leading to its lysis.

Furthermore, we found that nutrient availability dynamically regulates ixotrophy through insertion sequences (ISs) that toggle the expression of predation-associated genes. This adaptive mechanism enables *Aureispira* to optimize energy expenditure depending on environmental conditions.

Our work provides the first detailed view of ixotrophy at the molecular level, revealing how an interplay between complex cellular machineries enable bacterial predation and nutrient uptake. These insights enhance our understanding of microbial interactions in aquatic environments, highlighting ixotrophy's role in ecosystem dynamics and microbial population control.

S-38* Are two competing symbionts occupying the same spatial niche in the honeybee gut?

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The composition and distribution of the gut microbiota is shaped by competitive interaction. Recently, our lab discovered that *Frischella perrara* and one strain of *Gilliamella apicola* - two honey bee gut symbionts - have an antagonistic interaction in the ileum, a part of the hindgut. *F. perrara* carries a Type VI Secretion System (T6SS) that regulates the gut exclusion of *G. apicola.* T6SSs are contact-dependent nanoweapons that deliver toxic effectors into competing microbes, promoting inter-bacterial competition. As this form of competition requires direct physical contact between bacterial cells we hypothesize that *F. perrara* and *G. apicola* are found within the same location in the gut. The aim of this work is to determine the biogeography of *F. perrara* and *G. apicola* in the gut, providing insights into the spatial dynamics underlying their interaction.
To address this, microbiota-depleted bees were colonized with *G. apicola* or *F. perrara*. Seven days after colonization, the ileum was dissected and bacterial loads within the gut were quantified by counting colony-forming units (CFUs). Moreover, for half of the samples, the anterior and posterior parts of the ileum were further separated to have a finer scale resolution of bacterial location. Interestingly, both bacteria were mainly located in the anterior part of the ileum. To have a better characterization of the biogeography of both symbionts at the microscopic scale, bacterial strains carrying plasmids encoding fluorescent proteins were generated: *G. apicola* expressing E2-Crimson, and *F. perrara* expressing GFP. We are currently colonizing microbiota-depleted bees with these strains, and the ileum will be examined using confocal microscopy to determine bacterial localization.
Demonstrating that *Frischella* and *Gilliamella* niches overlap will open new perspectives on the mode of action of the *F. perrara* T6SS in the honey bee gut, contributing to the understanding of the molecular mechanisms regulating competition.

S-39 Modulating gene expression and protein secretion in the bacterial predator Bdellovibrio bacteriovorus

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The predatory bacterium *Bdellovibrio bacteriovorus* kills other bacteria, thrives in diverse environments, and holds great potential to address major challenges in medicine, agriculture and biotechnology. As a bacterial predator it represents an alternative to traditional antimicrobial strategies to combat multidrug-resistant bacterial pathogens and prevent food waste, while the multitude of predatory enzymes it produces hold potential for biotechnological applications. While *B. bacteriovorus* is secreting an extensive arsenal of hydrolytic enzymes, the secretion process is sparsely explored. Currently, limited availability of versatile genetic tools and secretion assays constrain both fundamental studies and bioengineering of *B. bacteriovorus*. We developed a molecular toolbox for *B. bacteriovorus* by systematically tuning gene expression and secretion of a reporter protein. We investigated functional native and synthetic promoters from the Anderson library with varying expression levels and demonstrated their efficacy in driving expression of the fluorescent reporter protein mScarletI3 at both the population and single-cell level. Additionally, we evaluated different ribosomal binding sites (RBS) to fine-tune gene expression. To examine secretion, we established a novel protocol to quantify extracellular release of a Nanoluc luciferase reporter protein in *B. bacteriovorus* using different native Sec-dependent signal sequences. Overall we support the research community with a toolbox addressing existing limitations in *B. bacteriovorus* engineering, supporting tailored applications and fundamental studies of this predator in microbial ecology, agriculture, biotechnology and medicine.

S-40 The natural diversity of E. coli transporter-dependent capsules

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Bacterial capsules are critical for interactions with phages, antibiotics, the host immunity and vaccines. Despite the relevance of *Escherichia coli* in the current antimicrobial resistance scenario, the full diversity of its capsular polysaccharides remains largely unknown.

To address this gap, we generated a genotype-to-serotype map by sequencing 35 reference strains for transporter-dependent capsular serotypes. Using this map as a starting point, we analysed >37,000 *E. coli* genomes, identifying 85 distinct capsule biosynthesis loci, 50 of which novel. We mapped gene function by combining sequence- and structure-based approaches, uncovering a much larger functional diversity than previously reported and revisiting previous assumptions on serotype-sugar associations, for which we provide experimental evidence via mass spectrometry.

Leveraging this updated catalog, we developed a hidden Markov model-based typing tool, kTYPr, which we applied to a newly curated collection of > 24,000 *E. coli* genomes, unique in scale, global distribution, metadata resolution and genome dereplication, thereby overcoming known biases of public *E. coli* genome collections. We demostrated that capsules greatly extend the ecological diversity of *E. coli*, with novel types enriched in undersampled, non-human-associated environments. In humans, we assessed the impact of capsule types on *E. coli* invasiveness, using a globally distributed *E. coli* carriage dataset of > 2,600 human gut metagenomes as control. We revealed an extensive overlap in the capsular repertoire of commensal and invasive strains and, in addition to known associations, we identified four types never linked to *E. coli* disease before, including two with newly defined genetic architecture.

Altogether, these results advance our understanding of *E. coli* surface diversity and ecology, providing a foundation for targeted antimicrobial strategies targeting *E. coli* capsules via phages, drugs or vaccines.

S-41

Role of a new operon in cell wall homeostasis and antibiotic resistance in Pseudomonas aeruginosa

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The peptidoglycan (PG) cell wall is a dynamic, cross-linked polymer that surrounds most bacterial cells and is essential for maintaining bacterial shape and preventing lysis from osmotic pressure. During growth and division, PG is continuously remodeled, requiring a precise balance between synthesis and degradation. PG synthesis relies on SEDS proteins, as well as, key targets of beta-lactam antibiotics, Penicillin-Binding Proteins (PBPs). The opportunistic pathogen *Pseudomonas aeruginosa* resists beta-lactams through AmpC, a chromosomally encoded beta-lactamase whose expression is tightly regulated in response to PG homeostasis.

In this study, we identified mutations in a previously uncharacterized operon that contribute to antibiotic resistance in *P. aeruginosa*. We show that one protein encoded by this operon directly interacts with FtsI (PBP3), which, with the SEDS protein FtsW, drives septal PG synthesis. Notably, we found that FtsN, an essential divisome protein that activates septal cell wall synthesis by FtsWI, is not required in the absence of the operon. These results suggest that the operon negatively regulates PG synthesis by FtsWI at the division site.

Furthermore, the absence of this operon leads to beta-lactam resistance through increased AmpC beta-lactamase production and alters aminoglycosides and cationic peptides resistance via mechanisms that remain unknown. Thus, understanding the precise role of this operon in both septal PG synthesis and antibiotic resistance is crucial for developing new antibacterial treatments and strategies, particularly in the context of increasing antimicrobial resistance.

M-15 Diagnosis in mycology: new tests, new strategies?

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S-42 Impact of milbemycin oxime on fluconazole resistance in Candida auris

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Background
Candida auris is a pathogenic yeast that can develop multiple antifungal resistances in particular to azoles (e.g. fluconazole). Milbemycin oxime (MO) potentiates the effect of fluconazole (FLC) against *Candida* spp. by inhibiting ABC transporters, such as Cdr1, which is involved in azole drug efflux.

Objectives
This study aimed at assessing the interaction of MO and FLC against clinical (n=4) and laboratory-generated (n=4) *C. auris* isolates with different mechanisms of azole resistance.

Methods
Interactions of MO and FLC were assessed by checkerboard assays and defined as synergistic, indifferent or antagonistic according to the fractional inhibitory concentration index (FICI) values. The fluorescent substrate rhodamine 6g (R6G) was used to measure ABC transporter activity in the absence or presence of MO.

Results
A synergistic interaction between MO and FLC was observed against most isolates including those harboring Cdr1-independent mechanisms of azole resistance (e.g. *ERG11* mutations). The highest synergism was observed in a laboratory-generated strain overexpressing *CDR1*, while the interaction was indifferent in a strain lacking *CDR1*. R6G experiments confirmed the inhibitory effect of MO on ABC transporters.

Conclusions
MO could represent an interesting adjunctive therapy against azole-resistant *C. auris*, in particular those with *CDR1* overexpression.

S-43 Emergence of terbinafine-resistant Trichophyton indotineae strains in a Swiss dermatology laboratory

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Aims

Trichophyton indotineae, also referred to as *T. mentagrophytes* genotype VIII, is an emerging dermatophyte species characterised by persistent dermatomycoses and widespread antifungal resistance. This study aims to analyse terbinafine-resistant strains of *T. indotineae* currently isolated in a swiss microbiology laboratory specialised in dermatology.

Methods

Seventeen strains isolated in our laboratory and identified as *Trichophyton indotineae* were tested for their resistance to terbinafine. The MIC determination was performed on microplate according to the EUCAST method with minor modifications (Sensititre plate, Thermo Fisher Scientific Inc., Waltham, Massachussetts, USA). The same isolates were analysed by the DermaGenius® Resistance Multiplex real-time PCR kit (PathoNostics B.V., Maastricht, The Netherlands) and by sequencing of the squalene epoxidase gene to highlight the points of mutations which confer this resistance.

Results

Of the 17 strains, 7 were identified as sensitive to terbinafine with a MIC value <= 0.5-1 ug/ml. All these strains were defined as wild strains by the Dermagenius kit. Changes in amino acids of the squalene epoxidase gene were limited to $Ala^{448}Thr$, $His^{440}Tyr$ and $Phe^{415}Leu$. The ten remaining isolates defined as resistant showed MICs of 1, 2, 4, 16 and even >16 ug/ml. Six resistant strains had a MIC of 16 or > 16ug/ml with a mutation $Phe^{397}Leu$ detected by the Dermagenius kit and confirmed by sequencing. The other four strains were defined as wild type by the kit but had different mutations by sequencing, $Gln^{408}Leu$, $Phe^{415}Cys$ or $Phe^{415}Val$.

Conclusion

The emergence of terbinafine-resistant *Trichophyton indotineae* is now a reality in Switzerland. Screening on a plate containing 0.2ug/ml of terbinafine is very helpful. The most resistant strains can be detected using the DermaGenius® Resistance Multiplex real-time PCR kit, this method being easier to set up than sequencing. Sequencing of the squalene epoxidase gene may reveal other mutations that appear predictive of other MIC values.

S-44 FKS1 genotyping of Candida albicans strains with borderline anidulafungin MIC according to 2024 revised EUCAST Breakpoints

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Background

In December 2024, EUCAST published version 11 of its clinical breakpoint guidelines, lowering the resistance threshold for *Candida albicans* to anidulafungin from >0.03 mg/L to >0.016 mg/L. When using the MICRONAUT-AM susceptibility testing system interpreted under EUCAST criteria, this revision—based on updated MIC distributions and pharmacodynamic data—may lead to an overestimation of resistance. Such misclassification could compromise antifungal stewardship by promoting inappropriate therapeutic choices.

Objectives

This study aims to determine whether *C. albicans* isolates with MICs (as defined by EUCAST or CLSI) between 0.5 and 0.016 mg/L harbor mutations in the *FKS1* gene hot-spot regions known as responsible for echinocandin resistance. The goal is to assess the clinical relevance of adopting the revised EUCAST breakpoints.

Methods

A total of 40 isolates will be analyzed. Fifteen prospective isolates with MICRONAUT-AM anidulafungin MICs of 0.03 or 0.06 mg/L will undergo *FKS1* hot-spot sequencing. Additionally, twenty-five retrospective isolates from the FUNGINOS cohort, already sequenced for the *FKS1* hot-spots and tested by YeastOne (CLSI criteria), will be retested using MICRONAUT-AM to ensure consistency. Genetic analysis will focus on known resistance-associated mutations in *FKS1*.

Results

Among the 24 first strains analysed, 8 have an anidulafungin MIC between of 0.03 mg/L, one of 0.06 mg/L. The others have an MIC of 0.016 mg/L. None of them exhibited mutations in the FKS1 hotspots.

Conclusions

These preliminary results indicate a low risk of underestimating resistance with the new breakpoints. However, sequencing the remaining strains with higher anidulafungin MICs is necessary before forming a definitive stance on adopting the revised EUCAST thresholds.

S-45 In Silico Evaluation of Ribosomal Amplicon Targets for Fungal Identification: Comparative Performance of ITS, LSU and SSU Regions

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Introduction
Accurate fungal identification is essential for both clinical diagnostics and environmental studies. The internal transcribed spacer (ITS) region of ribosomal DNA is the most widely used molecular marker in fungal taxonomy, owing to its high sequence variability and universality across the fungal kingdom. However, other ribosomal regions such as 18S (SSU) and 26S (LSU) are also used in sequencing-based approaches. This study aimed to evaluate and compare the taxonomic performance of different ribosomal targets (ITS1, ITS2, full ITS, SSU, LSU) using in silico methods.

Method
In silico PCR simulations were performed using published primers for ITS1 and ITS2 (White et al., 1990; Illumina protocol) and routine clinical primers for SSU and LSU. Two reference databases were used: Eukaryome v1.8 (470,470 sequences covering ITS, SSU, and LSU regions) and UNITE v10 (93,085 curated ITS sequences focused on fungal taxonomy). Amplicon sizes ranged from 202 bp (SSU) to 309 bp (LSU), and taxonomic assignments were
performed using the zAMP tool.
Three datasets were analyzed: 187 complete fungal genomes from NCBI, 145 Aspergillus genome and with the creation of a section-level taxonomy using 725 species, and 72 clinically relevant fungal pathogens from the CHUV strain collection.
Sensitivity was calculated at each taxonomic rank (kingdom to species).

Results
1. Test I: complete fungal genomes from NCBI
   Full ITS achieved the highest species-level sensitivity (0.85 with Eukaryome; 0.84 with UNITE), followed by ITS2 (0.73) and ITS1 (0.69) with Eukaryome. LSU and SSU showed poor resolution beyond the family level. Main limitations were failed amplification or incorrect assignments among closely related species.
2. Test II Aspergillus species:
   Species-level sensitivity was 0.742–0.744 for Eukaryome ITS full/ITS1 and 0.694 for UNITE ITS full. LSU and SSU performed poorly (0.171 and 0.010). Section-level classification was more accurate, with 0.83–0.85 sensitivity for ITS regions.
3. Test III: CHUV clinical pathogens
   After correction of misassignments, species-level sensitivity exceeded 0.8 for Eukaryome ITS full/ITS1/ITS2 and UNITE ITS full. However, 23 species failed to amplify (19 across all targets), mostly due to missing ITS regions in incomplete genome assemblies.

Conclusion
Amplicon sequencing targeting the ITS region—particularly ITS full or ITS2—offers robust and reliable fungal identification, outperforming SSU and LSU. The use of specialized databases and accurate primer design significantly improves taxonomic resolution.
In silico analyses highlighted both the potential and the limitations of current genome resources, particularly the absence of target regions in draft assemblies.
Based on these results, a follow-up project will focus on experimental validation of fungal detection using PCR followed by Sanger or next-generation sequencing (NGS).

M-16 Unfolded protein response during viral infections

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Persistent viruses can cause long-term illnesses and pose a serious risk to people with weakened immune systems. Human adenovirus (AdV) is one such virus that can be deadly in these individuals. Our previous work1 has shown that AdV persistence relies on a cellular stress response called the unfolded protein response (UPR) in the endoplasmic reticulum (ER)2,3. Specifically, the UPR sensor IRE1α is activated by the viral glycoprotein E3-19K, which depends on E1A for its expression and forms a complex with IRE1α in the ER lumen. Once activated, IRE1α splices to activate a transcription factor X-box binding protein-1 (XBP1), which then binds to the E1A promoter and boosts E1A gene expression. This, in turn, increases the production of E3-19K, creating a self-sustaining cycle (feed-forward loop). This loop helps AdV replicate more efficiently and maintain a long-term infection even in the presence of the immune activator interferon. However, the role of E3-19K in viral persistence could not be fully assessed as E3-19K deleted virus still partly activated IRE1-XBP1 through an alternative pathway. Preliminary data suggests that this happened via E1A prior to the expression of E3-19K. We aim to discover how E1A modulates IRE1α activity before E3-19K expression to trigger an unusual form of IRE1α activation and support lytic and persistent viral replication.

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M-17 **Molecular characterization of clinical influenza B virus isolates in human lung explants**

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S-46/P-78 Tracing the tropism: How seasonal and avian influenza A viruses target epithelial cells across the human airway

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Influenza A virus (IAV) enters the human host via the respiratory tract, with the airway epithelium serving as the primary site of infection. While previous studies have demonstrated that human (hIAV) and avian (aIAV) strains exhibit distinct cellular tropism within the human epithelium, contradictory results have been described and the underlying determinants of cell type specificity remain poorly understood. Moreover, it is unclear how these patterns vary along different regions of the respiratory tract.
To address these questions, we used differentiated primary human airway epithelial cell cultures derived from both the upper (nasal) and lower (bronchial) respiratory tract to model the complexity and heterogeneity of the airway epithelium in vitro. The cultures were infected with either seasonal hIAV or aIAV strains. The infections were assessed at the level of viral mRNA expression through single-cell RNA sequencing and at the protein level via flow cytometry. Our analysis revealed that ciliated and secretory cells are the primary targets of infection. Strikingly, in bronchial cells, hIAV infects both cell types without a strong preference for either, whereas aIAV displays a significant tropism for ciliated cells. Further, we found that preferential targeting is established during the viral binding stage, despite both cell types displaying similar levels of the viral attachment receptor sialic acid in both α2,3- and α2,6-linkages. To further probe the glycan-mediated mechanisms of viral binding, we performed N-glycomics using mass spectrometry on sorted ciliated and secretory cells. This analysis revealed preliminary differences in surface sialylated glycan complexity and structures between the cell types, which might explain the observed tropism.
Our findings provide new insights into the cell-type specificity of seasonal and avian IAV across the human airway. Understanding these glycan-dependent interactions is key to elucidating the molecular barriers that limit aIAV infection in humans.

S-47 Distinct Transcriptomic Signature of HIV-positive Lymph Node Dendritic Cells during Chronic HIV infection

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Background

Understanding how HIV infection influences the localization and function of Lymph Node (LN) Dendritic Cells (DCs) is essential for defining mechanisms of viral persistence and immune dysregulation during chronic HIV infection.

Results

To characterize the influence of HIV infection on LN myeloid DCs, the single cell transcriptomic profiles of myeloid DCs isolated from inguinal LNs of HIV uninfected (N=3), untreated viremic HIV-infected individuals (N=3) and ART treated HIV-infected individuals (N=3) were analyzed and compared. Notably, HIV replication was most notably associated with increased proportion of cells harboring HIV transcripts (28% *vs.* 18%) and HIV transcript-load per cell within $CCR7^{hi}$ LN DCs relative to $CCR7^{lo}$ DCs. In addition, meta clustering analyses revealed an enrichment of HIV transcript-positive cells within a population of $CCR7^{lo}$ DCs (i.e. Cluster 3; C3) as compared to all other cell clusters ($P<0.05$). Interestingly, this population harbored significantly high expression of genes involved in cell cycle progression and early differentiation (MKI67, TOP2A, DNMT1) as compared to any other cell cluster (N=9; $P<0.05$), possibly indicating to a population of proliferative, precursor-like DCs. These observations were further confirmed through 1) mass cytometry-based analyses of Ki67 expression on LN DCs and 2) phylogenetic analysis of individual HIV-1 proviral genomes with HIV integration site analyses performed on *ex vivo* sorted LN $CCR7^{hi}$ and $CCR7^{lo}$ LN DCs. Interestingly, HIV-transcript-positive cells *versus* HIV-transcript-negative cells within C3 revealed a spatial segregation: transcript-positive cells preferentially expressed genes supporting localization to T-B cell regions (GPR183 and GRB2) as compared to transcript-negative cells that harbored genes associated with migration to T cell zones (IGSF6, PLXNC1, FYB1, FAM26F) ($P<0.05$).

Conclusion

These findings suggest that untreated chronic HIV infection most notably reshapes $CCR7^{lo}$ population enriched for HIV transcripts and capable of clonal expansion—that preferentially localize to T-B cell border regions, potentially promoting HIV transmission to activated Tfh cell precursors.

M-18 Ureaplasma & Mycoplasma infection: clinical significance

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S-48 Diagnosis and antimicrobial susceptibility testing of Neisseria gonorrhoeae in four different laboratories from 2014 to 2024

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Background

Neisseria gonorrhoeae is a significant global public health concern due to its increasing prevalence and multidrug resistance. Accurate and timely diagnosis is critical for treatment to avoid complications and dissemination.

Gonococcal culture, which allows antimicrobial susceptibility testing (AST), is increasingly replaced by molecular tests because of higher sensitivity and rapidity.

This study aims to evaluate the diagnostic approaches by different laboratories (Bioanalytica, Dianalabs, MCL, Dr. Risch) across various Swiss regions as well as positivity rates and resistance rates of *N. gonorrhoeae* isolates from 2014 to 2024.

Methods

Patient data and laboratory diagnostic methods were analyzed across four different laboratories over a six and ten-year period. Different diagnostic approaches were used: initial PCR, initial culture and reflex culture for PCR positive samples. AST was performed on cultured isolates by determination of minimum inhibitory concentrations (MICs).

Results

Diagnostic strategies varied, with one laboratory performing systematic gonococcal cultures on all genital samples and PCR on specific request and three laboratories conducting specific gonococcal cultures mainly following PCR positivity. The positivity rate for *N.gonorrhoeae* PCR between 2014-2024 was stable varying from 1.2-2.5%. For routine genital culture diagnosis (initial culture), the detection rate varied from 0.1-0.3%. AST performed on 1107 isolates revealed a significant increase in resistance rates from ca. 50% in 2014 to above 70% in 2024 against ciprofloxacin. Resistance to cefixime has been described since 2014 and remained stable (<5%). The prevalence of ceftriaxone-resistant isolates (MIC > 0.125 mg/L) remained low (n=6 isolates).

Conclusion

The majority of laboratories in this study (n=3) use PCR as the first line test for the diagnosis of *N. gonorrhoeae* infections and do not use systematic *N. gonorrhoeae* culture for genital specimen. Positivity rates did not significantly increase between 2014 and 2024. AST is still necessary for resistance surveillance, as multi-resistant strains have been described worldwide.

S-49*/P-80* differential transcriptional analysis of chlamydia trachomatis following iron chelation

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The life cycle of *Chlamydia trachomatis* (CT) includes an intracellular, biphasic developmental sequence. Additionally, in response to stressors such as IFN-γ, temperature changes or iron deprivation, the bacteria can survive, within the host, in a reversible persistent state. However, the precise mechanisms leading to the transition from replicative, reticulate bodies (RBs) to aberrant bodies (ABs) have not been characterized.

By comparing the transcriptomes of RBs and ABs, we have identified differentially expressed genes predicted to be part of a two-component regulatory system (TCS). TCSs are used by bacteria to sense and respond to their environment. AB formation was induced in CT-infected cells by depleting iron using the chelator 2,2'-bipyridyl (BPDL). RT-qPCRs were performed to quantify RNA levels and to assess gene expression during normal growth and persistence. In parallel, immunofluorescence microscopy was carried out to compare gene expression with morphological changes associated with AB. Results show that TCS genes were strongly downregulated in ABs compared to RBs. When BPDL stress was removed from ABs, expression levels of TCS genes returned to those found in control infections. Downregulation in gene expression varied based on the stressor applied, indicating a relationship between different stress stimuli and TCS gene regulation.

In parallel, we also characterized the expression of genes upregulated during persistence, namely the three genes of the *trpRBA* operon and genes encoding IncD, IncE, IncF, and IncG proteins. Incs are required for biosynthesis and establishment of the bacterial inclusion. Unexpectedly, preliminary results showed that expression of Incs was dependent on the host cell line. To further explore the role of the TCS during persistence, we will overexpress the TCS proteins and assess their impact on chlamydial morphology during persistence.

Uncovering the biological mechanisms that trigger the development of persistent bacteria may provide key insights into the processes underlying chronic chlamydial infections.

S-50 Deciphering the Intracellular Trafficking of Chlamydia trachomatis to Improve Therapeutic Targeting

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Chlamydia trachomatis is the most reported bacterial sexually transmitted infection worldwide, with rising incidence and significant long-term reproductive health consequences. As an obligate intracellular pathogen, *C. trachomatis* has developed specialized strategies to invade and survive within host cells. After entering cells, *C. trachomatis* avoids lysosomal degradation by circumventing the conventional Rab5/Rab7-mediated endosomal maturation process. Instead, they form non-acidic, Lamp1-positive inclusions via a pathway that relies on Rab14. This alternative trafficking route is not well understood, especially during the early stages of infection, making it difficult to develop effective intracellular therapies. The Rab14-dependent pathway is also required for cationic cell-penetrating peptides (CPPs) to be endocytosed and trafficked into cells. Because CPPs utilize the same endocytic route as *C. trachomatis*, they offer a unique opportunity to study these non-canonical trafficking processes and to develop targeted drug delivery strategies. We employ high-throughput genetic and proteomic screening to map early host-pathogen interactions and identify new trafficking regulators. At the same time, we evaluate the ability of CPP-antibiotic conjugates to access intracellular inclusions through these shared pathways, aiming to advance new approaches for treating both acute and persistent intracellular infections.

S-51 Impact of genital bacteria on human sperm physiology

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Male infertility is a growing health concern, with up to 50% of cases classified as idiopathic. Recent studies suggest that genital microbiota may influence sperm function, offering a potential explanation for some idiopathic cases. In this study, we examined how selected genital bacteria affect human spermatozoa physiology, focusing on viability and mitochondrial membrane potential—two key indicators of sperm function.

Using culture-based methods, we have established a collection of seminal bacteria that have been isolated from seminal fluid. Interestingly, several isolates were able to replicate in seminal fluid. Purified spermatozoa were exposed in vitro to fluorescently-labelled bacteria, and their physiological responses were assessed by flow cytometry and confocal microscopy at multiple time points (0h, 24h, 48h). We observed diverse bacterial adhesion profiles to spermatozoa, ranging from low to high attachment. Bacterial attachment significantly impacted sperm viability, with some species inducing over 50% sperm mortality. Mitochondrial membrane potential was also altered by bacterial presence, with some strains inducing possibly premature hyperpolarization, potentially affecting sperm motility and fertilization capacity.

Our findings underscore the physiological consequences of genital bacterial interaction with sperm and provide novel experimental evidence of how seminal microbiota can modulate sperm function and supports further investigation into their role in male infertility.

M-19 The ecology and evolution of small bacterial communities

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Understanding how microbial communities in natural ecosystems assemble and evolve is crucial, as these communities greatly affect us and our environment. But since studying eco-evolutionary dynamics in natural systems is extremely challenging, in my lab we use small bacterial communities as model systems. I will give an overview of the work in our lab. I will first talk about how four species of bacteria interact to degrade pollutants in industrial waste waters, how these interactions are shaped by the environment over short and evolutionary timescales, and how we can use the principles of group selection to breed new communities from scratch for more efficient bioremediation. I will then present more recent work that generalises on these findings to see whether we can predict and control ecological and evolutionary dynamics in other contexts as well.

S-52

Evaluation of Bacillus thuringiensis var. israelensis spillover from mosquito-treated areas to agricultural fields

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Bioinsecticides based on the bacterium *Bacillus thuringiensis* var. *israelensis* (Bti) are widely used for mosquitoes and black flies control due to the production of proteins toxic to Culicidae and Simuliidae larvae. Among others, Bti is in close phylogenetic relationship with the food-contaminant *B. cereus s.s.* and the agricultural bioinsecticides *B. thuringiensis* var. *aizawai* and *kurstaki*, which are members of the *B. cereus* group (BCG).
In contexts where mosquito control treatments are in proximity of agricultural fields, the potential spillover of Bti into the food chain needs to be closely investigated. Even though the safety of Bti for humans and non-target organisms have been extensively described, Bti spillover from treated areas to adjacent sites is currently poorly quantified. Moreover, the impact of Bti relative to the overall BCG biodiversity in food production should be carefully evaluated.
Soil samples at distances ranging from 0 to >3000 m from regularly Bti-treated areas were collected before and after yearly Bti treatments in the region of the Piano di Magadino (Ticino, Switzerland), where mosquito control with Bti and agricultural fields are in close proximity. Soil Bti concentrations were quantified by real-time q-PCR to evaluate the effect of distance and recent treatment on Bti persistence. Moreover, strains belonging to the BCG were isolated from the soil samples on selective agar to assess overall BCG occurrence.
Bti concentration was significantly correlated with decreasing distance and was detected up to 850 m from the nearest treated surface. However, few meters were enough to decrease the Bti concentration down to levels that made it minor compared to the overall BCG diversity. Thus, this real case scenario study showed negligible risk of Bti spillover into the food chain.

S-53* Evaluating the importance of pyoverdine in two potato-associated Pseudomonas strains on their anti-Phytophthora activity

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The oomycete *Phytophthora infestans* has been causing detrimental yield losses over the last 200 years. Still today, it is difficult to fight it with conventional pesticides. That is one of the reasons why *biocontrol* is an attractive alternative to fight this pathogen. *Pseudomonas* strains have been known to produce a large arsenal of secondary metabolites that are useful to combat several crop diseases. Next to more direct biocontrol traits such as antibiotics and toxins, *siderophores* are a highly interesting type of biocontrol trait present in *Pseudomonas*.

In this study, we focus on two *Pseudomonas* strains, R32 and R47, which have been previously characterized as successful antagonists against *P. infestans in vitro* and *in planta*. Both are able to produce *pyoverdine*, a high affinity iron chelator produced by many fluorescent *Pseudomonads*. We were interested in the role of pyoverdine in the respective biocontrol activity of both strains towards *P. infestans.*

For this purpose, we created pyoverdine mutants by knocking-out *pvdE*, which encodes a periplasmic transporter for ferribactin, pyoverdine's precursor in the wild-type background, but also in a HCN-deletion background for both strains. These mutants were then tested for antagonistic activity against *P. infestans* in several *in vitro* assays and *in planta* with a leaf disc assay.

For *in vitro* mycelium growth inhibition, pyoverdine and subsequently its loss appeared irrelevant to the activity of both strains. Looking at the pathogen's spore development, the role of pyoverdine in biocontrol activity proved to be minor. *In planta*, the pyoverdine single mutant of R47 was less active than the wild-type. This was the only result indicating that pyoverdine has a direct role in the antagonism against the oomycete.

Our findings suggest that pyoverdine may have a role outside of direct iron competition, but more towards *iron sensing* and subsequent regulation in our strains.

S-54 Nutrients and flow shape the cyclic dominance games between Escherichia coli strains

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Evolutionary game theory has provided various models to explain the coexistence of competing strategies, one of which is the rock-paper-scissors (RPS) game. A system of three *Escherichia coli* strains—a toxin-producer, a resistant, and a sensitive—has become a classic experimental model for studying RPS games. Previous experimental and theoretical studies, however, often ignored the influence of ecological factors such as nutrients and toxin dynamics on the evolutionary game dynamics. In this work, we combine experiments and modeling to study how these factors affect competition dynamics. Using 3D-printed mini-bioreactors, we tracked the frequency of the three strains in different culturing media and under different flow regimes. Although our experimental system fulfilled the requirements of cyclic dominance, we did not observe clear cycles or long-term coexistence between strains. We found that both nutrients and flow rates strongly impacted population dynamics. In our simulations, we explicitly modeled the release, removal and diffusion of toxin. We showed that the amount of toxin that is retained in the system is a simple indicator that can predict competition outcomes across broad parameter space. Moreover, our simulation results suggest that high rates of toxin diffusion might have prevented cyclic patterns from emerging in our experimental system.

S-55 Microbial communities influencing methane cycling in temperate Swedish peatlands

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Northern peatlands are significant organic carbon pools, naturally acting as carbon dioxide (CO_2) sinks but emitting methane (CH4) as a product of the anoxic decomposition of organic matter driven by complex microbial communities in soil. This study investigates the unexplored microbial communities of pristine peatlands in Värmland, central Sweden, (Björsmossen [BM], Norra Romyren [NR], Lungsmossen [LM] and Havsjömossen [HM]) with a particular focus on microorganisms involved in CH4 cycling. Peat samples up to 60 cm depth were subjected to biogeochemical measurements and 16S rRNA amplicon sequencing to compare the biogeochemistry and microbial diversity across the sites. Additional deep samples up to 700 cm depth were retrieved and subjected to amplicon sequencing to shed light on the microbial communities across the entire peat layer. This revealed similar biogeochemical conditions (temperature, acidic pH, high CO_2 to CH4 ratios) and some consistent dominant microbial phyla across sites that may play a fundamental role across peatland locations. BM and NR had the highest species diversity (alpha diversity), which generally decreased with soil depth. Among the deep samples those from BM and LM, collected at 540 and 700 cm of depth respectively, were dominated by archaea, mainly Crenarchaeota, comprising many uncultivated lineages (e.g., Bathyarchaeia and Methanomethylicia). Seven microbial taxa were identified as potentially involved in CH4 cycling, comprising aerobic methanotrophic bacteria and methanogenic archaea, but no anaerobic methanotrophic archaea. Potential methanotrophs made up around 20% of the microbial community in the top 10 cm across sites, but significantly decreased in abundance below 30 cm sampling depth. Potential methanogens belonging to Halobacterota were found at all depths but were most abundant at 30 cm (LM) with 68% of relative abundance. Additionally, elevated CO_2 to CH4 ratios measured at three sites at these depths suggest that there is an unidentified microbial process causing the low CH4 levels relative to CO_2 in these layers. These findings provide unprecedented insights into the taxonomic composition and potential metabolism of microbial communities inhabiting temperate northern peatlands of the Värmland region, highlighting their correlation with peat biogeochemistry. Upcoming metagenomic and metatranscriptomic data will help clarify their metabolic roles and involvement in CH4 cycling.

M-20 An epithelial perspective on cellular crosstalk in the intestinal mucosa

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The intestinal mucosa forms an integral barrier between our bodies and the outside world. Being exposed to a huge variety of metabolites and microbes taken up with our food or stably colonizing the intestine, it is permeable for essential nutrients while serving as a protective barrier to prevent microbial translocation. A tight balance between the intestinal epithelium, immune cells in the underlying tissue and the intestinal microbiota present in the lumen maintains intestinal homeostasis. The impairment of this balance can trigger disease, such as infection, metabolic disorders, and chronic inflammation (e.g. inflammatory bowel disease). Our work focuses on understanding how these key players interact to maintain intestinal homeostasis, with a special interest in the intestinal epithelium. The intestinal epithelium is in direct contact with the microbiota colonizing the intestinal lumen (e.g. sensing via pattern recognition receptors, uptake of metabolites), yet we know little about the molecular basis of epithelium-microbiota crosstalk and the impact of commensal bacteria on epithelial barrier function. We make use of state-of-the-art in vivo and advanced organoid-based in vitro models, (single cell) omics techniques and bioengineering approaches, to map mucosal cellular crosstalk and dissect the underlying molecular mechanisms in mouse and human.

S-56* Characterization of mitochondria-targeting Legionella pneumophila effectors

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The facultative intracellular bacterium *Legionella pneumophila* replicates in amoebae and macrophages and can cause a life-threatening pneumonia called “Legionnaires’ disease”. *L. pneumophila* translocates more than 300 distinct “effector proteins” into the host cell during infection. These effectors have been shown to manipulate nearly every major cellular pathway and organelle of the host. One such target is the mitochondrion, where *L. pneumophila* effectors mimic, modify, or modulate mitochondrial components, such as the F_0F_1 ATPase or ATP/ADP translocases. Although the functions of most *L. pneumophila* effectors remain unknown, the sheer number and diversity of effectors suggests that a substantial portion may target mitochondria. Indeed, the combination of bioinformatic and unbiased proteomics approaches has led to the identification of novel mitochondria-targeting effectors.

This project investigates novel mitochondria-targeting *Legionella* effector proteins that impair host cell respiration and promote bacterial replication. Preliminary data show that these toxins localize to mitochondria and disrupt their function. Some of these effector proteins structurally resemble known secreted bacterial toxins. This work will dissect the mode of action of these effector proteins using biochemical, genetic, and imaging approaches to evaluate their impact on host cell mitochondria. Additionally, the study seeks to identify host interaction partners and assess their role in infection. The insights thus gained will advance our understanding of host-pathogen interactions and mitochondrial manipulation and may yield highly specific biomolecular probes with potential biotechnological and/or clinical applications.

S-57 Deciphering the Non-Canonical Endocytic Trafficking of Chlamydia trachomatis: A Rab14-Dependent Pathway

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Chlamydia trachomatis is an obligate intracellular pathogen that evades host degradation pathways by entering and replicating within a membrane-bound compartment called the inclusion. Unlike many intracellular pathogens, *C. trachomatis* does not rely on the canonical Rab5/Rab7 endosomal maturation cascade. Instead, our recent findings demonstrate that *C. trachomatis* traffics via a Rab14-dependent route, bypassing degradative lysosomal compartments and targeting non-acidic, Lamp1-positive vesicles.
Using CRISPR/Cas9 knockout cell lines and dominant-negative Rab constructs, we show that Rab5 and Rab7 are dispensable for *C. trachomatis* entry and inclusion formation. Instead, we observe robust colocalization of bacterial inclusions with Rab14 and EEA1, even in the absence of Rab5. These findings suggest that early endosomes marked by EEA1 can form independently of Rab5, contributing to an alternative endocytic pathway exploited by *C. trachomatis*.
To uncover host factors regulating this Rab14-dependent route, we performed Rab14 pull-downs followed by mass spectrometry, identifying a network of candidate proteins whose depletion significantly reduced *C. trachomatis* infectivity in HeLa cells. In parallel, a high-throughput compound screen identified over 90 molecules that reduce infection rates, including drugs unrelated to classical antimicrobial mechanisms.
Altogether, these results reveal a previously underappreciated trafficking route that is critical for *C. trachomatis* intracellular survival. By characterizing this Rab14-dependent pathway and its associated host factors, our work opens new avenues for understanding bacterial pathogenesis and identifying therapeutic targets to disrupt infection at early stages.

S-58 Goblet cell invasion by Pseudomonas aeruginosa promotes breaching of human respiratory epithelia

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P. aeruginosa surface colonization entails complex behavior including adherence, virulence induction and dissemination. Infection studies are hampered by the lack of experimental models that faithfully recapitulate the physiology of human tissue and, at the same time, offer the experimental power to investigate the infection process with high temporal and spatial resolution. We established an *in vitro* 3D lung infection model from human stem cells, with air-liquid interface in a Transwell. Immunocytochemistry- and histology staining confirmed that the architecture and cellular composition of the tissue closely resembles the human bronchial epithelium. Using live cell microscopy, we demonstrate that the tissue recapitulates lung functions such as production of mucus and cilia beating. We utilize the upper airway tissues to visualize and quantify *P. aeruginosa* lung infection with unprecedented spatial and temporal resolution. These studies provide a detailed mechanistic frame for how human pathogens overcome the mucus barrier and rapidly spread on mucosal tissue and how they combine internalization into specialized cell types and collective behavior to rapidly and effectively breach the barrier function of the lung epithelium. Our results establish lung Transwells as versatile *in vitro* model to study bacterial infections and drug response in a human-like environment.

S-59*/MT-04*/P-12* ImmunoPhage: Targeting Persistent Infections with Engineered Immunomodulatory Bacteriophages

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Persistent infections arise when bacteria exploit vulnerabilities in the immune response and resist clearance. While conventional therapies focus on the eradication of pathogens, immunomodulatory approaches aim to enhance the host's defenses. Immunomodulatory treatments using cytokines are a promising addition to the therapeutic repertoire, though their clinical success in infectious diseases has been limited.

Bacteriophages (phages) are another emerging approach against bacterial infections. Previously, it was shown that phage therapy can be enhanced by engineering antimicrobial effectors into the phage genomes. Here, we aim to integrate immunomodulation with phage therapy by encoding cytokines, and generating so-called *ImmunoPhages*. Once ImmunoPhage infects a bacterial pathogen at the site of the infection, cytokines are expressed and released alongside progeny phages. This approach offers a potentially safer, more targeted method of immunotherapy, compared to systemic delivery of cytokines. When combined with phage therapy, it can address key limitations of natural phages, including targeting phage-resistant, intracellular, and quiescent bacterial populations.

Our first generation of ImmunoPhages are designed to treat recurrent urinary tract infections (UTIs), where *E. coli* evades the immune response by forming intracellular bacterial communities and quiescent reservoirs deep within the epithelium. We demonstrate the production of functional chemokines, interleukins, and interferons in clinical *E. coli* isolates using this approach, leveraging phages from two distinct viral families. Ongoing studies are evaluating the efficacy of ImmunoPhage in a mouse UTI model.

In summary, ImmunoPhages combine the antibacterial effect of phages with targeted immunotherapy and offer a customizable, antibiotic-free strategy for persistent and chronic infections.

M-21 40 years of forest tree diagnostics and advisory service – interesting emergence patterns for pine foliage diseases

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Swiss Forest Protection (WSS) was established as a diagnostic and advisory service in 1983 as part of the initiatives addressing the « Waldsterben » problem. Despite several reorganizations, the service continued to operate, and diagnostic results were stored in a database comprising almost twenty thousand records. This exceptional time series was analyzed to investigate changes in the pine foliage pathology landscape over time. Pine trees are distributed globally and are essential ecological as well as economic contributors to forests. However, they are susceptible to numerous pathogens that reduce their growth rates or even threaten their survival.

Out of more than 1000 pine records, this study addresses the initial occurrence and evolution in abundance of various pine pathogens. We will first retrace the emergence and introduction of *Diplodia* tip blight of pines *(Diplodia sapinea),* along with the regulated pine diseases *Dothistroma* and *Lecanosticta* needle blight in Switzerland. Furthermore, we will discuss both the emergence of new pine pathogens and the decrease in inquiries regarding common pine pathogens, such as *Lophodermium* and *Gremmeniella*. Lastly, we will focus on a few rare pine diseases and their occurrences. Our study highlights the value of long-term collections and databases for a better understanding of epidemiological processes.

S-60* Identifying biocontrol traits and boosting them through amino acid adjuvants

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Chemical pesticides and copper-based products are used to protect plants in current agricultural practices. However, these substances have a negative impact on the environment and also on human health. Therefore, solutions with less negative impact are being studied. One of these is biocontrol, the use of living organisms to fight plant pathogens. *Pseudomonas, Bacillus* and *Streptomyces* strains are known for their high biocontrol potential. However, while their efficacy is well documented under laboratory conditions, their performance in the field remains limited. The objective of this study was to investigate whether the addition of specific amino acids could enhance the biocontrol activity of four bacterial strains belonging to genera *Pseudomonas, Bacillus* and *Streptomyces* against *Phytophthora infestans*, the causal agent of late blight in *Solanaceae* plants. The focus was on the improvement of biocontrol-related traits and siderophore production, rather than solely on bacterial growth. All twenty proteinogenic L-amino acids were tested *in vitro* by measuring the mycelial growth of *P. infestans* in the presence of biocontrol agents and individual amino acids. Each amino acid exhibited a different influence on biocontrol efficacy. Some, such as arginine and lysine, led to a significant reduction in mycelial growth of the pathogen and also enhanced siderophore production in the studied *Pseudomonas* and *Streptomyces* strains. Building on these results, future work will extend this approach to other developmental stages of the pathogen, particularly focusing on spore release and germination. In addition, metabolomic analyses will be conducted to identify the molecules involved in the increased biocontrol activity. These findings suggest that the use of specific adjuvants can improve biocontrol performance of beneficial bacteria, offering a promising strategy to enhance the effectiveness of biological control in field applications.

S-61 Genetic variation among progeny shapes symbiosis in a basidiomycete with poplar

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Forest trees rely on ectomycorrhizal (ECM) fungi for acquiring scarce resources such as water and nutrients. However, the molecular mechanisms governing ECM traits remain inadequately understood, particularly the role of intraspecific fungal variation in root-tip colonisation and trophic interactions. This study examined six ECM traits using Pisolithus microcarpus, an ECM fungus capable of forming ECM rootlets in poplar. A collection of 40 sibling monokaryons and their parental dikaryon was analysed through genome and transcriptome sequencing to examine quantitative trait loci, gene expression, and mating-type loci. These findings revealed a pronounced phenotypic continuum in poplar root colonisation by sibling monokaryons, ranging from incompatible to fully compatible strains. Genetic recombination among monokaryons was demonstrated, and genomic regions potentially involved in ECM-fungal traits were identified. Transcriptomic analysis revealed greater differentiation of transcriptomic profiles between fungal strains than between fungal tissues, and uncovered tissue-specific functional responses for ECM and free-living mycelia. Poplar exhibited distinct transcriptomic responses when interacting with different sibling monokaryons and the parental dikaryon. Allele sorting at 11 mating-type loci confirmed the species' heterothallic tetrapolar system. This study advances understanding of the genetic and transcriptomic mechanisms underlying ECM symbioses, highlighting intraspecific fungal diversity's role in forest ecosystem functioning.

S-62 Agroforestry through the eye of the Stametsian model – The co-culture of fruit trees and morel mushrooms

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Agriculture undergoes a rising pressure such as biological impoverishment of soils and climate change. Old farming methods like agroforestry could mitigate the impact of these problems in a sustainable approach. New methods like trees-fungi co-culture could contribute to promote the growth of resilient trees for future farming systems. Indeed, mycorrhized plants have been shown to better overcome meteorological extremes (Roberti et al., 2025). Likewise, fungal inoculated soils tend to better retain water and nutrients (Stamets, 2000). In our case the interaction between fruit trees and morel is investigated. A mycorrhiza-like interaction is expected to happen between the two organisms.

S-63 Electrical Signalling in Fungal Mycelia: Past, Present, and Future

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Electrical signalling is a fundamental mechanism for integrating environmental stimuli and coordinating biological responses across living systems. While extensively characterized in animals and plants, its role in fungi has remained relatively elusive. Early investigations revealed action potential-like signals and localized electrical currents in filamentous fungi, hinting at a potential communication system within and across hyphal networks. Recent advances have reignited interest in fungal electrophysiology, driven by hypotheses surrounding mycorrhizal-mediated plant communication and the development of fungal-based electronic materials.

To advance in the understanding of the function of electrical signalling, we present a novel methodological framework using printed circuit boards with embedded differential electrodes, combined with Faraday shielding and frequency-domain analysis, to reliably detect extracellular voltage fluctuations in mycelial networks. These signals, shown to correlate with fungal growth and respond to biocidal treatments, suggest a biological origin and a role in environmental adaptation.

With this presentation, we aim to establish a foundation for standardized, reproducible research in fungal electrical signalling. We highlight key challenges and opportunities for advancing our understanding of fungal communication, with implications for ecology, bioelectronics, and systems biology.

M-22 Ex vivo modelling of tissue resident antimicrobial responses in precision cut lung slices

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Abstract: Tissue resident host responses to microbial infections in the respiratory tract rely on the interaction of a multitude of cell types. Modelling the complexitiy of these responses in tissue culture is challenging. Precision cut lung slices (PCLS) are volume defined organ discs maintaining the cellular complexity and 3D architecture of lung tissue and are arguably the most realistic cell culture model of the respiratory tract. We compare here the transcriptional antimicrobial response after influenza A virus (IAV) or S. pneumoniae (Spn) challenge in human (hPCLS) and murine PCLS (mPCLS) ex vivo with that of an in vivo infection. Notably, antiviral responses are clearly distinglishable from antibacterial responses in this ex vivo model. Our data further reveal an expected reduced response in mPCLS from naïve animals, notably a complete absence of type II interferon. These tissues lack resident lymphoid T memory effector cells and cannot recruit immune cells from the blood. Surprisingly, mPCLS from immune experienced animals do not respond more pronounced to IAV challenge, while still controlling IAV replication better. In contrast, hPCLS provide a broad antiviral response after IAV challenge, including type I, II and III interferons and proinflammatory cytokines (notably IL-1b). Blocking these cytokines reduces in tissue communication, suggesting a functional cross-talk. Our data suggest that hPCLS are a sophisticated model to study early antiviral host responses relevant for human lung pathology.

M-23 Decoding the mechanisms of virus infection using advanced human models

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The ongoing global outbreak of monkeypox virus (MPXV), the causative agent of mpox and driven by clades IIb and Ib, has been linked not only to characteristic mucocutaneous symptoms but also to severe complications such as encephalitis and adverse pregnancy outcomes. To investigate MPXV's impact on vulnerable human tissues, we employed two complementary human-based experimental models targeting the placenta and brain. First, we used an ex vivo human placental explant model to assess the potential for vertical transmission. Placental biopsies exposed to clade IIb MPXV showed selective infection of trophoblast cells, including the syncytiotrophoblast and cytotrophoblast layers, while stromal components remained unaffected. Infection led to disruption of the syncytiotrophoblast barrier, altered epithelial organization, and a reliance on direct cell-to-cell spread. Transcriptomic analysis confirmed remodeling of the placental microenvironment, upregulation of growth factor signaling and structural genes, limited inflammation, and increased expression of MEG3, a gene associated with placental dysfunction. These findings suggest MPXV can compromise placental integrity and pose a risk for congenital transmission. Second, we applied a human neural organoid model to study MPXV's neurotropic properties. Clade IIb MPXV efficiently infected neural progenitors, neurons, and astrocytes, forming intracellular viral factories and spreading via cell-to-cell contact, confirmed using a recombinant fluorescent reporter virus. Infected neurites exhibited bead-like swellings, features of neurodegeneration, preceding cell death, as visualized through live-cell imaging. Host response analysis revealed signatures of disrupted neuronal homeostasis and neurodegenerative pathways, with limited antiviral responses. Notably, treatment with tecovirimat significantly reduced viral replication in neural organoids. Together, these human-based models demonstrate MPXV's capacity to infect and damage placental and neural tissues, providing mechanistic insights into congenital complications and mpox-associated encephalitis. This dual-tissue approach underscores the value of human models in studying emerging viral threats and supports their use in evaluating targeted antiviral interventions.

S-64

Establishing a phage collection as a first step towards a new strategy for the management of non-typhoidal Salmonellosis (NTS).

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Acute non-typhoidal salmonellosis is the second most common cause of gastroenteritis in Switzerland. Antibiotics are not recommended due to limited efficacy and the perturbation they cause to the gut microbiota, a natural barrier to the infection. Thus, untreated carriers represent an important reservoir for transmission to vulnerable people, in whom the infection can become life-threatening. Using bacteriophages, specific bacterial viruses, is proposed as a decolonization strategy for carriers. A collection of Salmonella clinical isolates was assembled in collaboration with NENT, UZH. Bacteriophages were isolated from local wastewaters. Their host ranges were characterized through efficiency of plating (EOP) assays. *In silico* analyses of the phage genomes allowed evaluation of their suitability for clinical intervention. We retrieved eight genetically distinct lytic phages. None carried genes coding for known virulence or antimicrobial resistance determinants. The collection efficiently infected and lysed 99% of 104 *Salmonella* clinical isolates from the six most dominant serovars (Enteritidis, Typhimurium, Monophasic Typhimurium, Napoli, Infantis, and Derby). However, *S.* Infantis and *S*. Derby displayed high resistance to individual phages, suggesting presence of specific and highly effective anti-phage mechanisms in these serovars. First phage training experiments suggested possibility to increase virulence of some phages. Genetically different Salmonella phages with clinical potential were isolated from Swiss wastewaters. These phages cover a broad spectrum of dominant serovars, *S.* Infantis and *S*. Derby being the exceptions. Next steps towards clinical application are i) to evaluate their *in vitro* efficacy, ii) to produce few promising candidates through the Swissmedic authorized CHUV phage manufacturing pipeline and to iii) evaluate their efficacy *in vivo* in a preclinical model of *Salmonella* infection.

S-65 Impact of viral morphology on infectivity of influenza A virus in airborne transmission

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Airborne transmission of respiratory viruses, including influenza A virus (IAV), poses significant challenges to public health. However, the mechanisms governing viral inactivation in aerosols remain poorly understood. IAVs exhibit morphological variability, ranging from 100 nm spherical virions to micron-long filaments, depending on growth conditions. While laboratory-passaged strains typically produce uniform spherical particles, clinical isolates often generate pleomorphic populations. Although virion morphology is believed to influence viral transmissibility, the mechanisms of how viral morphology affects the airborne transmission of IAV are yet to be delineated. In this study, we generated spherical and filament-producing IAVs that are genetically similar but differ in morphology to investigate the impact of virion shape on aerosol stability and infectivity in human airway epithelium. We combined the comparison of the stability of these morphological variants in both bulk solutions and aerosol systems to examine how physicochemical aerosol properties, such as elevated solute concentration and acidic pH, affect airborne viral survival. Both variants exhibited similar stability in effloresced saline aerosols at 25% relative humidity (RH); however, filament-producing viruses showed enhanced stability in aqueous saline aerosols at 85% RH. Their inactivation patterns at high RH were consistent with results from 26× PBS solution, which mimics the water activity of equilibrium aerosols. In contrast, filament-producing viruses exhibited accelerated decay in acidic conditions, both in pH 5 solution and in acidified aerosols. These results suggest that pleomorphic IAV isolates, with functionally versatile viral populations, may enhance airborne stability under diverse environmental conditions. Additionally, filamentous IAVs confer an infectivity advantage under mucosal immune pressures, including neutralizing IgA and IgG antibodies, and mucus inhibition in primary human airway epithelial cultures. Overall, our study reveals that the pleomorphic nature of IAV is a strategic adaptation that enhances viral fitness during airborne transmission by increasing stability and infectivity under diverse extracellular pressures

M-24 Update on Trichophyton indotineae

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This update underscores the growing clinical relevance of *Trichophyton indotineae* and highlights the need for enhanced diagnostic capacity and awareness of antifungal resistance in dermatophytes.
Trichophyton mentagrophytes genotype VIII, now designated *T. indotineae,* has emerged over the past decade as a significant global health concern. Originally identified as the predominant cause of dermatophytosis in India, *T. indotineae* has since been reported in numerous countries, including several cases in Switzerland. The organism presents unique diagnostic and therapeutic challenges.
Clinically, *T. indotineae* causes widespread tinea corporis, cruris, and occasionally capitis or genital infections. Lesions are often extensive, dry, and minimally inflammatory, with central clearing frequently absent. The species shows a high rate of terbinafine resistance and occasional reduced susceptibility to itraconazole, limiting the efficacy of standard first-line treatments. Relapses are common and may occur despite apparent clinical resolution.
Diagnosis is complicated by the limited specificity of commercial PCR assays, which often report the broader *T. interdigitale/ mentagrophytes* complex. Reliable species identification and resistance profiling require ITS sequencing and antifungal susceptibility testing – techniques currently available only in specialized laboratories.
A retrospective analysis from Zurich (2014–2024) identified 33 confirmed cases of *T. indotineae,* with a significant proportion involving patients with travel links to South Asia. Published treatment data support the use of oral terbinafine (2 × 250 mg) or itraconazole (2 × 100 mg) for a minimum of 6–8 weeks, often longer, combined with topical antifungals. Despite high recurrence rates, re-treatment with initially effective agents is usually successful.

M-25 **Neoehrlichia mikurensis**

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M-26 Artificial intelligence in microbiology

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Artificial Intelligence (AI) is transforming medical microbiology by accelerating data interpretation, enhancing diagnostic precision, and enabling new forms of digital decision support. This lecture will present an overview of how AI tools, especially large language models (LLMs) and machine learning (ML) algorithms, are being implemented across the diagnostic workflow - from pre-analytical triage and culture plate image analysis to interpretation of resistance patterns and genomic data.

Prospective studies at our institute have evaluated the performance of multiple LLMs, including ChatGPT, GPT-4, and domain-specific models (e.g., medical pre-trained Llama2), for their ability to support diagnostic stewardship. Results show that while generalized models perform well, domain-specific training and continuous benchmarking remain critical for clinical use. In antimicrobial susceptibility testing (AST), computer vision-based systems are now capable of interpreting inhibition zones and reading culture plates with accuracy nearing expert-level performance. However, full automation still requires human oversight due to regulatory, quality, and ethical considerations.

The talk will also address key challenges: fragmented datasets, lack of interoperability, commercial dependencies, and the underrepresentation of low- and middle-income countries in AI training data - all of which contribute to biased outputs. Furthermore, the vast computational and energy demands of modern AI systems raise environmental and sustainability concerns.

Microbiology laboratories, with their high degree of standardization and ISO-regulated frameworks, offer a robust environment to develop and validate AI applications. Nonetheless, successful integration requires a multidisciplinary approach involving microbiologists, data scientists, clinicians, and policymakers. Education and continuous critical evaluation will be essential as we move toward AI-augmented laboratory medicine.

The future of microbiology will not be defined by data volume alone, but by our ability to translate AI-generated insights into meaningful clinical action: responsibly, equitably, and with scientific rigor. For this sufficient funding of diagnostic labs are necessary.

S-66*/P-82* batch effects and data representation in maldi-tof ms data for machine learning driven identification of microorganisms

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Introduction

MALDI-TOF MS is a cornerstone of rapid microbial identification. Machine learning (ML) models applied to MS data promise to improve specificity, antimicrobial resistance (AMR) and virulence prediction, which potentially reduces turnaround times and optimized treatments. However, these ML models struggle with generalizability, as their performance declines when applied on different machines or over extended periods. Two factors contribute: (i) batch effects and (ii) inconsistent spectral quality. Considering that data representation enhances ML model effectiveness, optimizing data representation strategies may offer a needed solution.
We aimed to explore batch effects in MALDI-TOF MS data and evaluate current data representation strategies to improve model generalizability.

Methods

We analysed fifty clinical isolates of the closely related *Enterobacter cloacae* group on four different MALDI-TOF MS machines, with four technical replicates per machine and three different operators, generating 2400 spectra. Species identity and genetic diversity were confirmed using whole genome sequencing (WGS). Spectral differences across machines and replicates were assessed in relation to phylogenetic strain relationships, and spectral clustering was used to explore grouping patterns among spectra. We applied post-acquisition m/z calibration using genus-specific ribosomal protein markers to correct peak shifts and compared spectra.

Results

In the dataset, we had *E. hormaechei*, *E. ludwigii*, and *E. sichuanensis, E. kobei*, *E. cloacae* and *E. roggenkampii sensu stricto.* Preliminary results show that subspecies were more distinctly mapped in the t-SNE plot post-calibration. Spectra with fewer than 15 detected ribosomal markers mapped together despite being generated from different strains, operators, and machines; this pattern, visible before calibration, formed a distinct cluster after the calibration step.

Conclusion

Our results highlight the value of both noise reduction and the application of quality control. Low-quality spectra can affect reproducibility and lead to misidentification. Establishing exclusion criteria based on spectral quality is essential for reliable species identification and accurate downstream analyses.

S-67 Validation of digital slide scanning and artificial intelligence (AI) assisted interpretation for the detection of intestinal parasites in human stool specimens

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Background

Infections with intestinal parasites are relatively rare in low- or non-endemic regions and the testing volume is disproportionately high with >98% of negative microscopy results. Manual light microscopy is the gold standard for the detection of intestinal helminth and protozoan infections, although this method suffers from sensitivity, is time-consuming and highly reliant of the expertise and training of the microscopist. Thus, there is an urgent need for a standardised and traceable, high- or low- throughput and cost-effective diagnostic approach to replace the labor-intensive manual microscopic stool examination.

Methods

We performed a clinical validation of the Grundium Ocus 40 digital microscopy (DM) scanner combined with a CNN model for the detection of intestinal protozoa and helminth eggs/larvae in concentrated wet-mount stool samples compared to manual microscopy as the reference standard to screen human fecal wet mount slides for presence or absence of a set of parasites at slide level. The DM/CNN approach was validated on a reference sample collection (n=100) confirmed by manual microscopy and clinical samples (n=208) from patients with suspected infection with intestinal parasites that were submitted to our laboratory for diagnostic analysis.

Results

The DM/CNN approach showed similar positive and negative slide level agreement compared to conventional light microscopy. Dilution series of reference samples revealed slightly lower sensitivity for the DM/CNN system and the Grundium Ocus 40 digital scanner revealed problems finding the focal plane at higher dilution levels. Using the 208 clinical samples, the Cohens Kappa coefficient for agreement of DM/CNN and light microscopy was 0.899.

Conclusion

Our data indicate that the Grundium Ocus 40 combined with a CNN model for wet mount stool samples is reliable for routine diagnostics but needs further optimization for specific organisms. Confirmation of positive samples is required by either manual reviewing of images on a screen or by microscopic examination.

S-68*/P-73* nanomur: innovative nanomotion technology for antibiotic susceptibility testing - a game-changer for urinary tract infections?

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Background and study objective

Urinary tract infections (UTIs) are very prevalent and can be life-threatening in case of complication like sepsis. Currently, urine antibiotic susceptibility testing (AST) exceeds 30 hours, mainly due to culture, potentially leading to suboptimal antibiotic treatment. This complicates UTI management, especially amid rising antibiotic resistance.
The NANOMUR study aims to reduce the time-to-results for urine AST through innovative “nanomotion” technology and machine learning, bypassing the need for culture.

Methods

Turbid urine samples undergo the routine diagnostic workflow (gold standard) and nanomotion devices, testing for ceftriaxone and ciprofloxacin. Performance is evaluated using a confusion matrix. Time-to-results for both methods are also compared.

Results

204 experiments were conducted this far, 172 eligible after data cleaning. Nanomotion performance was calculated for monomicrobial samples (81 experiments). The accuracy was 82.72% (67/81), sensitivity was 85.92% (61/71), and specificity was 60% (6/10). Compared to monomicrobial samples, polymicrobial samples including one Gram-negative and one Gram-positive bacteria (55 experiments) showed no significant difference in nanomotion performance.
Nanomotion’s mean time-to-results was 4 hours 24 minutes, significantly lower ($p < 0.0001$) than the routine mean for monomicrobial or polymicrobial samples – respectively 37 hours 18 minutes and 56 hours 18 minutes.

Conclusion and perspectives

Nanomotion exhibited good accuracy and sensitivity but lacked specificity. However, the significant time gain of nanomotion could be a game-changer in patient management. The next phase of the study will include experiments at 37°C, allowing us to decrease the time-to-results even further and to assess if performance can be improved that way.

S-69*/P-77* A journey to antimicrobial discovery: roadblocks and interesting side quests

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The remarkable outer membrane of Gram-negative bacteria presents a significant barrier to antibiotic action. To overcome this, we aimed to develop antimicrobial nanobodies targeting the essential outer membrane protein BamA. Exploiting the β-barrel assembly machinery's crucial role in bacterial survival, we immunized alpacas to raise BamA-specific nanobodies. Using NestLink technology, we identified a small panel of high-affinity cell-surface binders. However, these initial nanobodies lacked antibacterial activity.

To elicit inhibitory nanobodies targeting a vulnerable BamA conformation, a subsequent immunization campaign yielded a next generation of high-affinity binders, again devoid of direct antibacterial activity. Intriguingly, one candidate synergized with the sensitizer PMBN, affecting bacterial fitness. Further investigation revealed a novel, previously undescribed effect of PMBN, potentially indicating a vulnerability in the BamA target and offering insights into outer membrane dynamics. This unexpected finding provides a rational foundation for future antimicrobial development.

Concurrently, we are intensifying our search for direct antimicrobial nanobodies by implementing a functional assay within our screening pipeline. This dual approach – understanding target vulnerabilities and directly screening for activity – represents our ongoing strategy to circumvent the outer membrane barrier and discover novel antibacterial agents.

M-27 Viral Control of Biosignatures in Anoxic Worlds

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Viruses are key players in microbial ecology, not only for their roles in host mortality but also for their capacity to reprogram host metabolism and reshape biogeochemical cycles. While the impacts of viruses that infect oceanic cyanobacteria (cyanophages) are well-documented, their influence on anoxygenic phototrophic bacteria in oxygen-depleted environments remains largely unexplored.

Our research program establishes a novel workflow to investigate phage control over biosignature-producing anoxygenic phototrophs in stratified, anoxic, and sulfidic aquatic systems, which serve as modern analogs of the early Earth's oceans. By integrating metagenomics, metatranscriptomics, and cross-infection mesocosm experiments, we assess how the presence of phages shapes microbial metabolism, sulfur cycling, and nutrient demand.

Across systems, we observed low viral transcription within dense microbial plates, indicative of lysogeny or suppressed replication. Nonetheless, viral genomes recovered from these environments encoded a wide range of auxiliary metabolic genes (AMGs) involved in sulfur oxidation, pigment biosynthesis, and photosynthesis, pointing to the potential for non-lytic metabolic reprogramming. In mesocosm experiments, the addition of phages reshaped sulfur redox dynamics and selectively altered the uptake of biologically active elements (e.g., Cu, P, Mg), while chemically inert elements remained unaffected.

Together, these findings suggest that phages can modulate microbial function and alter the chemical signatures of anoxygenic phototrophs. As this framework expands across diverse host-virus systems and environments, we aim to gain a deeper understanding of how viruses contribute to the formation and interpretation of microbial biosignatures. This research provides valuable insights for microbiology and astrobiology, enhancing our ability to interpret geochemical signals of past life on Earth and guiding the search for life in anoxic extraterrestrial settings.

S-70 Investigating the ecology and temporal dynamics of Microcoleus species in a Swiss river

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Toxic benthic cyanobacteria, particularly *Microcoleus* species, have been implicated in domestic animal fatalities, primarily affecting dogs, worldwide[1]. Research has focused on tools enabling toxin detection, genetics, or monitoring schemes for these toxic species[2-4]. However, the ecology of *Microcoleus* cyanobacteria remains underexplored. Therefore, this study aims to better understand the benthic microbial communities involving *Microcoleus*, potential interactions between species, and abiotic factors that might drive community structure. Over the course of a year, we collected benthic sediments from a Swiss river associated with canine intoxication. We extracted total environmental DNA and sequenced the microbial communities to identify dominant cyanobacteria. Additionally, we investigated potential anatoxin-a production using an Oscillatoriales-specific PCR targeting the *anaC* gene. Our results revealed that microbial communities appeared to be influenced more by site-specific factors rather than temporal changes. Bacterial communities were predominantly composed of cyanobacteria and proteobacteria. *Microcoleus* species were consistently present across all sites, showing significant correlations with other cyanobacteria, such as *Potamolinea*, and proteobacteria, such as *Rhodobacter*. Eukaryotic communities were mainly dominated by algae, with relatively high abundances of diatoms, rotifers, and nematodes. *Microcoleus* species also presented significant correlations with eukaryotic organisms, particularly *Pinnularia* diatoms. Certain abiotic factors, such as conductivity, sodium and calcium ions, followed similar seasonal trends as *Microcoleus* dynamics. Interestingly, no positive signal for *anaC* was detected in the benthic communities, suggesting the potential presence of both toxic and non-toxic species co-occurring. This research enhances our understanding of the ecology of *Microcoleus* species in lotic systems. However, further studies are necessary to improve species identification resolution, distinguishing between toxic and non-toxic *Microcoleus* species, to gain a deeper understanding of these cyanobacteria.

S-71* Anthropogenic Impact on AMR Dynamics in Switzerland's Aare River

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The global spread of antimicrobial resistance (AMR) is a serious public health concern, driven by widespread antibiotic use and the environmental release of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). While wastewater treatment plants (WWTPs) are important sources of anthropogenic AMR input, large rivers, which serve as vital water resources, contribute to downstream dissemination. However, the drivers and dynamics of AMR propagation along entire river systems remain poorly understood.

As Switzerland's longest and one of its largest rivers, the Aare plays an important role for the 'water castle of Europe'. This study examines the impact of WWTP discharges, particularly those receiving high loads of hospital effluent, on ARG distribution along the 288 km of the Aare river-continuum. Unlike previous studies, this study also included pristine upstream locations and moderately impacted environments.

Using quantitative PCR targeting 14 ARGs linked to resistance to eight clinically relevant antibiotic classes, we conducted a high-resolution spatial survey, complemented by 16S rRNA amplicon sequencing, to assess shifts in the riverine resistome and microbiome. The high spatial resolution revealed a progressive increase in ARG abundance along the river, driven by WWTP effluents enriched with ARGs. On average, effluents exhibited nearly 4'000-fold higher ARG concentrations compared to upstream levels, resulting in downstream ARG enrichments in the Aare River ranging from 0.4-fold to 141-fold. Additional ARG inputs from major tributaries such as the Reuss and Limmat further sustained elevated levels in the lower reaches. Interestingly, ARG levels decreased after sections where Aare passed through lakes.

This study establishes the first detailed baseline for ARG prevalence along a large river system from nearly untouched headwaters to pollution-affected lower reaches. Situated in the upper reaches of the Rhine watershed, these findings provide critical insights into AMR dynamics in aquatic environments and inform future monitoring and mitigation strategies.

S-72 Drivers of ecological success for Escherichia coli vary among human gut microbiome samples from different individuals

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Gut microbial community composition varies from one person to another. Potentially, this means the ecological interactions experienced by individual strains or species also vary among microbiomes of different people. However, testing this directly in human microbiomes and identifying associated ecological drivers is challenging. Here, we use replicated anaerobic microcosms to quantify variability of population growth for a key commensal species among microbiome samples from different individuals, and to identify underlying intra- and interspecific interactions. In a reciprocal transplant experiment, both absolute and relative growth perfomance of different *Escherichia coli* strains varied among gut microbiome samples from different healthy individuals. This was partly explained by intraspecific competition: ecological success of individual *E. coli* strains was associated with displacement of resident conspecifics. However, the determinants of *E. coli* growth varied among samples from different individuals. In one microbiome sample with a distinctive taxonomic composition, culture acidification by resident microbes impaired growth of all *E. coli* strains. These results suggest that inter-individual microbiome variation translates to variable ecological interactions with incoming bacteria, impacting the relative success of different strains and thereby helping to explain the structure and diversity of human microbiomes.

S-73 Distribution of Wood-Ljungdahl Pathway in global metagenomes

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The Wood-Ljungdahl Pathway (WLP) is an ancient anaerobic carbon fixation metabolism found in both archaea and bacteria. This pathway plays a critical role in energy conservation across diverse ecosystems and is closely linked to methanogenesis and acetogenesis. However, we have a limited grasp on the diversity and environmental distribution of microorganisms that use the WLP and the role that extrachromosomal elements play in augmenting this metabolism. In this study, we leveraged an extensive dataset of 120,765 metagenomes spanning diverse biomes to identify genes associated with the WLP, methanogenesis, and acetogenesis in microbial genomes and extrachromosomal elements. Combining sequence and structural homology searching, we detected genes associated with these pathways in 151,318 MAGs that encoded either a partial (50% of the pathway + CODH/ACS gene) or complete WLP. These MAGs capture a broad taxonomic diversity, covering 81 phyla and 211 classes. Additionally, we detected circular extrachromosomal elements harboring WLP genes across biomes, including marine and freshwater systems, groundwater, hot springs, permafrost, and the human gut. This study presents a comprehensive global atlas of microbial genomes and extrachromosomal elements encoding WLP, acetogenesis and methanogenesis.

M-28 The cellular function of the Penicillin Binding Protein 1b (PBP1b)

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A multi-protein system called the divisome promotes bacterial division. This apparatus synthesizes the peptidoglycan (PG) cell wall layer that forms the daughter cell poles and protects them from osmotic lysis. In the model Gram-negative bacterium *Escherichia* coli, PG synthases called class A penicillin-binding proteins (aPBPs) have been proposed to play crucial roles in division. However, there is limited experimental support for aPBPs playing a specialized role in division that is distinct from their general function in the expansion and fortification of the PG matrix. Here, we present *in situ* cryogenic electron tomography data indicating that the aPBP-type enzyme PBP1b is required to produce a wedge-like density of PG at the division site. Furthermore, atomic force and live cell microscopy showed that loss of this structure weakens the division site and renders it susceptible to lysis. Surprisingly, we found that the lipoprotein activator LpoB needed to promote the general function of PBP1b was not required for normal division site architecture or its integrity. Additionally, we show that of the two PBP1b isoforms produced in cells, it is the one with an extended cytoplasmic N-terminus that functions in division, likely via recruitment by the FtsA component of the divisome. Altogether, our results demonstrate that PBP1b plays a specialized, LpoB-independent role in *E. coli* cell division involving the biogenesis of a PG structure that prevents osmotic rupture. The conservation of aPBPs with extended cytoplasmic N-termini suggests that other Gram-negative bacteria may use similar mechanisms to reinforce their division site.

S-74* **Mycobacterium tuberculosis leucine aminopeptidase (Rv2213) activates a promising anti-TB hit compound**

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Tuberculosis (TB) remains the top infectious disease killer worldwide. The rise of drug-resistant TB strains challenges global control efforts and highlights the urgent need for novel therapeutics with distinct mechanisms of action. Motivated by this therapeutic gap, we are investigating the mode of action of a novel anti-TB compound, O1, identified from a Roche chemical library. O1 selectively inhibits MTB complex growth at low micromolar levels without human cell toxicity. Resistance mapping revealed mutations in a non-essential gene, Rv2213, indicating that Rv2213 activates O1 as a prodrug. Deletion and complementation of the Rv2213 homologue in *M. bovis* BCG confirmed its role in susceptibility. Biochemical assays characterised Rv2213 as an M17 leucine aminopeptidase hydrolysing N-terminal amino acids. We resolved the hexameric structure of Rv2213 at 2.2 Å by cryo-electron microscopy, providing a structural basis to rationalise O1-resistance-associated mutations, particularly those distant from the active or cofactor-binding sites.

Using untargeted LC-MS, we identified a dominant enzymatic product from O1 hydrolysis. Ongoing real-time NMR studies aim to characterise the product structure and kinetics of this reaction. In parallel, we are resolving the Rv2213-O1 complex to define the enzyme-compound interaction. To uncover the final essential target of the O1-derived product, we introduced a multi-copy vector expressing the Rv2213 homologue in BCG and selected for O1-resistant mutants under high compound pressure. This approach aims to isolate mutations in the essential target of the O1-derived active product, which are typically rare due to essentiality.

This work uncovers a novel enzymatic activation pathway of a promising anti-TB compound, offering mechanistic and structural insights that inform rational drug design. These findings advance the development of next-generation therapeutics against multidrug-resistant TB and contribute to broader efforts in combating antimicrobial resistance.

S-75*/ P-83* The role of ribosomal hibernation factors for Legionella pneumophila starvation, virulence, and intracellular persistence

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Ribosomal hibernation downregulates energy-intensive translational activity in many bacteria upon encounteringadverse conditions. However, little is known about this process in *Legionella* pneumophila, an amoeba-resistantbacterium found in technical and natural water systems. *L. pneumophila* replicates in amoebae and also infectshuman alveolar macrophages, thus causing a severepneumonia termed Legionnaires' disease.
L. pneumophila possesses two different putative hibernation promoting factors (HPFs), which likely compete for the same ribosomal binding site: a homolog of the "long-HPF", which promotes the formation ofinactive 100S dimers, and a homolog of RaiA, whichinactivates ribosomes as 70S monomers. We found that *L. pneumophila* missing the RaiA homolog indeed forms more inactivated 100S dimers. Furthermore, we identified a homolog of the ribosomal silencing factor RsfS, which binds the 30S subunit and thus inhibitsribosome assembly, as well as the conserved GTPase HflX, which rescues stalled 70S ribosomes and putatively splits 100S dimers and inactivated 70S monomers.
Ribosomal hibernation factors contribute to long-term starvation survival of *L. pneumophila*, and both their loss and overexpression significantly prolong the lag time during regrowth in rich media. Moreover, all *L. pneumophila* strains lacking one or more ribosomal hibernation factors are impaired for virulence, and the overexpression of HPFs intensifies the virulence phenotype immensely. All mutants are taken up less efficiently by their natural host *A. castellanii*, in agreement with an initial delay and/or reduction in intracellular replication.
The mutants also form fewer persisters (non-growers) during the infection cycle, indicating that ribosomal hibernation regulates intracellular phenotypic heterogeneity. Interestingly, *L. pneumophila* strains lacking individual ribosomal hibernation factors tend to form less motile, *flaA*-expressing bacteria at the late stages of infection, and conversely, the loss of both HPFs significantly increases the emergence of the transmissive, flagellated sub-population. Taken together, the ribosomal hibernation factors of *L. pneumophila*regulate starvation, virulence, and persistence of the pathogen.

S-76 Targeting the outer membrane of Gram-negative bacteria to potentiate the effect of antimicrobial peptides

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Antimicrobial peptides (AMPs) are promising alternatives to antibiotics, but their clinical application is often hindered by suboptimal pharmacokinetics properties and toxicity. We recently observed that *Escherichia coli* mutants lacking outer membrane (OM) biogenesis genes are hypersensitive to AMPs. OM is an asymmetric membrane containing lipopolysaccharides (LPS) at its surface and in which outer membrane proteins (OMPs) are inserted by the BAM protein complex. Essential proteins from the BAM complex or involved in LPS biogenesis are promising drug targets. We thus hypothesized that combining AMPs with OM biogenesis inhibitors (OMBIs) may potentiate their activity.

We both used already described OMBIs and performed in silico predictions to identify novel OMBIs targeting non-essential components of the BAM complex. Candidate molecules were selected according to the predicted affinity to their target, to their size and solubility. OMBIs were tested in combination with a selection of AMPs in checkerboard and time-kill assays on both laboratory strains and clinical isolates of *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Several OMBIs, including three in silico predicted molecules, showed synergism with some AMPs. Toxicity of the most efficient combinations was assessed, and their in vivo efficiency was tested in a *Galleria mellonella* infection model.

Further research is now needed to improve newly described OMBIs efficacy, solubility and pharmacokinetics properties. Modifications of these molecules may lead to the development of efficient potentiators of AMP activity, allowing to decrease the concentrations of AMPs needed to treat bacterial infections.

S-77 Dual RNA-seq identifies druggable factors supporting Streptococcus pneumoniae fitness during influenza virus superinfection

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Secondary bacterial infections following influenza A virus (IAV) infection, particularly with *Streptococcus pneumoniae*, remain a major clinical challenge. They have been and persist as a cause of morbidity and mortality during influenza pandemics and seasonal epidemics. The bacterial adaptation to the IAV-altered lung environment is still poorly understood. To investigate this, we performed *in vivo* dual RNA sequencing in a mouse model of IAV–*S. pneumoniae* superinfection, analyzing transcriptional changes in both host and pathogen. Across two different *S. pneumoniae* strains and time points post-IAV infection, we identified a conserved bacterial gene signature, including genes involved in maltose metabolism, iron acquisition, competence, and notably, genes encoding the alcohol dehydrogenases AdhE and AdhA. Their upregulation was consistently observed early after infection, suggesting a rapid bacterial response to the primed environment. While individual knockouts for *adhE* or *adhA* did not affect bacterial fitness during superinfection, a double-knockout strain lacking both genes showed a strong fitness defect in IAV-primed mice, but not in mice infected with *S. pneumoniae* alone. These findings indicate that *adhE* and *adhA* function redundantly and are specifically required for adaptation to the IAV-modified lung. To test the translational relevance of this pathway, we treated mice with fomepizole, a FDA approved broadly active alcohol dehydrogenase inhibitor. Fomepizole significantly reduced *S. pneumoniae* lung burden in superinfected, but not singly infected, mice. Altogether, our results identify *adhE* and *adhA* as critical for *S. pneumoniae* adaptation during superinfection and highlight alcohol dehydrogenase inhibition as a potential therapeutic strategy. Ongoing work aims to define the host-derived signals inducing *adhE/adhA* expression and to elucidate how these enzymes support bacterial fitness in the primed lung.

M-29 Dissecting the genetic machinery of aryl hydrocarbon receptor (AhR) agonist production in the skin commensal yeast Malassezia

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The lipophilic yeast *Malassezia* is by far the most abundant member of the skin mycobiome, representing over 90% of all commensal skin fungi. While being a commensal fungus, *Malassezia* has also been associated with some skin disorders like seborrheic dermatitis (SD), atopic dermatitis (AD) and pityriasis versicolor (PV). Recent studies also report an involvement of *Malassezia* species in extracutaneous diseases such as Crohn's disease. Our understanding of the interaction between *Malassezia* and the host remains incomplete, both in the healthy and in the diseased skin. Previous studies found that *Malassezia furfur* converts tryptophan into brown-pigmented indoles that activate aryl hydrocarbon receptor (AhR) signaling in human keratinocytes. To elucidate the biochemical pathway responsible for AhR ligand production in *M. furfur,* we used a combination of random and targeted mutagenesis coupled with transcriptomics and metabolomics. We developed a genetic screen that allowed the isolation of mutants impaired in the production of the characteristic brown indoles in media supplemented with tryptophan as the sole nitrogen source, and defective AhR activation in human keratinocytes. The availability of M. furfur mutants and ex vivo and in vivo models allows to establish the mechanism by which *Malassezia* AhR agonists affect the antifungal response, with implications for therapeutical approaches targeting the tryptophan metabolic pathway in the fungus or AhR signaling in the host.

S-78 Interplay Between Malassezia spp. and Candida auris in Skin Colonization

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Background
The skin's mycobiome, predominantly composed of *Malassezia* species, plays a pivotal role in maintaining microbial homeostasis. Skin colonization by *C. auris*, an emerging global health concern, is a critical risk factor for nosocomial invasive infection caused by this fungus. Recent studies have suggested that the presence of the commensal yeast *Malassezia* may facilitate *C. auris* skin colonization. Here, we aim at establishing the mechanisms underlying the interspecies interaction contributing to dysbiosis.

Methods
The crosstalk between *Malassezia* spp. and *C. auris* was tested using mono- and co-colonization models on HaCaT human keratinocytes and murine ear skin. Fungal adhesion, cytokine secretion, and cytotoxicity were assessed *in vitro*. *In vivo*, skin fungal loads, tissue inflammation, cellular infiltrates, and cytokine gene expression were quantified.

Results
Exposure of keratinocytes with both fungi individually or together confirmed the limited capacity of *C. auris* to induce a host response, and uncovered that *C. auris* suppresses inflammation induced by another stimulus. As such, *C. auris* curbed the *Malassezia*-induced IL-6 secretion from keratinocytes, while modulating *Malassezia*'s adhesion to the host. In turn, *Malassezia* spp. and its soluble factors mitigated cytotoxicity caused by *C. auris* in keratinocytes. *In vivo*, co-colonization led to a significant increase in *C. auris* fungal loads compared to mono-colonization, while *M. sympodialis* colonization did not change by the presence of *C. auris*. Reminiscent of what we observed *in vitro*, tissue inflammation, neutrophil recruitment, and IL-1β induction was reduced in co-colonization conditions compared to *M. sympodialis* mono-colonization.

Conclusion
Our findings suggest that the mutual interactions between both fungi creates an environment that enables stable colonization of *C. auris* in the skin, opening new options for targeting the skin mycobiome as a strategy to prevent *C. auris* colonization and subsequent invasive infection. Further research is needed to elucidate the mechanisms underlying the inter-fungal skin interactions.

S-79 Fungal highways as drivers of microbial diversity in soils

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In complex and heterogeneous environments, the hyphae of filamentous fungi and oomycetes are known to provide a support for the dispersal of other microorganisms. The use of these “fungal highways” is regulated by the interplay of both physical and biological factors. Therefore, the ability of different species to establish fungal highways can vary and must be confirmed experimentally. Several devices can be used to test this in specific pairings. However, these methods are usually time-consuming and cannot be applied at a large scale and in an intermediate throughput format. In this study, we used 3D printing to develop an experimental tool for the evaluation of bacterial dispersal on hyphal networks. Different materials and designs were tested to produce a “crossing bridge” that the fungal/oomycete-bacterial couples would use to move across two physically separated environments. The performance of various designs was compared using multiple species until the identification of an optimal topology. The final design allows the simultaneous testing of fungus/oomycete-bacteria couples and the inclusion of any culturing media. The comparison of a high and low-nutrient media showed that bacterial transport was more effective under low-nutrient conditions, even though fungal growth was slower. This study provides an easy-to-implement approach for evaluating the effective transport of bacteria by fungi and fungi-like hyphal networks.

S-80 Harnessing fungi and their interactions with bacteria and metals for unconventional mining applications

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Bacterial-fungal interactions (BFI) are essential for ecosystem functioning. In soil, these interactions promote and maintain the biogeochemical cycling of numerous elements. This involves processes which have direct influences on metal/element solubility. Collectively, they can be summarized as mobilization and immobilization processes whether they increase or decrease metal/element

solubility, respectively. Important ecosystem functions result from these processes in the context of BFIs, from enhanced nutrient bioavailability to plants or to the alleviation of the toxicity of harmful elements such as heavy metals. As a result, understanding BFIs in a biogeochemical context have also consequences on the use of bacteria and fungi in biotechnology, as these interactions affect the behaviour, physiology and ecology of both partners and in turn their influence on metal/element solubility.

In this presentation, we will focus on a concrete example using the field of biomining of geothermal fluids. In particular, we are considering industrial geothermal fluids that are obtained via drilling the subsurface and pumping hot fluids to the surface in order to obtain heat and/or transform heat into electricity. Recent research has demonstrated that these fluids contain a mixture of elements, including critical raw materials (CRM) at concentrations of economic interest. This is in particular the case of Lithium (Li). As Europe is currently dependent on other countries for the supply of this CRM, there is a timely need to find ways to exploit these resources. In addition, geothermal fluids also host microbial communities consisting of both prokaryotes and fungi. In this presentation, we will highlight the challenges related to the exploitation of the geothermal resources considering them into a biogeochemical perspective. We will also present a case-study consisting into the research path to implement a biomining approach by using combined fungal oxalogenesis and bacterial oxalotrophy. By harnessing the natural interactions that exist between microbes, along with their ability to act as chemical reactors, an ecological, economical, and ethical strategy for the biorecovery of metals could be developed for the field of biomining, with further applications for environmental bioremediation.

M-30 Resilience and protective immunity against African swine fever depends on host-environment interactions

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African swine fever (ASF) is a lethal hemorrhagic disease for domestic pigs and wild boars and the current epizootic is a major threat for animal health and the global pork industry. The development vaccine candidates against ASF virus (ASFV) is challenging since only live attenuated vaccine candidates show some efficacy and safety concerns remain due to insufficient attenuation and risks of recombination. To identify factors that regulate immune responses to ASFV leading to protective immunity against a virulent challenge, we investigated resilience and adaptive immune responses to infection with the moderately virulent ASFV field strain Estonia 2014 specific pathogen-free (SPF) pigs and in conventional farm pigs, which differ in their gut microbiome and their basal immune activation status.
SPF pigs were more resilient to intramuscular and oronasal infection with the ASFV Estonia 2014 strain compared to farm pigs, which showed increased fever and clinical signs. SPF and farm pigs that survived the infection with the Estonia 2014 infection were rechallenged with the virulent ASFV Armenia 2008 strain. SPF pigs were fully protected against disease and showed little or no viremia upon re-challenge. In contrast, farm pigs developed high viremia, high proinflammatory cytokine responses, severe clinical signs, and 40% reached humane endpoints. Our findings suggest that limited prior immune exposure to other pathogens and/or the microbiome composition of SPF pigs promotes resilience to infection with a moderately virulent ASFV strain promotes protective adaptive immune responses upon rechallenge.

M-31 The co-inhibitory receptor TIGIT promotes tissue protective functions in T cells

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The co-inhibitory receptor TIGIT suppresses excessive immune responses in autoimmune conditions while also restraining anti-tumor immunity. In viral infections, TIGIT alone does not affect viral control, but has been shown to limit tissue pathology. However, the underlying mechanisms are incompletely understood. We found TIGIT+ T cells to not only express an immunoregulatory, but also a tissue repair gene signature. Specifically, upon viral infection TIGIT directly drives expression of the tissue growth factor amphiregulin (Areg), which is strongly reduced in the absence of TIGIT. We identified regulatory T cells (Tregs), but not CD8+ T cells, as the critical T cell subset mediating these tissue-protective effects. In Tregs, TIGIT engagement upon TCR stimulation induces the transcription factor Blimp-1, which then promotes Areg production and tissue repair. Thus, we uncovered a novel, non-classical function of the co-inhibitory receptor TIGIT, wherein it not only limits immune pathology by suppressing the immune response, but also actively fosters tissue regeneration by inducing the tissue growth factor Areg in T cells.

M-32 SARS-CoV-2-specific mucosal immunity reduces infection susceptibility and virus shedding

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The nasal mucosa is the primary site of virus entry and shedding for SARS-CoV-2. An effective mucosal immunity has the potential to protect against infection or reduce viral shedding. Many mucosal vaccines against SARS-CoV-2 are under development, but there are no mucosal immune correlates of protection against infection and virus shedding to help guide vaccine development and benchmark mucosal vaccines.
Here, we investigate how pre-existing mucosal immunity influences susceptibility to SARS-CoV-2 infection and infectious virus shedding, by prospectively following 320 participants for 17 months (01/23-05/24). We repeatedly collected paired nasal and serum samples and determined viral load kinetics of PCR-confirmed SARS-CoV-2 infections. Additional infections were identified by significant increase between pre- and post-season SARS-CoV-2-specific mucosal and/or serum antibody levels.
Individuals that had PCR- or serologically confirmed SARS-CoV-2 infections had significantly lower pre-existing spike-specific secretory IgA levels, compared to individuals whose infection was detected by increase in mucosal antibodies or remained uninfected during the study. These differences were not observed for pre-existing spike-specific serum antibodies. Furthermore, elevated pre-existing binding and neutralising mucosal but not serum antibody levels were associated with reduced viral shedding. Finally, we show that SARS-CoV-2 infection elicits a broader neutralizing mucosal antibody response compared to serum.
Our findings indicate that mucosal antibodies can decrease the risk of SARS-CoV-2 infections and reduce virus shedding, highlighting the need to develop efficient mucosal vaccines.

POSTERFLASH & POSTERS
P-01 – P-09

PF-01*/P-01* A bacterial cell wall repair and modification system to resist host antibacterial factors

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Pathogenic bacteria have acquired the ability to resist antibacterial defense mechanisms of the host. Streptococci are common in animal microbiota and include opportunistic pathogens like Group A Streptococcus (GAS) and *Streptococcus pneumoniae* (pneumococcus). While the conserved streptococcal S protein has been identified as a key factor in GAS virulence, its exact function is unclear. Here, we show that the pneumococcal S protein is crucial for resisting against host-derived antimicrobials by coordinating cell wall modification and repair. Specifically, we show that S proteins are septally localized through their transmembrane domain and contain an extracellular peptidoglycan (PG) binding LysM domain which is required for its function. Protein-protein and genetic interaction studies demonstrate that the pneumococcal S protein directly interacts with a PG synthase, class A penicillin binding protein PBP1a, and the PG deacetylase PgdA. Single-molecule experiments reveal that the fraction of circumferentially moving PBP1a molecules is reduced in the absence of S protein. Consistent with an impaired PBP1a function, streptococci lacking S protein exhibit increased susceptibility to cell wall targeting antibiotics and altered cell morphologies. We show that pneumococci lacking the S protein cannot persist transient penicillin treatment, are more susceptible to the human antimicrobial peptide LL-37 and to lysozyme, and show decreased virulence in zebrafish and mice. Our data supports a model in which S proteins regulate PBP1a activity and play a key role in coordinating PG repair and modification. This cell wall 'sentinel' control system provides defense against host-derived and environmental antimicrobial attack.

PF-02*/P-02* **Direct bacterial headspace sampling for rapid online volatilomics analysis in medical microbiology diagnostics**

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Background: The ability to detect and identify bacterial pathogens from volatile emissions is an emerging approach for rapid diagnostics of bacterial and fungal pathogens. It can reduce the time-to-result for antibiotic susceptibility testing (AST) from 48h to just below a few hours. With the emergence of high-resolution analytical techniques like gas chromatography-mass spectrometer, ion mobility spectrometer, and secondary electrospray ionization spectrometer, quantitative analysis of the headspace biomarker concentrations has become available, and promising results were obtained for the identification of the bacteria and AST. However, MS-based devices are too complicated and expensive. To translate volatilomics into a powerful tool for rapid diagnostics in primary care, we need compact and affordable devices utilizing nanosensors to detect volatile organic compounds (VOCs). Sampling of the headspace volume plays a crucial role for reliable measurements.
Methods: We define the requirements of reproducible sampling for reliable volatilomic studies and design a headspace sampling system for microbiological analysis with chemoresistive sensors (e.g. selective for acetone) and mass spectrometry for comparative analyses. The sampling parameters were optimized and various clinical isolates were used to analyse the VOCs.
Result: We present our bacterial headspace sampling system for the detection of volatilome signatures and tracking of the dynamic volatilome profiles. This versatile system is tailored to accurately sample the headspace volume of the growth media while providing optimal incubation conditions for longitudinal monitoring. Key aspects of headspace sampling are discussed through preliminary *Escherichia coli* measurements, the focus being on the acetaldehyde concentrations as the main indicator of the growth phase. We demonstrate that different classes of VOCs (alcohols, ketones, aromatics,…) can be measured and bacteria proliferation can be monitored by measuring the headspace acetaldehyde concentration over the incubation period.
Conclusion: We demonstrate a proof-of-principle device to measure volatilome components, which eventually could be used for rapid detection of pathogens.

PF-03*/P-03* **Genetic variants of tetX linked to tetracycline susceptibility in Mycobacterium abscessus complex.**

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Mycobacterium abscessus complex (MABC) is a highly drug-resistant non-tuberculous mycobacterium and a leading cause of pulmonary infections in individuals with cystic fibrosis (CF). The intrinsic resistance of MABC to most antibiotics, coupled with the poor efficacy and severe side effects of current treatment regimens, presents a major clinical challenge. Current therapies often rely on third-generation tetracyclines like tigecycline, which—despite their in vitro activity—cause significant gastrointestinal toxicity, limiting their long-term use. In this context, the identification of strain-specific drug susceptibility profiles is essential for designing personalized treatment regimens that are both more tolerable and more effective.

Our study focuses on the role of naturally occurring polymorphisms in the *tetX* gene, which encodes a flavin-dependent monooxygenase responsible for inactivating first- and second-generation tetracyclines (e.g., tetracycline, doxycycline, minocycline). Whole-genome sequencing and drug susceptibility testing of Swiss MABC clinical isolates revealed distinct *tetX* alleles associated with differential susceptibility to tetracyclines. Specifically, SNPs in the codons causing aminoacidic substitution D454G and G455E were found to alter the functionality of TetX, leading to a loss of resistance in some strains. Notably, the D454G mutation was associated with a loss of resistance, resulting in increased susceptibility to tetracycline, doxycycline and minocycline, whereas the G455E mutation was linked to inducible resistance to tetracycline and minocycline, suggesting a functional but regulated TetX activity.

These findings were validated through heterologous expression, site-directed mutagenesis, and MIC profiling in isogenic mutants. Our data show that MABC resistance is not uniform and that *tetX* variants can predict susceptibility to better-tolerated oral tetracyclines.

Screening for *tetX* polymorphisms could enable personalized treatment with first- or second-generation tetracyclines, reducing reliance on toxic drugs like tigecycline. This approach offers a clear advantage in patient care by minimizing side effects. Integrating *tetX* genotyping into diagnostic workflows would support tailored, cost-effective therapy for MABC infections, particularly in CF patients.

PF-04*/P-04* **Role of the transcription factor Wor2 in biofilm formation of Candida auris**

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Candida auris is an emerging fungal pathogen with the ability to develop antifungal resistance and to cause nosocomial outbreaks. *C. auris* is able to adhere to abiotic surfaces and form biofilms, which contributes to interhuman transmission and virulence. In this study, we assessed the role of the zinc cluster transcription factor Wor2 (B9J08_002136) in biofilm formation of *C. auris*.
Two *WOR2* mutants were constructed in the wild-type strain IV.1 : *WOR2*^{HA} (hyperactivation of *WOR2*) and *wor2*Δ (deletion of *WOR2*). Phenotypic growth of the mutants was assessed, and their biofilm forming capacity was measured by crystal violet assays.
The downstream effectors of Wor2 were investigated by RNA sequencing, comparing *WOR2*^{HA} to IV.1. The function of these genes was further examined through their overexpression under the control of *ADH1* promoter at their native loci.
The mutants did not exhibit any growth defect. Compared to the IV.1 strain, *WOR2*^{HA} reduced biofilm formation while *wor2*Δ increased biofilm formation. Transcriptomic analyses revealed significant downregulation of *ALS4112* and *SCF1* (two important adhesins) in *WOR2HA.* Overexpression of *ALS4112* and *SCF1* in the IV.1 background showed increased biofilm production. In the *WOR2HA* background, overexpression of *SCF1* could restore biofilm capacity, which was not the case for *ALS4112* overexpression.
This study demonstrates for the first time that Wor2 acts as repressor of biofilm formation in *C. auris*, probably by down regulation of Als4112 and Scf1, with Scf1 being the main downstream effector.

PF-05*/P-05* Phage-derived endolysins as precision tools against vancomycin-resistant enterococci

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Antibiotic-resistant infections are on the rise, posing a major public health challenge. Gram-positive pathogens such as *Enterococcus faecalis* and *Enterococcus faecium* are of particular concern, especially in healthcare settings. As the efficacy of conventional antibiotics continues to decline and the pipeline for new drugs remains limited, alternative therapeutic approaches are urgently needed.

Endolysins, bacteriophage-derived peptidoglycan hydrolases, offer a targeted and efficient mechanism for bacterial lysis. These enzymes can induce rapid cell death, by cleaving conserved bonds within the peptidoglycan layer. Their ability to act exogenously on Gram-positive bacteria, which lack an outer membrane, makes them especially attractive for treating enterococcal infections.

To evaluate their potential, we conducted a systematic analysis of native and engineered endolysins targeting *Enterococcus spp*. The panel included enzymes with diverse enzymatically active domains (EADs) - glycosidases, amidases, and endopeptidases - each recognizing distinct peptidoglycan bonds. These catalytic domains are coupled with structurally diverse cell wall binding domains (CBDs), including α-helices, β-sheets, and α-β-sandwich architectures. Chimeric constructs were also generated through rational domain swapping, connected via flexible linkers to optimize activity and stability.

Our results demonstrate that endolysins exhibit potent bactericidal activity against *Enterococcus* spp., including vancomycin-resistant strains in clinically relevant environments. The modularity of their architecture enables precise engineering for enhanced specificity and function. Moreover, the conserved nature of their peptidoglycan target reduces the likelihood of resistance development.

Together, these findings underscore the therapeutic potential of endolysins as next-generation antimicrobials. Their structural diversity, robust killing efficacy, and low propensity for resistance position them as strong candidates for clinical development in combating multidrug-resistant infections.

PF-06*/P-06* Antimicrobial susceptibility testing of Capnocytophaga canimorsus: toward evidence-based treatment guidelines

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Introduction

Capnocytophaga canimorsus is a rare zoonotic pathogen transmitted through close contact with dogs and cats (typically bites or licks), capable of causing life-threatening infections in immunocompetent and -compromised individuals. Despite frequent empirical use of β-lactam antibiotics (often combined with β-lactamase inhibitors) as first-line therapy, no standardized antimicrobial susceptibility testing (AST) method or EUCAST clinical breakpoints exist for this pathogen. Our study aims to fill this gap by determining the minimal inhibitory concentration (MIC) distributions and epidemiological cutoff values (ECOFFs) for key antibiotics, paving the way for evidence-based guidelines and management of *C.* canimorsus infections.

Methods

AST was performed using agar dilution reference method, adapted with Heart Infusion Agar with 5% sheep blood (HIA-SB) to support the fastidious growth requirements of *C.* canimorsus. Each batch included quality control strains (*C. canimorsus* ATCC 35979, *E.* coli ATCC 25922, *S. aureus* ATCC 29213) and tested 15 antibiotic concentrations (0.016-128 mg/L) per agent (penicillin, amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam). To date, 115 isolates (clinical and animal-derived) from 26 centers across 12 different countries have been analyzed, with a target 250 isolates for robust MIC distributions. ECOFFs were estimated visually using EUCAST-recommended criteria.

Results

For penicillin and amoxicillin, we observed distinct wild-type (WT) and non-WT (resistant) populations, enabling preliminary visual estimation of ECOFFs (≤8mg/L). In contrast, amoxicillin-clavulanic acid showed less distinct separation, though MICs were consistently reduced 5-12 fold in 14 confirmed β-lactamase-producing isolates. Piperacillin-tazobactam demonstrated the most promising activity so far, with no apparent non-WT population among tested isolates, suggesting generally high susceptibility to this combination.

Conclusions and Outlook

Further testing is underway to validate these promising preliminary findings, including experiments using alternative EUCAST-recommended media. Our objective is to establish robust ECOFFs and susceptibility profiles for *C.* canimorsus, providing clinicians with critical guidance for empirical treatment following animal bites.

PF-07/P-07 Where global warming stands on plasmids-encoded resistance transfer?

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Background
Encouraged fossil fuel consumption and carbon dioxide (CO_2) emissions are increasing systematically in the last decades playing an important role in the global warming chain. The Global Climate report and the World Meteorological Organization stated that 2024 was the hottest year on record as warming hits 1.54°C above pre-industrial era. Plasmid-encoded antimicrobial resistance is a global public health concern, thus this study aimed to evaluate whether increases in temperature could affect the rate of plasmid conjugation frequencies (CF).

Materials
Escherichia coli carrying either an IncX3-bla_{NDM-5} or IncL-bla_{OXA-48} scaffolds were submitted to conjugation under increasing temperatures (22°C, 27°C, 31°C, 33°C, 37°C, and 40°C). The nalidixic-resistant strain *E. coli* JM109 was used as recipient. Selections of donors plus transconjugants were done in 1 mg/L of meropenem for IncX3-bla_{NDM-5} and 50 mg/L of temocillin for IncL-bla_{OXA-48} scaffolds. Selection of transconjugants only were performed with the addition of 50 mg/L of nalidixic acid to the meropenem or temocillin, depending on the strain.

Results
The IncX3-bla_{NDM-5} strain showed increased CF at 33°C (6.2–47.8-folds), 37°C (7–18.3-folds), and 40°C (3.7–28.6-folds) compared to lower temperatures (22°C, 27°C, and 31°C). Similarly, the IncL-bla_{OXA-48} strain showed increases at 31°C (27.7–45.1-fold), 33°C (11–17.8-fold), 37°C (6.6–10.7-fold), and 40°C (8.8–14.3-fold) compared to 22°C and 27°C.

Conclusions
Significant increases in CF could be observed in higher temperatures such as 37°C and 40°C when compared with plasmid transfer at lower temperatures like 22°C and 27°C. These findings to be confirmed with a variety of clinical isolates and resistance plasmids underlines the role of global warming in promoting high levels of plasmid exchange, and consecutively, the dissemination of critically important resistance genes in public health.

PF-08/P-08 The BioPreparedness Biobank: A One Health Resource for Secure Microbial Preservation and Controlled Research Access

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The BioPreparedness Biobank, a core activity of the Multidisciplinary Center for Infectious Diseases (MCID) at the University of Bern, is a One Health platform supporting the collection and preservation of microorganisms from human, animal, and environmental sources. This integrated approach enables research on infectious diseases, antimicrobial resistance, and emerging zoonotic threats.

The biobank provides secure, long-term storage for high-consequence pathogens under clearly defined conditions. Researchers can deposit microbial materials while retaining full ownership; access for research purposes is only granted with the depositor's explicit consent, formalized through a Material Transfer Agreement (MTA).

In addition to its biobanking function, the infrastructure includes a synthetic genomics platform capable of assembling viral genomes in yeast hosts. This enables the design of specific mutations or genetic markers under stringent biosafety conditions. The biobank also provides access to curated bacterial and viral collections through collaborations with the Institute of Virology and Immunology (IVI) and Spiez Laboratory, complementing its in-house holdings.

All operations are carried out within a biosafety level 3 (BSL-3) environment and supported by an automated –80 °C storage system, a dedicated Biobank Information Management System (BIMS), and a validated quality management system. The quality and reliability of these processes were confirmed through the NORMA certification awarded by the Swiss Biobanking Platform (SBP), following a comprehensive on-site audit.

By combining secure storage, high biosafety standards, advanced synthetic biology capabilities, and transparent governance, the BioPreparedness Biobank serves as a valuable resource for the microbiology research community, supporting responsible access to critical microbial resources while safeguarding depositor rights.

PF-09*/MT-02*/P-09* **diet and stress in vervet monkeys: the role of the gut microbiome**

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The gut microbiome functions as a key interface between host and environment, influencing physiological and behavioural processes. In both humans and animals, its composition is shaped by intrinsic and extrinsic factors – diet, stress, habitat and seasonality. Among these, diet plays a central role in health and stress resilience, with many species – including primates – engaging in self-medication behaviour. Vervet monkeys (*Chlorocebus pygerythrus*), with an omnivorous diet, a complex social structure, including matriarchy and hierarchy, and different habitats such as wild, urban, and captive, offer a valuable model to study these interactions. These factors influence stress and food choices, while shaping the gut microbiome. Yet, the links between diet, stress, and gut microbiome remain unclear. This study aims at investigating how diet, social group, habitat, rank, and seasonality affect gut microbiome composition, diversity and function.

Fecal samples have been collected from female vervet monkeys across wild, urban and captive habitats in KwaZulu-Natal (South Africa) since 2018 to the present. 321 out of 5176 collected samples were selected based on group, seasonal and longitudinal representation, and sequenced by shotgun metagenomics (Illumina™ NovaSeq). Taxonomic profiles obtained with mOTUs and diversity metrics will be analyzed cross-sectionally and longitudinally in relation to the factors of interest. Functional profiles will be obtained using zShoMan pipeline, based on KEGG, CAZyme, and eggNOG.

Preliminary analysis of 35 samples from 8 wild vervet monkeys revealed significant seasonal variation in gut microbiome species richness (wet and dry seasons) and evenness (autumn compared to spring and summer). Additionally, microbiome composition showed significant variability across group, season, and rank. These findings highlight the importance of considering environmental and individual factors in primate gut microbiome research.

MY THESIS IN 180 SECONDS & POSTERS P-09 – P-20

MT 01*/P-10* Culturomics insights into phenotypic antimicrobial resistance evolution in the gut microbiota of allogeneic stem cell transplant patients

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Allogeneic stem cell transplant (allo-HSCT) recipients are at high risk for bacterial infections and receive frequent antibiotics, disrupting the gut microbiota and driving antimicrobial resistance (AMR). Despite its clinical relevance, the *in vivo* dynamics of resistance remain poorly understood. Here, we aim to explore the prevalence and resistance dynamics of gut bacteria in these patients at a single-strain resolution.

We collected weekly stool samples from 100 allo-HSCT patients from pre-transplant to 6 months post-transplant. Using a culturomics-based approach under aerobic conditions, we isolated 20 random colonies per sample from COS, MacConkey, and UriSelect agar. Colonies were identified by MALDI-TOF and biobanked. Phenotypic AMR of *Escherichia coli* was assessed by EUCAST disc diffusion using six antibiotics (amikacin, ceftriaxone, levofloxacin, cefepime, piperacillin/tazobactam, and amoxicillin/clavulanate).

Preliminary data from six patients included 7'815 isolates representing 65 species. The most frequent were *E. coli* (36.5%), *Enterococcus faecalis* (22.8%), and *E. faecium* (16.1%), showing evidence that interspecies dominance and selection pressure shape microbial community structure. Among 695 *E. coli* isolates, a total of 4'169 inhibition zone measurements revealed dynamic changes in antibiotic susceptibility. In one patient, the median diameter of the levofloxacin inhibition zone dropped from 31 mm (sensitive; interquartile range [IQR] 31.0–31.50mm) to 6mm (resistant; IQR 6.00–6.00mm) after one week.

This study provides culturomics-based insights into AMR evolution in single bacterial isolates within the gut microbiota during allo-HSCT. Our findings highlight dynamic, strain-level adaptations to antibiotic pressure, underscoring the potential of this approach to uncover critical patterns in AMR evolution. As the study progresses, expanded sampling, phenotypic and genotypic testing across a larger patient cohort will further enhance these insights.

PF-09*/MT-02*/P-09* **Diet and stress in vervet monkeys: the role of the gut microbiome**

Dr. Daulet Amerzhanov [1], Prof. Claire Bertelli [1], Dr. Sedreh Nassirnia [1]

1.Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

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MT-03*/P-11* Bacterial Rocket Science: Serovar-Specific Teichoic Acids Regulate ActA-Mediated Cell-to-Cell Spread in Listeria monocytogenes

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1.Institute of Food, Nutrition and Health, ETH Zurich, Switzerland

Listeria monocytogenes (Lmo) is a Gram-positive foodborne pathogen that poses a significant public health risk due to its ability to cause listeriosis, a disease with high mortality rates. Teichoic acids (TAs), including wall teichoic acids (WTAs) and lipoteichoic acids (LTAs), are critical components of the bacterial cell wall, contributing to both physiology and virulence. Previous studies have implicated glycosylation-deficient TAs in reduced virulence, potentially through effects on ActA, an actin assembly-inducing protein essential for actin-based motility and intercellular spread. In this study, we investigated how sugar-modified TAs influence ActA function during the cell-to-cell spread of *L. monocytogenes*. Using in-frame deletion mutants of TA biosynthesis genes, we characterized phenotypic changes during in vitro infection via immunofluorescence assays. Our results demonstrate that Galactose-decorated WTAs (Gal-WTAs), but not Galactose-decorated LTAs (Gal-LTAs), are critical for maintaining normal ActA activity in serovar 4b. Notably, in Serovar 1/2a strains, a similar dependency was observed for Rhamnose-decorated WTAs, underscoring the conserved yet serovar-specific role of WTA glycosylation in supporting ActA-mediated virulence. These findings provide new insights into the molecular interplay between cell wall architecture and virulence in Gram-positive pathogens and lay the groundwork for further studies into TA-mediated regulation of host-pathogen interactions.

S-59*/MT-04*/P-12* ImmunoPhage: Targeting Persistent Infections with Engineered Immunomodulatory Bacteriophages

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Persistent infections arise when bacteria exploit vulnerabilities in the immune response and resist clearance. While conventional therapies focus on the eradication of pathogens, immunomodulatory approaches aim to enhance the host's defenses. Immunomodulatory treatments using cytokines are a promising addition to the therapeutic repertoire, though their clinical success in infectious diseases has been limited.

Bacteriophages (phages) are another emerging approach against bacterial infections. Previously, it was shown that phage therapy can be enhanced by engineering antimicrobial effectors into the phage genomes. Here, we aim to integrate immunomodulation with phage therapy by encoding cytokines, and generating so-called *ImmunoPhages*. Once ImmunoPhage infects a bacterial pathogen at the site of the infection, cytokines are expressed and released alongside progeny phages. This approach offers a potentially safer, more targeted method of immunotherapy, compared to systemic delivery of cytokines. When combined with phage therapy, it can address key limitations of natural phages, including targeting phage-resistant, intracellular, and quiescent bacterial populations.

Our first generation of ImmunoPhages are designed to treat recurrent urinary tract infections (UTIs), where *E. coli* evades the immune response by forming intracellular bacterial communities and quiescent reservoirs deep within the epithelium. We demonstrate the production of functional chemokines, interleukins, and interferons in clinical *E. coli* isolates using this approach, leveraging phages from two distinct viral families. Ongoing studies are evaluating the efficacy of ImmunoPhage in a mouse UTI model.

In summary, ImmunoPhages combine the antibacterial effect of phages with targeted immunotherapy and offer a customizable, antibiotic-free strategy for persistent and chronic infections.

S-30*/MT-05*/P-13*

Uncovering global antimicrobial resistance in Capnocytophaga: insights from the largest genomic dataset to date

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Background

Certain *Capnocytophaga* species, most notably *C.* canimorsus, are common members of the oral microbiota of dogs and cats and can cause severe, sometimes life-threatening, infections in humans, typically following animal bites. Due to their slow and fastidious growth, species-level identification and antibiotic susceptibility testing are often delayed, leading clinicians to rely on empirical β-lactam therapy. However, emerging antimicrobial resistance (AMR) threatens the efficacy of these first-line treatments, while global data on AMR prevalence in *Capnocytophaga* remain sparse. We aimed to investigate distribution of AMR genes in *Capnocytophaga* isolates from human infections and domestic animals across 20 countries and match these findings with phenotypic testing.

Methods

We established the largest globally representative collection of (zoonotic) *Capnocytophaga* isolates to date (n=900; 536 human-clinical, 364 animal-oral) through the Global *Capnocytophaga* Consortium. This cohort includes isolates from 20 countries across 4 continents. Whole-genome sequencing (Illumina) was performed on 477 isolates. AMR genes were detected using AMRFinder+, ABRicate or AbriTAMR. Long-read sequencing (Oxford Nanopore) was performed on a subset (n=15) of isolates representing diverse geographies and resistance profiles.

Results

Acquired resistance genes were identified in 65/477 isolates (13.6%), spanning 13/20 countries, from both human (43%) and animal (57%) sources. These genes are associated with resistance to multiple antibiotic classes including β-lactams, aminoglycosides, macrolides, and tetracyclines.

Long-read sequencing revealed chromosomal integration of all detected AMR genes, even though plasmids were present in 6/15 screened isolates.

β-lactam resistance was detected in 36/477 (7.5%) isolates, predominantly involving the *blaOXA-347* gene (n=27). Two isolates harbored putative novel class D β-lactamase gene. Functional characterization experiments (via cloning and recombinant expression) and phenotypic susceptibility testing are underway to establish genotype-phenotype correlations.

Conclusion

This unprecedented dataset provides a critical baseline for AMR monitoring in *Capnocytophaga* and reveals a novel *blaOXA* gene with potential clinical relevance.

MT-06*/P-14* **Siderophore-mediated interactions between bacteria isolated from potato and Phytophthora infestans: exploring chemical communication underlying disease suppression.**

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Iron is an essential element for most organisms, but this trace element is often poorly available in soil. A common strategy for bacteria and plants to acquire this nutrient is through the secretion of siderophores, which are specialized metabolites that scavenge iron from the environment. Beyond iron acquisition, siderophores can also influence plant health and help to suppress phytopathogens. One such pathogen is the oomycete Phytophthora infestans, the causative agent of late blight in potato crops. The devastation caused by this phytopathogen remains a persistent threat, demanding constant vigilance and the development of new controlling strategies. This study investigates the response of a potato rhizosphere isolate, Pseudomonas donghuensis R32, which possesses strong antagonistic ability against P. infestans, to the presence of zoospores from Phytophthora infestans. Our results show an increased secretion of a particular siderophore, pyoverdine, during co-culture with Phytophthora infestans zoospores, suggesting a mechanism of iron competition or an induced defensive response. Our next step will be to screen a mutant bank in Pseudomonas R32 to identify the genetic elements involved in the upregulation of pyoverdine secretion in response to Phytophthora infestans. Furthermore, we conducted a screening of microorganisms isolated from the potato rhizosphere and phyllosphere to identify novel siderophore producers. This will allow us to explore the diversity of siderophore-producing microbes and to determine whether the response to chemical signals from P. infestans is specific to Pseudomonas or shared across different bacterial taxa. These two projects commonly assess the importance of siderophores in soil microbe interactions and their potential contribution to plant protection.

MT-07*/P-15* **Deficiency in peptidoglycan recycling promotes β-lactam drug sensitivity in Caulobacter crescentus**

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Bacterial cell growth and division are governed by constant remodeling of the peptidoglycan (PG) layer, achieved by cell wall hydrolases and cell wall synthesizing enzymes. Cell wall hydrolases are broadly classified as glycosidases or peptidases based on their chemical cleavage specificity. *Caulobacter crescentus*, a dimorphic model organism for studying bacterial cell cycle regulation, possesses a sophisticated peptidoglycan synthesis essential for maintaining cell wall integrity. Recent studies have revealed the involvement of the periplasmic soluble lytic transglycosylase, SdpA enzyme, in peptidoglycan remodeling in *C. crescentus*. The anhydromuropeptides produced through the activity of SdpA are transported into the cytoplasm by the permease AmpG in most gram-negative bacteria, which eventually leads to PG recycling. However, in *C. crescentus*, the PG recycling pathway has not yet been studied. We discovered that *C. crescentus* had an AmpG homolog, and an AmpG deletion mutant exhibited β-lactam sensitivity. The periplasmic fraction analysis revealed an accumulation of anhydromuropeptide *ampG* deletion mutants, indicating a critical role for *ampG* as a permease in anhydromuropeptide recycling. Furthermore, *C. crescentus* cells lacking both SdpA and AmpG showed severe growth defects. Quantification of soluble muropeptides via LC-MS analysis showed changes in levels of PG precursors (UDP-MurNAc, UDP-GlcNAc, and UDP-MurNAc-pentapeptide) in PG recycling mutants, suggesting that PG recycling products contribute towards *de novo* PG biosynthesis. In conclusion, this study reveals that defects in PG recycling play an important role in cell wall biogenesis and β-lactam resistance in *C. crescentus*.

S-14*/MT-08*/P-16*

Dismantling walls to grow - understanding the effect of physiological and environmental influences on L-form conversion during β-lactam treatment

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The bacterial cell wall and essential in hypoosmotic conditions for bacterial cell integrity. However, in hyperosmotic conditions, a subpopulation can shed the cell wall and convert to an amorphous, often avirulent L-Form state. This conversion is induced by cell wall targeting antibiotics such as β-lactams or other cell wall antagonists such as phage-derived endolysins. The bacterial L-Form can slowly proliferate in the presence of the β-lactams, and a population of L-Forms can revert frequently to the virulent walled state after antibiotic treatment is ended. While increased mutagenesis enables more efficient L-Form growth, the conversion is completely independent of genetic alterations. Over the decades of L-Form research, there have been multiple reports of L-Forms isolated from human patients. We previously showed that bacterial L-Forms can be induced in human host cells as well as in human urine by treatment with cell wall antagonists. However, it is currently not known which role L-Forms play exactly in antibiotic treatment failure and the mechanism of L-Form conversion remains elusive. Here we investigate the effects of cyclic di-AMP, the master regulator of cell wall homeostasis, on the ability of *Listeria monocytogenes* to convert to the L-Form state. We show that cyclic di-AMP is required for L-Form conversion and investigate the physiological role of cyclic di-AMP during β-lactam treatment in human host cells. We compare changes in L-Form conversion rates of *Listeria* populations to the general tolerance to β-lactam treatment and unravel the role of temperature in L-Form growth. L-Forms comprise an understudied field within antibiotic resilience and understanding the mechanisms of L-Form conversion will bring light to the role of the elusive L-Forms during antibiotic treatment failures in human patients and domestic animals.

MT-09*/P-17* Uncovering genus-wide genetic determinants of L-form conversion in Listeria

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1.Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland

L-forms are cell wall–deficient variants of normally walled bacteria that can proliferate indefinitely without peptidoglycan. Under exposure to cell wall–targeting agents such as β-lactam antibiotics, walled cells transiently convert into L-forms that can revert to the walled state once the stress is removed. However, with prolonged antibiotic exposure, these L-forms become stable and lose the ability to revert. This survival strategy is observed across diverse bacterial species and is thought to confer resistance to both antibiotic pressure and bacteriophage attack. Although genetic drivers of L-form conversion have been described in individual strains of *Listeria monocytogenes* and *Bacillus subtilis*, it remains unclear whether similar mechanisms apply more broadly across related taxa. In this study, we examined L-form switching in 13 strains representing six *Listeria* species by sequentially passaging cells under ampicillin stress. Whole-genome sequencing and single-nucleotide polymorphism (SNP) analysis of each L-form lineage against its parental walled state were performed to identify mutations associated with L-form conversion. Preliminary analysis of *L. fleischmannii* L-forms after one month of continuous antibiotic exposure uncovered non-synonymous SNPs in eight genes. These include *menD*, *tkt*, *irfF*, and *pdxS*, which encode proteins involved in menaquinone biosynthesis, pentose phosphate pathway flux, iron uptake, and pyridoxal-5′-phosphate cofactor production. The pattern of mutations suggests coordinated shifts in central metabolism, ion homeostasis, and membrane remodeling when peptidoglycan is absent. Ongoing sequencing of further passaged L-forms and comparative genomic analyses will clarify the extent to which these adaptive mutations are conserved across the *Listeria* genus, and enrichment analyses will identify key pathways associated with L-form conversion. Together, these insights will deepen our understanding of L-form–mediated stress adaptation and antibiotic tolerance.

MT-10*/P-18* **Ustilago maydis: a new model for understanding small GTPase-regulated actin assembly in endocytosis**

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Clathrin-mediated endocytosis (CME) is a conserved and dynamic process that involves the coordinated action of over 60 proteins. Across eukaryotes, a transient burst of branched actin assembly, driven by the Arp2/3 complex and its activators like WASP, provides the mechanical force necessary for membrane internalization during endocytosis. In mammalian cells, WASP activity is autoinhibited by its CRIB domain, and is relieved upon interaction with small Rho GTPases. In contrast, *Saccharomyces cerevisiae* WASP lacks this regulatory mechanism and functions independently of Rho GTPases. Instead, its activity is modulated by the fungal-specific endocytic proteins Sla1 and Bbc1. Sla1 and Bbc1 may functionally compensate for the lack of small Rho GTPase-mediated regulation in budding yeast. Basidiomycete fungi, like *Ustilago maydis* possesses both a CRIB-containing WASP and orthologs of Sla1 and Bbc1. This suggests the coexistence of both the mammalian-like and yeast-like regulatory paradigms for actin assembly during CME. Two fundamental questions are raised: how are the two regulatory mechanisms functionally integrated in a single organism, and what are the evolutionary steps that led to this convergence? To address this question, we employed a genome editing approach in *U. maydis*, with quantitative live-cell imaging. Our results reveal the co-existence of two WASP regulatory pathways in *U. maydis*: one mediated by the small GTPases Cdc42 and Rac1, and the other by the endocytic proteins Sla1 and Bbc1. Our findings suggest that *Ustilago maydis*, with its compact genome and amenability to high-resolution imaging, serves as a powerful model for studying the mechanism and evolutionary plasticity of Rho GTPase-WASP signaling pathways in endocytosis.

MT-11*/P-19* **Single-cell analysis of the switch from vegetative to filamentous growth in S.cerevisiae**

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Opportunistic pathogenic fungi pose a great threat to human health, particularly to immune compromised individuals. In pathogenic fungi like *C. albicans*, filamentation is associated with host-cell attachment, tissue invasion and virulence. Thus, there is a need to understand the molecular machinery causing the switch from vegetative to filamentous growth.
In the model organism *S. cerevisiae*, exposure to low-nutrients can trigger evolutionarily conserved signaling pathways that lead to the formation of filament-like structures called pseudohyphae. This growth pattern characterized by unipolar attached growth of elongated cells can be compared to filamentation in other fungi. Most of our knowledge on this process comes from agar invasion assays and colony morphology analysis. These studies allowed to identify the main players that control this morphological transition.
However, we still have a poor understanding of how the different signaling pathways PKA, TOR and f-MAPK mainly, interact to promote this cell fate transition. Therefore, we developed synthetic promoters responsive to downstream transcription factors of each pathway and developed a microscopy assay where we can follow the dynamic transition from vegetative to filamentous growth in single cells. Using an analog sensitive allele of the MAPK Kss1, we can monitor the cross-talk between the f-MAPK and the TOR and PKA pathways by monitoring the change in the output of the expression reporters in presence of the kinase inhibitor.

S-35*/MT-12*/P-20*

From seeding to post-harvesting: sugar kelp microbiome decoding for sustainable European aquaculture

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After rapid initial growth, the European seaweed aquaculture sector has entered a stagnation phase, presenting the unique opportunity to resolve scientific gaps crucial to sustainable and profitable sector advancement. Sugar kelp (*Saccharina latissima*) is a widely applied seaweed in food, cosmetics, bioremediation and more. During cultivation, early-life sugar kelp stages are glued onto ropes and eventually attach firmly using their holdfasts. Attachment is challenged by many factors, causing a >90% loss of unattached seeded sugar kelp. Research hints towards microbes playing an important role in early-life sugar kelp development, potentially fostering seeding attachment. Mechanical detachment of sugar kelp during harvest or detaching post-harvest remains pose additional economic and ecological risks. To limit these risks, the Dutch SEASEEDS project aims to improve cultivated sugar kelp attachment using a multidisciplinary approach, including research on allied microbiota. First, we characterized sugar kelp-associated bacterial and fungal communities by 16S and 18S rRNA gene amplicon sequencing. We collected sugar kelp and seawater samples throughout the cultivation cycle at the nearshore small-scale Oosterschelde site, seeded in by Hortimare B.V. and operated by The Seaweed Company. In turn, the microbial key players at sugar kelp detachment-prone cultivation stages (i.e., seeding, harvesting and post-harvesting) can be identified and potentially used to improve sugar kelp attachment. Secondly, we compare that study with sugar kelp samples from the first Dutch large-scale offshore farm, North Sea Farm 1, operated by the North Sea Farmers. Thirdly, we analyze sugar kelp samples from distinct European cultivation sites. These spatial comparisons allow to assess the generality of the observed microbial communities associated with cultivated sugar kelp. The entire monitoring efforts provides essential microbial insights that support the European seaweed cultivation sector advancement, promoting both economic viability and ecological sustainability.

POSTER PRESENTATIONS
P-01 – P-86

PF-01*/P-01* A bacterial cell wall repair and modification system to resist host antibacterial factors

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Pathogenic bacteria have acquired the ability to resist antibacterial defense mechanisms of the host. Streptococci are common in animal microbiota and include opportunistic pathogens like Group A Streptococcus (GAS) and *Streptococcus pneumoniae* (pneumococcus). While the conserved streptococcal S protein has been identified as a key factor in GAS virulence, its exact function is unclear. Here, we show that the pneumococcal S protein is crucial for resisting against host-derived antimicrobials by coordinating cell wall modification and repair. Specifically, we show that S proteins are septally localized through their transmembrane domain and contain an extracellular peptidoglycan (PG) binding LysM domain which is required for its function. Protein-protein and genetic interaction studies demonstrate that the pneumococcal S protein directly interacts with a PG synthase, class A penicillin binding protein PBP1a, and the PG deacetylase PgdA. Single-molecule experiments reveal that the fraction of circumferentially moving PBP1a molecules is reduced in the absence of S protein. Consistent with an impaired PBP1a function, streptococci lacking S protein exhibit increased susceptibility to cell wall targeting antibiotics and altered cell morphologies. We show that pneumococci lacking the S protein cannot persist transient penicillin treatment, are more susceptible to the human antimicrobial peptide LL-37 and to lysozyme, and show decreased virulence in zebrafish and mice. Our data supports a model in which S proteins regulate PBP1a activity and play a key role in coordinating PG repair and modification. This cell wall 'sentinel' control system provides defense against host-derived and environmental antimicrobial attack.

PF-02*/P-02* Direct bacterial headspace sampling for rapid online volatilomics analysis in medical microbiology diagnostics

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Background
The ability to detect and identify bacterial pathogens from volatile emissions is an emerging approach for rapid diagnostics of bacterial and fungal pathogens. It can reduce the time-to-result for antibiotic susceptibility testing (AST) from 48h to just below a few hours. With the emergence of high-resolution analytical techniques like gas chromatography-mass spectrometer, ion mobility spectrometer, and secondary electrospray ionization spectrometer, quantitative analysis of the headspace biomarker concentrations has become available, and promising results were obtained for the identification of the bacteria and AST. However, MS-based devices are too complicated and expensive. To translate volatilomics into a powerful tool for rapid diagnostics in primary care, we need compact and affordable devices utilizing nanosensors to detect volatile organic compounds (VOCs). Sampling of the headspace volume plays a crucial role for reliable measurements.

Methods
We define the requirements of reproducible sampling for reliable volatilomic studies and design a headspace sampling system for microbiological analysis with chemoresistive sensors (e.g. selective for acetone) and mass spectrometry for comparative analyses. The sampling parameters were optimized and various clinical isolates were used to analyse the VOCs.

Result
We present our bacterial headspace sampling system for the detection of volatilome signatures and tracking of the dynamic volatilome profiles. This versatile system is tailored to accurately sample the headspace volume of the growth media while providing optimal incubation conditions for longitudinal monitoring. Key aspects of headspace sampling are discussed through preliminary *Escherichia coli* measurements, the focus being on the acetaldehyde concentrations as the main indicator of the growth phase. We demonstrate that different classes of VOCs (alcohols, ketones, aromatics,…) can be measured and bacteria proliferation can be monitored by measuring the headspace acetaldehyde concentration over the incubation period.

Conclusion
We demonstrate a proof-of-principle device to measure volatilome components, which eventually could be used for rapid detection of pathogens.

PF-03*/P-03* **Genetic variants of tetX linked to tetracycline susceptibility in Mycobacterium abscessus complex.**

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Mycobacterium abscessus complex (MABC) is a highly drug-resistant non-tuberculous mycobacterium and a leading cause of pulmonary infections in individuals with cystic fibrosis (CF). The intrinsic resistance of MABC to most antibiotics, coupled with the poor efficacy and severe side effects of current treatment regimens, presents a major clinical challenge. Current therapies often rely on third-generation tetracyclines like tigecycline, which—despite their in vitro activity—cause significant gastrointestinal toxicity, limiting their long-term use. In this context, the identification of strain-specific drug susceptibility profiles is essential for designing personalized treatment regimens that are both more tolerable and more effective.

Our study focuses on the role of naturally occurring polymorphisms in the *tetX* gene, which encodes a flavin-dependent monooxygenase responsible for inactivating first- and second-generation tetracyclines (e.g., tetracycline, doxycycline, minocycline). Whole-genome sequencing and drug susceptibility testing of Swiss MABC clinical isolates revealed distinct *tetX* alleles associated with differential susceptibility to tetracyclines. Specifically, SNPs in the codons causing aminoacidic substitution D454G and G455E were found to alter the functionality of TetX, leading to a loss of resistance in some strains. Notably, the D454G mutation was associated with a loss of resistance, resulting in increased susceptibility to tetracycline, doxycycline and minocycline, whereas the G455E mutation was linked to inducible resistance to tetracycline and minocycline, suggesting a functional but regulated TetX activity.

These findings were validated through heterologous expression, site-directed mutagenesis, and MIC profiling in isogenic mutants. Our data show that MABC resistance is not uniform and that *tetX* variants can predict susceptibility to better-tolerated oral tetracyclines.

Screening for *tetX* polymorphisms could enable personalized treatment with first- or second-generation tetracyclines, reducing reliance on toxic drugs like tigecycline. This approach offers a clear advantage in patient care by minimizing side effects. Integrating *tetX* genotyping into diagnostic workflows would support tailored, cost-effective therapy for MABC infections, particularly in CF patients.

PF-04*/P-04* **Role of the transcription factor Wor2 in biofilm formation of Candida auris**

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Candida auris is an emerging fungal pathogen with the ability to develop antifungal resistance and to cause nosocomial outbreaks. *C. auris* is able to adhere to abiotic surfaces and form biofilms, which contributes to interhuman transmission and virulence. In this study, we assessed the role of the zinc cluster transcription factor Wor2 (B9J08_002136) in biofilm formation of *C. auris*.
Two *WOR2* mutants were constructed in the wild-type strain IV.1 : *WOR2*^{HA} (hyperactivation of *WOR2*) and *wor2*Δ (deletion of *WOR2*). Phenotypic growth of the mutants was assessed, and their biofilm forming capacity was measured by crystal violet assays.
The downstream effectors of Wor2 were investigated by RNA sequencing, comparing *WOR2*^{HA} to IV.1. The function of these genes was further examined through their overexpression under the control of *ADH1* promoter at their native loci.
The mutants did not exhibit any growth defect. Compared to the IV.1 strain, *WOR2*^{HA} reduced biofilm formation while *wor2*Δ increased biofilm formation. Transcriptomic analyses revealed significant downregulation of *ALS4112* and *SCF1* (two important adhesins) in *WOR2HA.* Overexpression of *ALS4112* and *SCF1* in the IV.1 background showed increased biofilm production. In the *WOR2HA* background, overexpression of *SCF1* could restore biofilm capacity, which was not the case for *ALS4112* overexpression.
This study demonstrates for the first time that Wor2 acts as repressor of biofilm formation in *C. auris*, probably by down regulation of Als4112 and Scf1, with Scf1 being the main downstream effector.

PF-05*/P-05* Phage-derived endolysins as precision tools against vancomycin-resistant enterococci

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Antibiotic-resistant infections are on the rise, posing a major public health challenge. Gram-positive pathogens such as *Enterococcus faecalis* and *Enterococcus faecium* are of particular concern, especially in healthcare settings. As the efficacy of conventional antibiotics continues to decline and the pipeline for new drugs remains limited, alternative therapeutic approaches are urgently needed.

Endolysins, bacteriophage-derived peptidoglycan hydrolases, offer a targeted and efficient mechanism for bacterial lysis. These enzymes can induce rapid cell death, by cleaving conserved bonds within the peptidoglycan layer. Their ability to act exogenously on Gram-positive bacteria, which lack an outer membrane, makes them especially attractive for treating enterococcal infections.

To evaluate their potential, we conducted a systematic analysis of native and engineered endolysins targeting *Enterococcus spp*. The panel included enzymes with diverse enzymatically active domains (EADs) - glycosidases, amidases, and endopeptidases - each recognizing distinct peptidoglycan bonds. These catalytic domains are coupled with structurally diverse cell wall binding domains (CBDs), including α-helices, β-sheets, and α-β-sandwich architectures. Chimeric constructs were also generated through rational domain swapping, connected via flexible linkers to optimize activity and stability.

Our results demonstrate that endolysins exhibit potent bactericidal activity against *Enterococcus* spp., including vancomycin-resistant strains in clinically relevant environments. The modularity of their architecture enables precise engineering for enhanced specificity and function. Moreover, the conserved nature of their peptidoglycan target reduces the likelihood of resistance development.

Together, these findings underscore the therapeutic potential of endolysins as next-generation antimicrobials. Their structural diversity, robust killing efficacy, and low propensity for resistance position them as strong candidates for clinical development in combating multidrug-resistant infections.

PF-06*/P-06* Antimicrobial susceptibility testing of Capnocytophaga canimorsus: toward evidence-based treatment guidelines

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Introduction

Capnocytophaga canimorsus is a rare zoonotic pathogen transmitted through close contact with dogs and cats (typically bites or licks), capable of causing life-threatening infections in immunocompetent and -compromised individuals. Despite frequent empirical use of β-lactam antibiotics (often combined with β-lactamase inhibitors) as first-line therapy, no standardized antimicrobial susceptibility testing (AST) method or EUCAST clinical breakpoints exist for this pathogen. Our study aims to fill this gap by determining the minimal inhibitory concentration (MIC) distributions and epidemiological cutoff values (ECOFFs) for key antibiotics, paving the way for evidence-based guidelines and management of *C.* canimorsus infections.

Methods

AST was performed using agar dilution reference method, adapted with Heart Infusion Agar with 5% sheep blood (HIA-SB) to support the fastidious growth requirements of *C.* canimorsus. Each batch included quality control strains (*C. canimorsus* ATCC 35979, *E.* coli ATCC 25922, *S. aureus* ATCC 29213) and tested 15 antibiotic concentrations (0.016-128 mg/L) per agent (penicillin, amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam). To date, 115 isolates (clinical and animal-derived) from 26 centers across 12 different countries have been analyzed, with a target 250 isolates for robust MIC distributions. ECOFFs were estimated visually using EUCAST-recommended criteria.

Results

For penicillin and amoxicillin, we observed distinct wild-type (WT) and non-WT (resistant) populations, enabling preliminary visual estimation of ECOFFs (≤8mg/L). In contrast, amoxicillin-clavulanic acid showed less distinct separation, though MICs were consistently reduced 5-12 fold in 14 confirmed β-lactamase-producing isolates. Piperacillin-tazobactam demonstrated the most promising activity so far, with no apparent non-WT population among tested isolates, suggesting generally high susceptibility to this combination.

Conclusions and Outlook

Further testing is underway to validate these promising preliminary findings, including experiments using alternative EUCAST-recommended media. Our objective is to establish robust ECOFFs and susceptibility profiles for *C.* canimorsus, providing clinicians with critical guidance for empirical treatment following animal bites.

PF-07/P-07 **Where global warming stands on plasmids-encoded resistance transfer?**

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Background
Encouraged fossil fuel consumption and carbon dioxide (CO_2) emissions are increasing systematically in the last decades playing an important role in the global warming chain. The Global Climate report and the World Meteorological Organization stated that 2024 was the hottest year on record as warming hits 1.54°C above pre-industrial era. Plasmid-encoded antimicrobial resistance is a global public health concern, thus this study aimed to evaluate whether increases in temperature could affect the rate of plasmid conjugation frequencies (CF).

Materials
Escherichia coli carrying either an IncX3-bla_{NDM-5} or IncL-bla_{OXA-48} scaffolds were submitted to conjugation under increasing temperatures (22°C, 27°C, 31°C, 33°C, 37°C, and 40°C). The nalidixic-resistant strain *E. coli* JM109 was used as recipient. Selections of donors plus transconjugants were done in 1 mg/L of meropenem for IncX3-bla_{NDM-5} and 50 mg/L of temocillin for IncL-bla_{OXA-48} scaffolds. Selection of transconjugants only were performed with the addition of 50 mg/L of nalidixic acid to the meropenem or temocillin, depending on the strain.

Results
The IncX3-bla_{NDM-5} strain showed increased CF at 33°C (6.2–47.8-folds), 37°C (7–18.3-folds), and 40°C (3.7–28.6-folds) compared to lower temperatures (22°C, 27°C, and 31°C). Similarly, the IncL-bla_{OXA-48} strain showed increases at 31°C (27.7–45.1-fold), 33°C (11–17.8-fold), 37°C (6.6–10.7-fold), and 40°C (8.8–14.3-fold) compared to 22°C and 27°C.

Conclusions
Significant increases in CF could be observed in higher temperatures such as 37°C and 40°C when compared with plasmid transfer at lower temperatures like 22°C and 27°C. These findings to be confirmed with a variety of clinical isolates and resistance plasmids underlines the role of global warming in promoting high levels of plasmid exchange, and consecutively, the dissemination of critically important resistance genes in public health.

PF-08/P-08 The BioPreparedness Biobank: A One Health Resource for Secure Microbial Preservation and Controlled Research Access

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The BioPreparedness Biobank, a core activity of the Multidisciplinary Center for Infectious Diseases (MCID) at the University of Bern, is a One Health platform supporting the collection and preservation of microorganisms from human, animal, and environmental sources. This integrated approach enables research on infectious diseases, antimicrobial resistance, and emerging zoonotic threats.

The biobank provides secure, long-term storage for high-consequence pathogens under clearly defined conditions. Researchers can deposit microbial materials while retaining full ownership; access for research purposes is only granted with the depositor's explicit consent, formalized through a Material Transfer Agreement (MTA).

In addition to its biobanking function, the infrastructure includes a synthetic genomics platform capable of assembling viral genomes in yeast hosts. This enables the design of specific mutations or genetic markers under stringent biosafety conditions. The biobank also provides access to curated bacterial and viral collections through collaborations with the Institute of Virology and Immunology (IVI) and Spiez Laboratory, complementing its in-house holdings.

All operations are carried out within a biosafety level 3 (BSL-3) environment and supported by an automated –80 °C storage system, a dedicated Biobank Information Management System (BIMS), and a validated quality management system. The quality and reliability of these processes were confirmed through the NORMA certification awarded by the Swiss Biobanking Platform (SBP), following a comprehensive on-site audit.

By combining secure storage, high biosafety standards, advanced synthetic biology capabilities, and transparent governance, the BioPreparedness Biobank serves as a valuable resource for the microbiology research community, supporting responsible access to critical microbial resources while safeguarding depositor rights.

PF-09*/MT-02*/P-09* **Diet and stress in vervet monkeys: the role of the gut microbiome**

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The gut microbiome functions as a key interface between host and environment, influencing physiological and behavioural processes. In both humans and animals, its composition is shaped by intrinsic and extrinsic factors – diet, stress, habitat and seasonality. Among these, diet plays a central role in health and stress resilience, with many species – including primates – engaging in self-medication behaviour. Vervet monkeys (*Chlorocebus pygerythrus*), with an omnivorous diet, a complex social structure, including matriarchy and hierarchy, and different habitats such as wild, urban, and captive, offer a valuable model to study these interactions. These factors influence stress and food choices, while shaping the gut microbiome. Yet, the links between diet, stress, and gut microbiome remain unclear. This study aims at investigating how diet, social group, habitat, rank, and seasonality affect gut microbiome composition, diversity and function.

Fecal samples have been collected from female vervet monkeys across wild, urban and captive habitats in KwaZulu-Natal (South Africa) since 2018 to the present. 321 out of 5176 collected samples were selected based on group, seasonal and longitudinal representation, and sequenced by shotgun metagenomics (Illumina™ NovaSeq). Taxonomic profiles obtained with mOTUs and diversity metrics will be analyzed cross-sectionally and longitudinally in relation to the factors of interest. Functional profiles will be obtained using zShoMan pipeline, based on KEGG, CAZyme, and eggNOG.

Preliminary analysis of 35 samples from 8 wild vervet monkeys revealed significant seasonal variation in gut microbiome species richness (wet and dry seasons) and evenness (autumn compared to spring and summer). Additionally, microbiome composition showed significant variability across group, season, and rank. These findings highlight the importance of considering environmental and individual factors in primate gut microbiome research.

MT 01*/P-10* Culturomics insights into phenotypic antimicrobial resistance evolution in the gut microbiota of allogeneic stem cell transplant patients

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Allogeneic stem cell transplant (allo-HSCT) recipients are at high risk for bacterial infections and receive frequent antibiotics, disrupting the gut microbiota and driving antimicrobial resistance (AMR). Despite its clinical relevance, the *in vivo* dynamics of resistance remain poorly understood. Here, we aim to explore the prevalence and resistance dynamics of gut bacteria in these patients at a single-strain resolution.

We collected weekly stool samples from 100 allo-HSCT patients from pre-transplant to 6 months post-transplant. Using a culturomics-based approach under aerobic conditions, we isolated 20 random colonies per sample from COS, MacConkey, and UriSelect agar. Colonies were identified by MALDI-TOF and biobanked. Phenotypic AMR of *Escherichia coli* was assessed by EUCAST disc diffusion using six antibiotics (amikacin, ceftriaxone, levofloxacin, cefepime, piperacillin/tazobactam, and amoxicillin/clavulanate).

Preliminary data from six patients included 7'815 isolates representing 65 species. The most frequent were *E. coli* (36.5%), *Enterococcus faecalis* (22.8%), and *E. faecium* (16.1%), showing evidence that interspecies dominance and selection pressure shape microbial community structure. Among 695 *E. coli* isolates, a total of 4'169 inhibition zone measurements revealed dynamic changes in antibiotic susceptibility. In one patient, the median diameter of the levofloxacin inhibition zone dropped from 31 mm (sensitive; interquartile range [IQR] 31.0–31.50mm) to 6mm (resistant; IQR 6.00–6.00mm) after one week.

This study provides culturomics-based insights into AMR evolution in single bacterial isolates within the gut microbiota during allo-HSCT. Our findings highlight dynamic, strain-level adaptations to antibiotic pressure, underscoring the potential of this approach to uncover critical patterns in AMR evolution. As the study progresses, expanded sampling, phenotypic and genotypic testing across a larger patient cohort will further enhance these insights.

MT-03*/P-11* Bacterial Rocket Science: Serovar-Specific Teichoic Acids Regulate ActA-Mediated Cell-to-Cell Spread in Listeria monocytogenes

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Listeria monocytogenes (Lmo) is a Gram-positive foodborne pathogen that poses a significant public health risk due to its ability to cause listeriosis, a disease with high mortality rates. Teichoic acids (TAs), including wall teichoic acids (WTAs) and lipoteichoic acids (LTAs), are critical components of the bacterial cell wall, contributing to both physiology and virulence. Previous studies have implicated glycosylation-deficient TAs in reduced virulence, potentially through effects on ActA, an actin assembly-inducing protein essential for actin-based motility and intercellular spread. In this study, we investigated how sugar-modified TAs influence ActA function during the cell-to-cell spread of *L. monocytogenes*. Using in-frame deletion mutants of TA biosynthesis genes, we characterized phenotypic changes during in vitro infection via immunofluorescence assays. Our results demonstrate that Galactose-decorated WTAs (Gal-WTAs), but not Galactose-decorated LTAs (Gal-LTAs), are critical for maintaining normal ActA activity in serovar 4b. Notably, in Serovar 1/2a strains, a similar dependency was observed for Rhamnose-decorated WTAs, underscoring the conserved yet serovar-specific role of WTA glycosylation in supporting ActA-mediated virulence. These findings provide new insights into the molecular interplay between cell wall architecture and virulence in Gram-positive pathogens and lay the groundwork for further studies into TA-mediated regulation of host-pathogen interactions.

S-59*/MT-04*/P-12* ImmunoPhage: Targeting Persistent Infections with Engineered Immunomodulatory Bacteriophages

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Persistent infections arise when bacteria exploit vulnerabilities in the immune response and resist clearance. While conventional therapies focus on the eradication of pathogens, immunomodulatory approaches aim to enhance the host's defenses. Immunomodulatory treatments using cytokines are a promising addition to the therapeutic repertoire, though their clinical success in infectious diseases has been limited.

Bacteriophages (phages) are another emerging approach against bacterial infections. Previously, it was shown that phage therapy can be enhanced by engineering antimicrobial effectors into the phage genomes. Here, we aim to integrate immunomodulation with phage therapy by encoding cytokines, and generating so-called *ImmunoPhages*. Once ImmunoPhage infects a bacterial pathogen at the site of the infection, cytokines are expressed and released alongside progeny phages. This approach offers a potentially safer, more targeted method of immunotherapy, compared to systemic delivery of cytokines. When combined with phage therapy, it can address key limitations of natural phages, including targeting phage-resistant, intracellular, and quiescent bacterial populations.

Our first generation of ImmunoPhages are designed to treat recurrent urinary tract infections (UTIs), where *E. coli* evades the immune response by forming intracellular bacterial communities and quiescent reservoirs deep within the epithelium. We demonstrate the production of functional chemokines, interleukins, and interferons in clinical *E. coli* isolates using this approach, leveraging phages from two distinct viral families. Ongoing studies are evaluating the efficacy of ImmunoPhage in a mouse UTI model.

In summary, ImmunoPhages combine the antibacterial effect of phages with targeted immunotherapy and offer a customizable, antibiotic-free strategy for persistent and chronic infections.

S-30*/MT-05*/P-13*

Uncovering global antimicrobial resistance in Capnocytophaga: insights from the largest genomic dataset to date

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Background

Certain *Capnocytophaga* species, most notably *C.* canimorsus, are common members of the oral microbiota of dogs and cats and can cause severe, sometimes life-threatening, infections in humans, typically following animal bites. Due to their slow and fastidious growth, species-level identification and antibiotic susceptibility testing are often delayed, leading clinicians to rely on empirical β-lactam therapy. However, emerging antimicrobial resistance (AMR) threatens the efficacy of these first-line treatments, while global data on AMR prevalence in *Capnocytophaga* remain sparse. We aimed to investigate distribution of AMR genes in *Capnocytophaga* isolates from human infections and domestic animals across 20 countries and match these findings with phenotypic testing.

Methods

We established the largest globally representative collection of (zoonotic) *Capnocytophaga* isolates to date (n=900; 536 human-clinical, 364 animal-oral) through the Global *Capnocytophaga* Consortium. This cohort includes isolates from 20 countries across 4 continents. Whole-genome sequencing (Illumina) was performed on 477 isolates. AMR genes were detected using AMRFinder+, ABRicate or AbriTAMR. Long-read sequencing (Oxford Nanopore) was performed on a subset (n=15) of isolates representing diverse geographies and resistance profiles.

Results

Acquired resistance genes were identified in 65/477 isolates (13.6%), spanning 13/20 countries, from both human (43%) and animal (57%) sources. These genes are associated with resistance to multiple antibiotic classes including β-lactams, aminoglycosides, macrolides, and tetracyclines.

Long-read sequencing revealed chromosomal integration of all detected AMR genes, even though plasmids were present in 6/15 screened isolates.

β-lactam resistance was detected in 36/477 (7.5%) isolates, predominantly involving the *blaOXA-347* gene (n=27). Two isolates harbored putative novel class D β-lactamase gene. Functional characterization experiments (via cloning and recombinant expression) and phenotypic susceptibility testing are underway to establish genotype-phenotype correlations.

Conclusion

This unprecedented dataset provides a critical baseline for AMR monitoring in *Capnocytophaga* and reveals a novel *blaOXA* gene with potential clinical relevance.

MT-06*/P-14* Siderophore-mediated interactions between bacteria isolated from potato and Phytophthora infestans: exploring chemical communication underlying disease suppression.

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Iron is an essential element for most organisms, but this trace element is often poorly available in soil. A common strategy for bacteria and plants to acquire this nutrient is through the secretion of siderophores, which are specialized metabolites that scavenge iron from the environment. Beyond iron acquisition, siderophores can also influence plant health and help to suppress phytopathogens. One such pathogen is the oomycete Phytophthora infestans, the causative agent of late blight in potato crops. The devastation caused by this phytopathogen remains a persistent threat, demanding constant vigilance and the development of new controlling strategies. This study investigates the response of a potato rhizosphere isolate, Pseudomonas donghuensis R32, which possesses strong antagonistic ability against P. infestans, to the presence of zoospores from Phytophthora infestans. Our results show an increased secretion of a particular siderophore, pyoverdine, during co-culture with Phytophthora infestans zoospores, suggesting a mechanism of iron competition or an induced defensive response. Our next step will be to screen a mutant bank in Pseudomonas R32 to identify the genetic elements involved in the upregulation of pyoverdine secretion in response to Phytophthora infestans. Furthermore, we conducted a screening of microorganisms isolated from the potato rhizosphere and phyllosphere to identify novel siderophore producers. This will allow us to explore the diversity of siderophore-producing microbes and to determine whether the response to chemical signals from P. infestans is specific to Pseudomonas or shared across different bacterial taxa. These two projects commonly assess the importance of siderophores in soil microbe interactions and their potential contribution to plant protection.

MT-07*/P-15* Deficiency in peptidoglycan recycling promotes β-lactam drug sensitivity in Caulobacter crescentus

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Bacterial cell growth and division are governed by constant remodeling of the peptidoglycan (PG) layer, achieved by cell wall hydrolases and cell wall synthesizing enzymes. Cell wall hydrolases are broadly classified as glycosidases or peptidases based on their chemical cleavage specificity. *Caulobacter crescentus*, a dimorphic model organism for studying bacterial cell cycle regulation, possesses a sophisticated peptidoglycan synthesis essential for maintaining cell wall integrity. Recent studies have revealed the involvement of the periplasmic soluble lytic transglycosylase, SdpA enzyme, in peptidoglycan remodeling in *C. crescentus*. The anhydromuropeptides produced through the activity of SdpA are transported into the cytoplasm by the permease AmpG in most gram-negative bacteria, which eventually leads to PG recycling. However, in *C. crescentus*, the PG recycling pathway has not yet been studied. We discovered that *C. crescentus* had an AmpG homolog, and an AmpG deletion mutant exhibited β-lactam sensitivity. The periplasmic fraction analysis revealed an accumulation of anhydromuropeptide *ampG* deletion mutants, indicating a critical role for *ampG* as a permease in anhydromuropeptide recycling. Furthermore, *C. crescentus* cells lacking both SdpA and AmpG showed severe growth defects. Quantification of soluble muropeptides via LC-MS analysis showed changes in levels of PG precursors (UDP-MurNAc, UDP-GlcNAc, and UDP-MurNAc-pentapeptide) in PG recycling mutants, suggesting that PG recycling products contribute towards *de novo* PG biosynthesis. In conclusion, this study reveals that defects in PG recycling play an important role in cell wall biogenesis and β-lactam resistance in *C. crescentus*.

S-14*/MT-08*/P-16*

Dismantling walls to grow - understanding the effect of physiological and environmental influences on L-form conversion during β-lactam treatment

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The bacterial cell wall and essential in hypoosmotic conditions for bacterial cell integrity. However, in hyperosmotic conditions, a subpopulation can shed the cell wall and convert to an amorphous, often avirulent L-Form state. This conversion is induced by cell wall targeting antibiotics such as β-lactams or other cell wall antagonists such as phage-derived endolysins. The bacterial L-Form can slowly proliferate in the presence of the β-lactams, and a population of L-Forms can revert frequently to the virulent walled state after antibiotic treatment is ended. While increased mutagenesis enables more efficient L-Form growth, the conversion is completely independent of genetic alterations. Over the decades of L-Form research, there have been multiple reports of L-Forms isolated from human patients. We previously showed that bacterial L-Forms can be induced in human host cells as well as in human urine by treatment with cell wall antagonists. However, it is currently not known which role L-Forms play exactly in antibiotic treatment failure and the mechanism of L-Form conversion remains elusive. Here we investigate the effects of cyclic di-AMP, the master regulator of cell wall homeostasis, on the ability of *Listeria monocytogenes* to convert to the L-Form state. We show that cyclic di-AMP is required for L-Form conversion and investigate the physiological role of cyclic di-AMP during β-lactam treatment in human host cells. We compare changes in L-Form conversion rates of *Listeria* populations to the general tolerance to β-lactam treatment and unravel the role of temperature in L-Form growth. L-Forms comprise an understudied field within antibiotic resilience and understanding the mechanisms of L-Form conversion will bring light to the role of the elusive L-Forms during antibiotic treatment failures in human patients and domestic animals.

MT-09*/P-17* Uncovering genus-wide genetic determinants of L-form conversion in Listeria

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L-forms are cell wall–deficient variants of normally walled bacteria that can proliferate indefinitely without peptidoglycan. Under exposure to cell wall–targeting agents such as β-lactam antibiotics, walled cells transiently convert into L-forms that can revert to the walled state once the stress is removed. However, with prolonged antibiotic exposure, these L-forms become stable and lose the ability to revert. This survival strategy is observed across diverse bacterial species and is thought to confer resistance to both antibiotic pressure and bacteriophage attack. Although genetic drivers of L-form conversion have been described in individual strains of *Listeria monocytogenes* and *Bacillus subtilis*, it remains unclear whether similar mechanisms apply more broadly across related taxa. In this study, we examined L-form switching in 13 strains representing six *Listeria* species by sequentially passaging cells under ampicillin stress. Whole-genome sequencing and single-nucleotide polymorphism (SNP) analysis of each L-form lineage against its parental walled state were performed to identify mutations associated with L-form conversion. Preliminary analysis of *L. fleischmannii* L-forms after one month of continuous antibiotic exposure uncovered non-synonymous SNPs in eight genes. These include *menD*, *tkt*, *irfF*, and *pdxS*, which encode proteins involved in menaquinone biosynthesis, pentose phosphate pathway flux, iron uptake, and pyridoxal-5′-phosphate cofactor production. The pattern of mutations suggests coordinated shifts in central metabolism, ion homeostasis, and membrane remodeling when peptidoglycan is absent. Ongoing sequencing of further passaged L-forms and comparative genomic analyses will clarify the extent to which these adaptive mutations are conserved across the *Listeria* genus, and enrichment analyses will identify key pathways associated with L-form conversion. Together, these insights will deepen our understanding of L-form–mediated stress adaptation and antibiotic tolerance.

MT-10*/P-18* Ustilago maydis: a new model for understanding small GTPase-regulated actin assembly in endocytosis

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Clathrin-mediated endocytosis (CME) is a conserved and dynamic process that involves the coordinated action of over 60 proteins. Across eukaryotes, a transient burst of branched actin assembly, driven by the Arp2/3 complex and its activators like WASP, provides the mechanical force necessary for membrane internalization during endocytosis. In mammalian cells, WASP activity is autoinhibited by its CRIB domain, and is relieved upon interaction with small Rho GTPases. In contrast, *Saccharomyces cerevisiae* WASP lacks this regulatory mechanism and functions independently of Rho GTPases. Instead, its activity is modulated by the fungal-specific endocytic proteins Sla1 and Bbc1. Sla1 and Bbc1 may functionally compensate for the lack of small Rho GTPase-mediated regulation in budding yeast. Basidiomycete fungi, like *Ustilago maydis* possesses both a CRIB-containing WASP and orthologs of Sla1 and Bbc1. This suggests the coexistence of both the mammalian-like and yeast-like regulatory paradigms for actin assembly during CME. Two fundamental questions are raised: how are the two regulatory mechanisms functionally integrated in a single organism, and what are the evolutionary steps that led to this convergence? To address this question, we employed a genome editing approach in *U. maydis*, with quantitative live-cell imaging. Our results reveal the co-existence of two WASP regulatory pathways in *U. maydis*: one mediated by the small GTPases Cdc42 and Rac1, and the other by the endocytic proteins Sla1 and Bbc1. Our findings suggest that *Ustilago maydis*, with its compact genome and amenability to high-resolution imaging, serves as a powerful model for studying the mechanism and evolutionary plasticity of Rho GTPase-WASP signaling pathways in endocytosis.

MT-11*/P-19* Single-cell analysis of the switch from vegetative to filamentous growth in S.cerevisiae

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Opportunistic pathogenic fungi pose a great threat to human health, particularly to immune compromised individuals. In pathogenic fungi like *C. albicans*, filamentation is associated with host-cell attachment, tissue invasion and virulence. Thus, there is a need to understand the molecular machinery causing the switch from vegetative to filamentous growth.
In the model organism *S. cerevisiae*, exposure to low-nutrients can trigger evolutionarily conserved signaling pathways that lead to the formation of filament-like structures called pseudohyphae. This growth pattern characterized by unipolar attached growth of elongated cells can be compared to filamentation in other fungi. Most of our knowledge on this process comes from agar invasion assays and colony morphology analysis. These studies allowed to identify the main players that control this morphological transition.
However, we still have a poor understanding of how the different signaling pathways PKA, TOR and f-MAPK mainly, interact to promote this cell fate transition. Therefore, we developed synthetic promoters responsive to downstream transcription factors of each pathway and developed a microscopy assay where we can follow the dynamic transition from vegetative to filamentous growth in single cells. Using an analog sensitive allele of the MAPK Kss1, we can monitor the cross-talk between the f-MAPK and the TOR and PKA pathways by monitoring the change in the output of the expression reporters in presence of the kinase inhibitor.

POSTER PRESENTATION

S-35*/MT-12*/P-20* **From seeding to post-harvesting: sugar kelp microbiome decoding for sustainable European aquaculture**

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After rapid initial growth, the European seaweed aquaculture sector has entered a stagnation phase, presenting the unique opportunity to resolve scientific gaps crucial to sustainable and profitable sector advancement. Sugar kelp (*Saccharina latissima*) is a widely applied seaweed in food, cosmetics, bioremediation and more. During cultivation, early-life sugar kelp stages are glued onto ropes and eventually attach firmly using their holdfasts. Attachment is challenged by many factors, causing a >90% loss of unattached seeded sugar kelp. Research hints towards microbes playing an important role in early-life sugar kelp development, potentially fostering seeding attachment. Mechanical detachment of sugar kelp during harvest or detaching post-harvest remains pose additional economic and ecological risks. To limit these risks, the Dutch SEASEEDS project aims to improve cultivated sugar kelp attachment using a multidisciplinary approach, including research on allied microbiota. First, we characterized sugar kelp-associated bacterial and fungal communities by 16S and 18S rRNA gene amplicon sequencing. We collected sugar kelp and seawater samples throughout the cultivation cycle at the nearshore small-scale Oosterschelde site, seeded in by Hortimare B.V. and operated by The Seaweed Company. In turn, the microbial key players at sugar kelp detachment-prone cultivation stages (i.e., seeding, harvesting and post-harvesting) can be identified and potentially used to improve sugar kelp attachment. Secondly, we compare that study with sugar kelp samples from the first Dutch large-scale offshore farm, North Sea Farm 1, operated by the North Sea Farmers. Thirdly, we analyze sugar kelp samples from distinct European cultivation sites. These spatial comparisons allow to assess the generality of the observed microbial communities associated with cultivated sugar kelp. The entire monitoring efforts provides essential microbial insights that support the European seaweed cultivation sector advancement, promoting both economic viability and ecological sustainability.

P-21* Characterizing the role of type IV pili in bacteriophage infections of Pseudomonas aeruginosa

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Pseudomonas aeruginosa is a major cause of hospital-acquired infections and a growing public health concern due to its frequent multidrug resistance. Bacteriophages, the viruses that infect and kill bacteria, offer a promising alternative to traditional antibiotics for treating resistant infections. The type IV pili of *P. aeruginosa* are multifunctional virulence factors that mediate adhesion, motility, and biofilm formation, while also serving as receptors for viral host recognition. However, a limited understanding of how phages target pili to initiate infections constrains both the rational application of phage therapy and our knowledge of bacterial adaptation and pathogenesis.

This project addresses this knowledge gap by isolating and characterizing novel pilus-targeting phages. Using a combination of genomic analyses, phenotypic assays, and structural and microscopy-based approaches, we aim to investigate how phages recognize and exploit type IV pili to infect bacterial host cells. Preliminary data have identified a tail fiber module shared among a diverse set of pilus-targeting phages, suggesting a conserved yet versatile tool for pilus utilization. Using CRISPR-Cas-based phage genome engineering, we have exchanged tail fiber genes between phages with differing host range and showed that different alleles of this module are necessary and sufficient for specific pilus recognition.

These findings advance our understanding of type IV pilus biology, phage-host interactions, and co-evolution, while also laying the groundwork for the rational design of phage therapies targeting multidrug-resistant *P. aeruginosa* strains.

P-22* Investigating the spatiotemporal dynamics of mycomembrane protein assembly

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Members of the order Mycobacteriales display a complex cell envelope composed of an inner membrane, a thick peptidoglycan cell wall, a second polysaccharide layer called arabinogalactan (AG), and an outer membrane consisting of trehalose-capped mycolic acids. This so-called mycomembrane constitutes an important permeability barrier against antibiotics, while containing a high number of mycolate outer membrane proteins (MOMPs) which play important roles in nutrient transport. How, when, and where these proteins are incorporated into the mycomembrane is unknown and has the potential to reveal potential drug targets against pathogenic Mycobacteria. Here, we developed a labeling strategy for the detection of surface exposed proteins based on ALFA-tag specific nanobodies. Using fluorescence live-cell microscopy, we found that MOMPs of the Mycobacterial species *Corynebacterium glutamicum* are incorporated at zones of growth, namely at the poles where new peptidoglycan is also incorporated. Furthermore, using FRAP we determined that MOMPs are non-diffusive, despite the mycomembrane itself being highly diffusive. Last, we observed that treating cells with an inhibitor for AG biosynthesis strongly inhibits the incorporation of new PorH, a porin-forming MOMP, into the mycomembrane. Biochemical data from our collaborators indicates that PorH is bound by arabinose, a major component of the AG. We further demonstrated that other MOMPs, such as ProtX, follow the same spatiotemporal insertion pattern and diffusion characteristics as PorH, suggesting a general trend in MOMP biogenesis. Taken together, these findings indicate that MOMPs are incorporated during de novo cell envelope synthesis and are anchored either directly to the AG-layer of the cell wall (as in the case of PorH) or through protein-protein interactions with non-diffusive substrates. Collectively, these results provide surprising new insights into the biogenesis of the mycomembrane and its unusual biology.

P-23* Investigating the single molecule displacement dynamics of cell wall hydrolases

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Understanding the regulation of cell wall biogenesis is a fundamental goal in bacterial cell biology. However, the coordination of PG synthesis and hydrolysis remains largely elusive at both the spatiotemporal and molecular scales. Elucidating new mechanisms of PG biogenesis may provide unexplored avenues for combating the rapidly increasing number of antibiotic-resistant bacterial infections.

All bacterial cells are surrounded by a peptidoglycan (PG) cell wall, a covalently crosslinked extracellular meshwork that is essential for withstanding internal turgor pressure and determines cell shape. Bacterial growth requires the tightly coordinated synthesis and hydrolysis of PG, facilitating the expansion and remodeling of the existing polymer.

Recent advancements in bacterial cell biology have led to new insights into the spatiotemporal assembly of the cell wall. This includes the association of many Penicillin-Binding Proteins (PBPs) with cytoskeletal elements such as MreB and FtsZ.

In contrast, for cell wall hydrolases—enzymes that cleave specific bonds within the PG meshwork—such regulatory mechanisms remain largely unknown, despite their essential role in PG biogenesis alongside synthases. Since these hydrolases are localized in the periplasmic space, they lack direct contact with cytoskeletal elements, raising the key question of how their activity is coordinated with cell wall synthases.

To address this question, we have engineered fusions of hydrolases including MltG and MepM with Halo-tags to perform Single Particle Tracking (SPT) experiments. This will reveal how the activity of these hydrolases is coordinated with cell wall synthases.

Preliminary results using Total Internal Reflection Microscopy and SPT have revealed key parameters of hydrolase molecular displacement, including the fraction of active molecules and the corresponding dwell times. Further experiments focusing on hydrolases in strains with genetic and chemical perturbations provide insight into the regulation of hydrolase activity at the single molecule level.

P-24 Selective Inhibition of Mycobacterium tuberculosis GpsI Unveils a Novel Strategy to Target the RNA Metabolism

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, responsible for approximately 1.3 million deaths per year. TB treatment comprises a six month long multidrug regimen The current challenge is the emergence and spread of multi-drug resistant (MDR) strains. One strategy to address the emergence of drug-resistant strains is to further fuel the drug development pipeline with new molecules with a novel and distinct mode of action.
Ribonucleases are currently studied as potential drug targets, but identification of high affinity inhibitors has failed so far. We recently carried out a whole-cell screening of a comprehensive chemical library containing 400'000 compounds for anti-mycobacterial activity. Based on potency, selectivity and toxicity, we identified 9 promising drug lead candidates[1]. The compound, 1-(4'-(2-phenyl-5-(**trifluor**omethyl)**oxa**zole-4-carboxamido)-[1,1'-biphenyl]-4-carboxamido)**cyclopentane**-1-**carboxylic acid** ,abbreviated here as X1, piqued our interest, due to its effectiveness in the low µM range against internalized *M. tuberculosis* (MTB), without exhibiting any host cell toxicity. WGS analysis of spontaneous compound X1 resistant Mtb showed three independent SNP in *gpsI* an essential gene involved in mRNA metabolism. We now aimed to confirm *gpsI* as target of X1 and investigate its mode of action, its mechanism of resistance and the basis for its specificity.
Here we demonstrate that X1 inhibits the RNA decay pathway. We provide the biochemical characterization of the drug target as well as the Cryo-EM structure of the ternary enzyme-inhibitor-substrate complex, which can be further used for inhibitor optimization. Transcription inhibitors like rifampicin are clinically approved antibiotics interfering with RNA polymerase. However, the emergence and spread of drug resistance limits its use. The identification of a potent RNAse inhibitor and its essential target is a promising step towards the development of a novel drug-class inhibiting RNA degradation. Our current work demonstrated a novel, underexplored drug target with a promising drug lead candidate X1, targeting specifically Mtb.

P-25 Unraveling how the human immune system inhibits therapeutic phages

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Over the last decade, bacteriophage therapy has re-emerged as a promising weapon in the fight against antibiotic-resistant infections. The patient's immune system is a factor that can influence the efficacy of phage therapy; however, our knowledge on the possible interactions between phages and human immunity is very limited. This project focuses on the complement system, a series of proteins present in blood serum and tissues which activate as a proteolytic cascade upon recognition of pathogens. My previous work has shown that the complement system can block adsorption of certain phages onto their hosts, delaying their infection process. Therefore, the aim of this project is to investigate what determines whether a phage is sensitive to inhibition by serum. We have explored the activity in human serum of a highly diverse set of phages against *Escherichia coli*, the BASEL collection, by monitoring bacterial turbidity and DNA staining of lysed bacteria in real time. This approach allowed us to detect subtle differences in phage infection dynamics, revealing that complement can delay viral infection across most different families of *E. coli* phages. Our next objective is to evolve these phages in the presence of complement to select mutants that are highly active in human serum. By comparing these phages to their ancestors, we hope to find potential complement targets on the phage capsid. Our ultimate goal is to generate therapeutic phages that perform better in human serum, either by directed evolution or genetic engineering. Finally, we aim to evaluate the activity of these phages in patient-mimicking micro-tissue models, potentially contributing to the creation of more effective phage cocktails.

P-26* Pathogen-Driven Modulation of Host Communication in the Amoeba Dictyostelium discoideum

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The facultative intracellular pathogen *Legionella pneumophila* naturally replicates in free-living amoeba. *Dictyostelium discoideum* is a highly motile amoeboid organism, widely recognized for its complex cell biology, including rapid cytoskeletal rearrangements, organelle trafficking, and intercellular communication. These features make *D. discoideum* a powerful model for uncovering evolutionarily conserved processes in eukaryotic cell biology. Upon infection with *L. pneumophila*, this model organism reveals striking host-pathogen interactions, especially at the level of organelle modulation, membrane dynamics, signal transduction and metabolic reprogramming.

In recent experiments, we observed the appearance of GFP signal in infected *D. discoideum* cells producing the endosomal marker 2xFYVE-mCherry after co-incubation with uninfected, GFP-labeled donor cells. Interestingly, the frequency and intensity of GFP signal acquisition showed a clear dependency on the multiplicity of infection (MOI) and varied with different durations of co-incubation followed by nutrient starvation. These observations point to an infection-dependent, dynamic cellular process that may involve the exchange of host-derived components under defined physiological conditions.

Taken together, these findings highlight *D. discoideum* as a promising system to study poorly understood, potentially conserved mechanisms of intercellular material exchange during infection. As an organism at the evolutionary boundary between unicellular and multicellular life, the combination of genetic tractability, live-cell imaging compatibility, and its unique infection biology features position *D. discoideum* as a powerful model to explore the interface between microbial manipulation and host cell plasticity.

P-27 Assembly Curator: rapid and interactive consensus assembly generation for bacterial genomes

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Introduction

Long-read sequencing technologies enable the generation of near-complete bacterial genome assemblies. However, no *de novo* assembler is perfect – issues like duplicated or missing plasmids, spurious contigs, and failures to circularize sequences remain common problems. Achieving optimal results still requires manual consensus generation. While tools like *Trycycler* simplify this process, they are labor-intensive and require command-line expertise. With the ability to sequence hundreds of datasets quickly and affordably, there is a growing need for faster, more accessible solutions.

Methods

Here, we present *Assembly Curator*, a platform that (i) imports multiple assemblies, (ii) clusters contigs, and (iii) facilitates interactive comparison and selection through a user-friendly graphical interface. The software has a plug-in system to enable the import of data produced by different assemblers. *Assembly Curator* enables on-the-fly calculation of dotplots and can submit contig subsequences directly to NCBI's Blast servers for approximate taxonomic identification, aiding in contamination assessment. It generates standardized and informative headers in FASTA files which are directly compatible with the NCBI annotation pipeline *PGAP*.

Results and Discussion

Assembly Curator enables the semi-automatic processing of hundreds of genomes in just a few hours, significantly reducing manual effort while maintaining high assembly completeness and accuracy.
Moreover, the browser-based UI enables biologists without programming skills but domain specific knowledge to perform or participate in the curation process. This can potentially lead to superior results.

P-28 Development of shuttle vector-based transformation systems for veterinary and zoonotic chlamydiae

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In veterinary medicine, the obligate intracellular bacteria *Chlamydia* (*C.*) *abortus, C. caviae* and *C. pecorum* are known to cause ovine enzootic abortion, conjunctivitis in guinea pigs and ocular/urogenital disease in koalas, respectively. Studying the biology of these bacteria has been challenging due to a dearth of genetic tools. This study aimed to establish stable transformation systems for *C. abortus, C. pecorum* and *C. caviae* by introducing shuttle vectors carrying constitutively expressed green fluorescent proteins. With the aim to select the most suitable green fluorescent protein for the tracking of chlamydiae *in vitro,* we further compared the fluorescence intensity of GFP to that of mNeonGreen. Shuttle vectors used for transformation comprised the native plasmid of the chlamydial species of interest, an *Escherichia coli* origin of replication (*ori*), a beta-lactamase (*bla*) or spectinomycin (*aadA*) resistance gene, and GFP or mNeonGreen placed under a *Neisseria meningitidis* promoter (nmP) for heterologous fluorescence expression. We compared the success of a *C. suis*-tailored transformation protocol (Protocol A) to an alternative protocol for *C. psittaci* and *C. trachomatis* (Protocol B), both of which employ calcium chloride for competence induction. We obtained stable transformants for *C. pecorum* and *C. caviae* using Protocols A and B, respectively, and we found that GFP is superior to mNeonGreen if used for heterologous expression in chlamydiae. In contrast, pre-incubation with trypsin-EDTA prior to the application of calcium chloride was needed to obtain transformants of *C. abortus*. The transformation efficiency of naturally plasmid-carrying avian strains was noticeably higher than that of plasmid-free ruminant *C. abortus*. Taken together, we established protocols for stable calcium-chloride mediated transformation for *C. pecorum* and *C. abortus* and expanded upon the genetic toolbox of *C. caviae.*

This work opens new avenues to broaden our understanding of these complex zoonotic bacteria.

P-29 T3SS effectors implicated in the early phase of the W. chondrophila developmental cycle

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Waddlia chondrophila is an emerging pathogen which causes adverse pregnancy outcomes in humans and abortion in ruminants. It is a strict intracellular bacterium that alternates between an extracellular infectious form (EB) and a replicative form (RB). The precise mechanisms involved in the transition from one form to the other have not been characterized yet.
Immediately after entry into the host cell, EBs must escape host defense mechanisms and establish their replicative niche, called an inclusion. For this purpose, bacteria secrete effectors in the host cell cytoplasm mainly via the Type 3 Secretion System (T3SS). We compared the transcriptomes of EBs and RBs, by RNA sequencing, to select genes which are highly upregulated in EBs, and we coupled this analysis with algorithms that predict T3SS-mediated secretion of the selected proteins. Hypothetical proteins Wcw_0260, Wcw_0429 and Wcw_1046 were selected for further studies.
RT-qPCR analysis along the bacterial life cycle confirmed that expression of the three genes is the highest during the early cycle, i.e. 0 to 6 hours post infection. Moreover, Wcw_0260 and Wcw_1046 were shown, in *Yersinia enterocolitica,* to be secreted by the T3SS.
To gain insight into their subcellular localization, the proteins were expressed in transfected HeLa cells. Wcw_0260 and Wcw_1046 localized to the plasma membrane, while Wcw_0429 associated with mitochondria. Interestingly, when overexpressed in *C. trachomatis,* Wcw_0260 induced the formation of aberrant bodies, which suggests a putative role for this protein in the establishment of the bacterial persistent form.
In the future, antibodies will be raised against these proteins and will be used in infected cells to assess secretion by immunofluorescence microscopy. Moreover, Co-IP will be performed to identify possible eukaryotic interactors, with the ultimate goal to define the role of Wcw_0260, Wcw_0429 and Wcw_1046 in adhesion/entry of EBs, bacteria survival, inclusion biogenesis or EB to RB transition.

P-30 disruption of undecaprenyl phosphate recycling suppresses ampC beta-lactamase induction in pseudomonas aeruginosa

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Undecaprenyl phosphate (UndP) is a lipid carrier essential for bacterial cell wall synthesis, responsible for transporting peptidoglycan (PG) precursors across the inner membrane. Members of the DedA superfamily were recently identified as UndP flippases that recycle this lipid carrier. Impairing UndP transport leads to the cytoplasmic accumulation of the PG precursor, UDP-MurNAc-pentapeptide (UDP-M5).

In *Pseudomonas aeruginosa*, resistance to beta-lactam antibiotics is primarily mediated by the chromosomally encoded beta-lactamase AmpC. Its expression is controlled by the transcription factor AmpR, which responds to intracellular PG fragments. Under normal conditions, AmpR binds the PG precursor UDP-M5 and represses *ampC*. During beta-lactam treatment or in mutants with disrupted PG recycling, such as the *ampD mutant*, alternative fragments (anhydromuropeptides) accumulate and activate AmpR, leading to high-level *ampC* expression and beta-lactam resistance.

We hypothesized that altering the balance of PG precursors by disrupting UndP recycling could reduce *ampC* expression even in derepressed strains. We show that deletion of *dedA4*, which encodes a predicted UndP flippase in *P. aeruginosa*, causes UDP-M5 accumulation and significantly reduces AmpC production and beta-lactam resistance in an *ampD* mutant. These findings demonstrate that PG precursor availability influences beta-lactamase regulation, identifying DedA4 as a potential therapeutic target. Inhibiting UndP recycling may represent a novel strategy to restore beta-lactam efficacy against resistant *P. aeruginosa* and other AmpC-producing pathogens.

P-31* MeSS and assembly_finder: A toolkit for in silico metagenomic sample generation

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Introduction

Numerous bioinformatics tools have been developed to taxonomically classify shotgun metagenomics data, highlighting the need to evaluate their performance (Ye *et al.*, 2019). To achieve this, in-silico data simulation offers a controlled environment for benchmarking, where ground truth parameters are known in advance (Gourlé *et al.*, 2019; Milhaven and Pfeifer, 2023). Among existing tools, CAMISIM is widely used (Fritz *et al.*, 2019). However, it is relatively slow and lacks support for latest long-read error profiles such as ONT R10.4.1. To address this, we introduce assembly_finder and MeSS, two user-friendly Bioconda packages designed to streamline genome retrieval and metagenome simulation

Methods

To assess MeSS, we simulated metagenomes based on 20 real samples from four body sites (buccal mucosa, throat, gut, and vagina) obtained from the Human Microbiome Project (Huttenhower *et al.*, 2012). Taxonomic profiles were generated using Kraken2 (Wood *et al.*, 2019) and Bracken (Lu *et al.*, 2017), and one reference genome per species was retrieved using *assembly_finder*. Simulations were conducted with MeSS and CAMISIM with shared parameters, producing 100bp paired-end reads based on the empHiseq2k error profile within a Nextflow (Di Tommaso *et al.*, 2017) pipeline. Community structure was compared using alpha and beta diversity metrics and visualized by NMDS ordination plots. Resource usage was benchmarked by simulating datasets with up to 2000 bacterial genomes.

Results

MeSS and CAMISIM generated communities closely resembling real metagenomes with preserved species relative abundance (PERMANOVA on Bray-curtis distance F= 0.08, p=1.000). MeSS outperformed CAMISIM in speed and memory efficiency, being approximately 18 times faster across samples with increasing number of genomes.

Conclusion

MeSS and *assembly_finder* provide a scalable, reproducible toolkit for simulating realistic metagenomes and benchmarking taxonomic classifiers. With many parameterization options and support for illumina, PacBio and ONT sequencing technologies, they enable customizable scenario-based simulations.

P-32 Characterisation of a novel metallo-beta-lactamase variant, HMB-3, identified in Pseudomonas asiatica

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Aims
The B1 subclass MBL, HMB-1, was described in 2017 from a clinical *Pseudomonas aeruginosa* isolate, first recovered in 2012 from a rectal swab in Germany and one other HMB variant, HMB-2, has been reported in *P. aeruginosa* in the USA.
Here, we describe the identification and characterisation of a novel HMB variant, HMB-3, found in a *P. asiatica* isolate in Switzerland.

Methods
A carbapenem-resistant isolate (Psa-HMB-3), was submitted to the Swiss National Reference Centre for Emerging Antibiotic Resistance for further investigation. Susceptibility testing was performed by disc diffusion, and MICs were determined by broth microdilution. The isolate was subject to whole genome sequencing (WGS) using both Illumina and Oxford Nanopore platforms. The bla_{HMB} allele was amplified and cloned into pCR-Blunt II-TOPO, before transformation into *E. coli* Top10. Site direct mutagenesis was performed on bla_{HMB-3} in plasmid pTOPO/HMB-3.

Results
Isolate Pas-HMB-3 was recovered from a tracheostoma wound swab of a 45-year male.
Pas-HMB-3 was resistant to all tested beta-lactams except for aztreonam. No carbapenemase genes were identified by routine laboratory tests (PCR, immunochromatographic tests). WGS identified the isolate as *P. asiatica*, a member of the *P. putida* group, and could be assigned to ST15. Subsequent analyses allowed the identification of a novel MBL encoding gene, HMB-3, differing from HMB-1 by 23 amino-acid substitutions. Susceptibility testing of recombinant *E. coli* strains showed that HMB-3 conferred resistance to most beta-lactams (except piperacillin (4 mg/L), aztreonam (0.125 mg/L) and cefiderocol (FDC) (2 mg/L). HMB-3 also conferred resistance to the beta-lactam/beta-lactam inhibitor combination cefepime/taniborbactam. Site direct mutagenesis was performed on bla_{HMB-3} identified that a single amino acid change, within active site loop 10, resulted in a FDC MIC reduction of 2 to 0.25 mg/L.

Conclusions
We described a novel HMB variant, HMB-3, capable of conferring resistance to most beta-lactams and with activity against FDC.

P-33* The Legionella effector RidL subverts the conserved, mitochondria-localizing large GTPase Vps1 in yeast

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The causative agent of Legionnaires' disease, *Legionella pneumophila*, is a facultative intracellular bacterium, which secretes more than 300 different effector proteins into eukaryotic host cells, where they subvert a plethora of cell biological processes. The effector protein RidL binds through a 29 kDa N-terminal fragment to the retromer coat complex subunit Vps29, thereby displacing the Rab7 GTPase activating protein (GAP) TBC1D5 from the retromer. The function of the C-terminal portion of RidL is not known. In this study, we used yeast as a model to further assess the mode of action of RidL. We show that RidL interacts via a 75 kDa C-terminal fragment with the conserved large fission GTPase Vps1 of *Saccharomyces cerevisiae*. The binding affinity was enhanced by the presence of a non-hydrolyzable GTP analogue, and the binding constant K_D was approximately 31.5 nM. RidL and a C-terminal fragment also bound to the Vps1_GG fragments of the yeasts *S. cerevisiae* and *Chaetomium thermophilum*, which comprise the GTPase and GTPase effector domain (GED) but lack the "stalk" domain of the large GTPase. Purified RidL and a C-terminal RidL fragment but not an N-terminal fragment reduced the GTPase activity of Vps1, and full length RidL inhibited Vps1 filamentation induced by a non-hydrolyzable GTP analogue. Finally, RidL and a C- but not an N-terminal fragment as well as Vps1 were enriched in subcellular mitochondrial fractions.
Taken together, the *Legionella* effector RidL interacts through its C-terminus with the conserved yeast large fission GTPase Vps1 on mitochondria. Our study validates the use of yeast as a model to functionally study the mode of action of bacterial effector proteins.

P-34 The Lausanne University Hospital GMP production pipeline for manufacture of therapeutic bacteriophages.

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Background
In June 2023, Swissmedic officially authorized the CHUV to produce therapeutic bacteriophages in accordance with Good Manufacturing Practices (GMP), to our knowledge as the first academic institution worldwide.

Methods
The purification process consists in i) the production of the lysate by co-incubating a production bacterial strain with a specific phage selected to target the strain infecting the patient; ii) the frontal filtration of the lysate followed by two centrifugations to obtain the bacteriophage concentrate; ii) the removal of endotoxins using a specific resin, and iii) the conditioning to obtain the final bacteriophage suspension (SBP). To verify that SBPs are within defined specifications, quality control tests are performed, including phage identification and purity tests (residual endotoxin, total residual bacterial DNA and proteins).

Results
Our process yields batches of GMP phages at 1E+08 PFU/mL - 1E+09 PFU/mL with injectable endotoxin levels and traces of residual bacterial DNA and proteins. While titers in saline of tested phages decreased by 4 log10 in 12 days, the addition of an undisclosed stabilizer led to increased stability to at least nine months. We began treating patients on a compassionate use framework in February 2024, with authorizations from the cantonal pharmacist in each case.

P-35

Clinical microbiology under pressure: evaluating the impact of false positives in BD MGIT 960 pyrazinamide susceptibility testing in Mycobacterium tuberculosis

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Background
Pyrazinamide (PZA) is a critical first-line drug in tuberculosis (TB) treatment. The Mycobacteria Growth Indicator Tube (MGIT) 960 system Becton Dickinson (BD) is the only WHO-recommended method for PZA drug susceptibility testing (DST). However, concerns about its accuracy have emerged. In 2024 we reported a rise in false positive (FP) PZA resistance to BD. In 2025, BD issued a global alert regarding an increase in FP results and announced the suspension of MGIT PZA kit production effective May 2025.

Objectives
We aimed to assess the extent of the FP PZA resistance, evaluate its diagnostic impact, and explore potential approaches to distinguish FP from true positive (TP) results.

Methods
We retrospectively analyzed 176 clinical *Mycobacterium tuberculosis* isolates tested for PZA susceptibility using MGIT 960 system between January 2023 and May 2025. Confirmatory testing included repeat MGIT assays and sequencing of *pncA* and *gyrB* genes; the latter identified *Mycobacterium bovis*, intrinsically resistant to PZA. Additionally, we evaluated the MGIT growth index and time to positivity (TTP) for potential predictive value.

Results
Between 2023 and 2025, 9% of isolates were confirmed as TP PZA-resistant mostly due to *M.bovis*. The FP rate showed an increasing trend: from 18% in 2023 to 33% in early 2025. Of 176 strains tested, 47 (27%) were FP, 16 (9%) TP, and 112 (64%) true negatives. The mean TTP was 7.2 days for TP and 5.7 days for FP, not statistically significant. The H37Rv reference strain yielded a FP rate of 25% (4/16 runs). Confirmatory testing increased laboratory workload but prevented reporting of FP results.

Conclusions
Our data highlight a high rate of FP PZA resistance results with the MGIT 960 system, with no robust phenotypic alternative currently available. Molecular confirmation through *pncA* and *gyrB* sequencing remains essential for ensuring diagnostic accuracy and guiding appropriate TB treatment decisions.

P-36 Report of a food-human genetic cluster of extended-spectrum beta-lactamase–producing Klebsiella pneumoniae ST14 within a defined spatiotemporal context

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Background and Objectives
Extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* (ESBL-*K. pneumoniae*) is a significant public health threat, classified by the WHO as a critical priority pathogen. While healthcare settings are recognized as reservoirs for transmission, the role of foodstuffs in the dissemination of ESBL-producing Enterobacterales (ESBL-PE) remains underexplored. This study investigates the genetic and epidemiological links between ESBL-*K. pneumoniae* ST14 isolates from food and human clinical samples in a defined spatiotemporal context.

Methods
A prospective study (ClinicalTrials.gov identifier: NCT03465683) was conducted in Basel, Switzerland (06/2017–06/2019), involving the collection of clinical, foodstuff, and environmental samples. Whole-genome sequencing was performed to assess genetic relatedness among ESBL-isolates using Illumina and Oxford Nanopore platforms. Core-genome multilocus sequence typing (cgMLST) and single nucleotide polymorphism (SNP) analyses were conducted to assess genetic relatedness. Antimicrobial resistance genes and plasmids were characterized and compared with international databases to evaluate genomic context.

Results
We identified a cluster of ESBL-*K. pneumoniae* ST14 isolates, including one from an alfalfa-cress sample and two from patients hospitalized at the University Hospital Basel. Genomic analyses revealed close genetic relationships (three and six cgMLST allelic differences, seven and 12 SNPs) between the food and the clinical isolates, suggesting a common ancestor. International genomic comparisons linked the isolates to two clinical strains from Germany and highlighted a distinctive plasmid carrying $bla_{\text{CTX-M-15}}$ and other resistance genes. The Swiss and German isolates differed from other *K. pneumoniae* ST14 genomes by at least 58 allelic differences.

Conclusions
This study supports foodstuffs as potential reservoirs for clinically relevant ESBL-*K. pneumoniae* and underscores the need for stringent hygiene practices across the food production chain. The genomic analyses support further investigation into foodborne transmission routes to mitigate the public health risks posed by antimicrobial resistance.

P-37

Impact of acquired broad-spectrum β-lactamases on susceptibility to oral cephalosporin (ceftibuten) and oral carbapenem (tebipenem) in combination with new β-lactamase inhibitors ledaborbactam and xeruborbactam in Escherichia coli.

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Aims

The spread of antimicrobial resistance in *Escherichia coli* has resulted in more prolonged hospital stays and difficult IV treatments for complicated urinary tract infections. However, new oral ß-lactam (BL) including oral cephalosporin, ceftibuten (CTB), and oral carbapenem, tebipenem (TEB), are currently being developed in combination with novel oral ß-lactamases inhibitors (BLI), avibactam (AVI), xeruborbactam (XERU) and ledaborbactam (LEDA). Hence, the aim of our research was to assess the impact of broad-spectrum β-lactamases in *E. coli* on susceptibility to the novel oral BLBLI combinations CTB-AVI, CTB-XERU, CTB-LEDA, TEB-AVI, TEB-XERU and TEB-LEDA.

Materials

Most representatives broad-spectrum ß-lactamases including class A (TEM-like, OXY-2, CTX-M-like, GES-like, BEL-like, SHV-like, PER-like, VEB-1, FRI-1, IMI-1, SME-1, NMC-A, KPC-like), class B (AIM-1, DIM-1, GIM-1, NDM-like, VIM-like, IMP-1, SPM-1), class C (DHA-1, CMY-like, ACT-like, ACC-1, FOX-5, MIR-17) and class D (OXA-like) were cloned into plasmid pUCp24 and replicated into isogenic *E. coli*. MICs were determined by broth microdilution for, CTB, TEB and their combinations with BLI. Concentrations of all BLI was fixed at 4 mg/mL. The susceptibility breakpoints for CTB-based and TEB-based combinations were respectively defined by ≤ 1 mg/ml and ≤ 4 mg/ml.

Results

The combinations CTB-AVI and CTB-LEDA, showed efficacy against most *E. coli* recombinant strains expressing Class A and D β-lactamases, but limited effectiveness against class C producers. TEB-AVI and TEB-LEDA were effective against strains producing Class A, C and D β-lactamases. TEB alone displayed a wider coverage than CTB-AVI and CTB-LEDA combinations against the *E. coli* collection. The combinations CTB-XERU and TEB-XERU showed low MIC values for almost all strains tested. Finally, TEB-XERU was the combination with the broadest spectrum and the highest efficacy against the recombinant strains, including metallo-β-lactamase producers;

Conclusions

This study highlighted the excellent performance of all newly developed oral BLBLI combinations against a broad spectrum of ß-lactamase producing *E. coli.*

P-38 Evaluation of the ELITech Meningitis Viral MGB Panel molecular assay

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Introduction
Common infectious etiologies of meningitis and encephalitis are Herpes simplex viruses (HSV) and Varicella Zoster virus (VZV). Rapid diagnosis and treatment are critical to minimize morbidity and mortality. We conducted a retrospective evaluation of the performance of the qualitative Meningitis Viral (MV) MGB molecular panel on cerebrospinal fluid (CSF). The study was extended to swab samples. The set up of an external calibration method for CSF viral quantification was also assessed.

Methods
A total of 7 frozen CSF, 30 swab samples from 35 patients and 20 external quality control (EQC) samples were analysed on the fully automated nucleic acid (NA) extraction and amplification BeGenius system for qualitative evaluation. Samples were previously tested on the Light Cycler (Roche) with the TibMolBiol HSV1/2/VZV molecular assay (Roche) after automated NA extraction on emag (bioMérieux). Reference material with known viral concentrations was used to established calibration lines. A qualitative evaluation of the results was determined by the Kappa coefficient and a quantitative correlation was assessed using the correlation coefficient r^2. The limit of detection (LoD) was also evaluated for the three viruses in the CSF.

Results
The qualitative evaluation showed that the results were concordant for the three viruses, demonstrating 100% of sensibility and specificity compared to the reference tests with a Kappa coefficient of 1. The quantitative evaluation reported a r^2 of 0.98 for HSV-1 and HSV-2 and 0.99 for VZV. The mean LoD for the three viruses was 350 copies/ml.

Conclusion
A high correlation was observed between the ELITech system and emag NA extraction and Light Cycler amplification. The MV MGB molecular panel exhibits high performances to detect and quantify HSV-1, HSV-2 and VZV in CSF and swab samples. Viral quantification in CSF samples can be extrapolated with the reference material with a linear regression equation.

P-39 In vivo development of high-level cefiderocol and ceftazidime-avibactam resistance in KPC-producing Klebsiella pneumoniae associated with mutations in KPC and the sensor histidine kinase, EnvZ

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Background
The β-lactam-β-lactamase inhibitor combination, ceftazidime-avibactam (CAZ-AVI), is used as a last-resort treatment for KPC-producing *Klebsiella pneumoniae* (KPC-Kp), however resistance has been frequently reported since its introduction, usually attributed to mutations within the bla_{KPC}. Here we describe a case where the treatment of CAZ-AVI-S/FDC-S KPC-Kp infection with CAZ-AVI, resulted in high-level FDC- and CAZ-AVI-R due to a combination of bla_{KPC} and *envZ* mutations.

Methods
Isolates were obtained from a 78-year-old male in the University Hospital "Città della salute e della scienza di Torino". During routine screening a CAZ-AVI- and FDC-S *K. pneumoniae* strain was isolated (KP1). The patient was treated with meropenem/vancomycin and CAZ-AVI/daptomycin/gentamicin. After 20 days, a CAZ-AVI- and FDC-R KPC-Kp (KP2) was isolated from thoracotomy wound pus.
Susceptibility testing was performed by broth microdilution. Isolates were subject to WGS. KPC alleles were cloned into the pTOPO-Blunt vector and expressed in *Escherichia coli* Top10. A complementation assay was performed by cloning the wildtype *envZ/ompR* into vector pACYC184, before transformation into KP2.

Results
KP1 and KP2 exhibited CAZ-AVI MICs of 8 and >256 mg/L, and FDC MICs of 2 and >256 mg/L. Both isolates were ST512 and harboured an identical resistance gene content, with the only one exception; KP1 harboured bla_{KPC-3} and KP2 harboured $bla_{KPC-121}$. SNP analyses identified that both strains were closely related, suggesting that KP2 was derived from KP1. Cloning and expression of both bla_{KPC-3} and $bla_{KPC-121}$ showed that the KPC variant was responsible for the high CAZ-AVI MICs but only resulted in a modest increase in FDC MICs (0.5 to 2 mg/L). WGS analyses identified a mutation within *envZ*, V147G, and complementation with the wildtype *envZ/ompR* resulted in a reduction of FDC MICs from >256 to 16 mg/L.

Conclusions
This study illustrated the contribution of both bla_{KPC} and *envZ* mutations to high-level CAZ-AVI and FDC resistance.

P-40 nanopore sequencing for enhanced bacterial pathogen identification in clinical diagnostics

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Aims
While 16S eubacterial PCR coupled with Sanger sequencing has been a standard for rapid and inexpensive culture-independent identification of bacterial pathogens in clinical samples, its ability to resolve polymicrobial infections is limited. Although Illumina next-generation sequencing offers superior resolution, it comes with higher costs and longer turnaround times. To overcome these drawbacks, we introduce a novel diagnostic approach utilizing 16S eubacterial PCR in conjunction with third-generation Oxford Nanopore Technologies (ONT) sequencing.

Methods
We assessed the diagnostic performance of 16S PCR-ONT sequencing by comparing it with Illumina-based 16S amplicon sequencing. This involved sequencing amplicons from more than 100 routine clinical samples. We evaluated the sensitivity of our approach by sequencing dilution series of two bacterial species to verify its diagnostic capabilities. The resulting ONT reads were analyzed with the LORCAN pipeline, while Illumina reads were processed using the short-read optimized *dada2* tool. Discrepant results were reviewed by an experienced infectiologist based on patient history and ancillary clinical results.

Results
Our results underscore the robust and accurate ability of ONT sequencing to capture the diversity of bacteria in clinical specimens. The combination of ONT sequencing and rapid data analysis allows for a turnaround time of less than one working day from DNA sample to bioinformatic results, while remaining cost-effective. The improved resolution of ONT compared to Sanger sequencing provides a distinct advantage in detecting pathogens in complex or low-DNA concentrated samples. Moreover, ONT sequencing achieves a taxonomic resolution similar to Illumina but with the practical benefits of smaller batch sizes and faster sequencing, while maintaining high clinical accuracy in bacterial species identification.

Conclusion
The combination of 16S eubacterial PCR and ONT sequencing marks a notable improvement in clinical microbial diagnostics, offering enhanced sensitivity and specificity compared to Sanger sequencing, as well as better cost-effectiveness and turnaround time than Illumina sequencing.

P-41

Evaluation of 4 PCR assays for detection of SARS-CoV-2, influenza A/B and Respiratory Syncytial Virus on Coyote Flash10

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Introduction

Rapid detection of respiratory diseases, such as coronavirus (SARS-CoV-2), influenza A/B virus (FluA/B) and respiratory syncytial virus (RSV) is important for the management of vulnerable patients and the organization of hospital services. The aim of this study was to compare the performance characteristics of different PCR kits from Coyote Bioscience Co (FlashDetect™ LyocartE: SARS-CoV-2, SARS-CoV-2&FluA&FluB, SARS-CoV-2&FluA&FluB&RSV and FluA&FluB&RSV Assay) with Xpert® Xpress Cov-FLU-RSV plus PCR kit from Cepheid or Cobas® SARS-CoV-2&influenza A/B or Cobas® SARS-CoV-2 PCR kits from Roche.

Methods

We retrospectively tested 92 nasopharyngeal samples with the Coyote Flash10 and the reference systems: 75 positive (21 RSV, 30 FluA/B, 24 SARS-CoV-2) and 17 negative. The positive samples tested exhibited Ct between 12 and 37, 40-54% of them with Ct above 30. Assay agreement was assessed by Cohen's kappa coefficient (KC). A comparison of the limit of detection of the Coyote Flash10 and the Cobas LIAT PCRs was performed by testing serial dilutions of several samples.

Results

For SARS-CoV-2 kit, we obtained a KC of 0.95. For the SARS-CoV-2&FluA&FluB kit, we obtained KC of 0.84 for SARS-CoV-2 and 0.91 for FluA/B. For the SARS-CoV-2&FluA&FluB&RSV, we obtained KC of 0.96 for SARS-CoV-2 and 0.97 for FluA/B and of 1 for RSV. For the FluA&FluB&RSV kit, we obtained KC of 0.96 for fluA/B and 1 for RSV. All the discordant results showed Ct above 30. The limits of detection determined by serial dilutions of positive samples were similar between the Coyote Flash10 and the Cobas LIAT for all kits tested.

Conclusion

Overall, the Coyote Flash10 exhibit similar performances than the reference tests Cobas LIAT and GenXpert. Discordant results were only observed with high Ct values which were over-represented in this study (40-54%) compared to the Cts observed in routine diagnostic (31.6% in 2024 at Admed)

P-42 Diagnostic algorithm for detection of carbapenemases and ESBLs in carbapenem-resistant Pseudomonas aeruginosa

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Background
Pseudomonas aeruginosa can acquire carbapenem resistance through several mechanisms, including genomic mutations causing the overexpression of efflux pumps, intrinsic AmpC β-lactamase, or reduced membrane permeability (porin loss). Alternatively, resistance can arise from the acquisition of plasmid-mediated carbapenemases and/or ESBLs. Unfortunately, carbapenemase/ESBL-producing-CRPA (CP/ESBL-CRPA) cannot be reliably distinguished from non-carbapenemase-producing-CRPA (non-CP-CRPA) solely on antimicrobial susceptibility testing results of conventional β-lactam-antibiotics. However, these two groups exhibit different activity profiles when tested against novel β-lactam/β-lactamase inhibitor (BL/BLI)-combinations. We developed and validated a simple diagnostic algorithm to screen and confirm carbapenemase and ESBL production in CRPA.

Methods
We determined disc diffusion growth inhibition zones and gradient strip minimal inhibitory concentration (MIC) values for 136 whole-genome-sequenced, international CRPA isolates. This collection included 70 metallo-β-lactamase (MBL)-producing isolates, 1xGES-5 producer, 1xKPC-2 producer, 12xESBL producers, and 53xAmpC-hyperproducing isolates. The antibiotics tested were ceftolozane-tazobactam (C-T), ceftazidime-avibactam, imipenem-relebactam (I-R), meropenem-vaborbactam, cefepime-enmetazobactam (C-E), and aztreonam-avibactam, the latter tested only by E-test. Additionally, we employed a lateral flow immunoassay (Carba-5, NG-Biotech) to confirm MBL production and conducted double disc synergy testing (DDST) to enhance ESBL detection.

Results
C-T was the most effective screening antibiotic for distinguishing MBL and ESBL producers from AmpC-hyperproducing CRPA, achieving 100% sensitivity for both MBL and ESBL producers. I-R reliably confirmed MBL production in C-T positive CRPA isolates, with 92.8% sensitivity and 100% specificity. Incorporating Carba-5 into the phenotypic algorithm improved sensitivity for confirming MBL production to 100%. For the remaining C-T positive but I-R negative isolates, C-E showed 75% sensitivity and 78.6% specificity for detecting ESBL production. Additionally, the DDST confirmed ESBL production in 6 out of 9 ESBL producers

Conclusion
We established a simple, cost-effective, and reliable diagnostic algorithm that enables screening and confirmation of carbapenemase and ESBL production in CRPA. This diagnostic algorithm supports rapid identification of resistance mechanisms, enabling timely and targeted treatment interventions.

P-43

Evaluation of commercial susceptibility testing methods for in vitro determining daptomycin susceptibility in vancomycin-resistant-Enterococcus faecium

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Background

Daptomycin is an important treatment option for infections caused by vancomycin-resistant-*Enterococcus faecium* (VRE*fm*). AST requires fixed Ca^{++}-concentrations (50mg/L). As this is difficult to control in Mueller-Hinton-agar, disk diffusion is not suitable. Beyond commercial BMD methods, gradient diffusion strips are widely used, ensuring consistent Ca^{++}-concentrations within the strip. However, variability in AST-results across methods has been observed, especially at borderline susceptibilities. We compared the performance of various commercial BMD methods and the E-test®.

Methods

We tested 89 VRE*fm* clinical isolates with borderline daptomycin susceptibility. The EUCAST and CLSI method with manual BMD in Mueller-Hinton-broth supplemented with 50mg/L Ca^{++} served as the reference method for MIC-determination. We compared the following commercial BMD-methods: (i) Sensititre EUVENC (ThermoFisher), (ii) Phoenix (BD), (iii) VITEK-2 (bioMérieux), (iv) UMIC (Bruker), and E-test® (bioMérieux). Isolates were categorized based on standard-BMD-MICs and the CLSI CBPs (S ≤4 mg/L, R >4 mg/L). We assessed Categorical agreement (CA), essential agreement (EA), bias, very Major Errors (vME), and Major Errors.

Results

Most isolates (77/89,86.5%) exhibited MICs within ±1 fold-dilution of the CLSI CBP. Phoenix showed the best performance, with EA=92% and a CA=69%, and vMEs observed in 22 cases (25.3%). EUVENC exhibited an EA=87.6%, a CA=74.2%, and a vME rate of 24.7%. UMIC achieved an EA=74.2%, a CA=70.8%, and a vME rate of 27%. E-test® showed EA=68.5%, CA=66.3% and a vME rate of 32.6%. VITEK-2 performed the lowest, with an EA=67.4%, CA=64%, and a vME rate of 36%. All methods showed high negative biases (≤-57.3%).

Conclusion

None of the methods met ISO-20776-2:2021 criteria (EA/CA≥90%), and all showed significant negative biases, indicating a tendency to underestimate MICs. Commercial BMD methods performed best while VITEK-2 and E-test® were unreliable in detecting elevated MICs. AST variability near breakpoints must be considered when interpreting daptomycin susceptibility for VREfm.

P-44 In vitro activity of zosurabalpin against carbapenem-resistant-A. baumannii (CRAB) clinical isolates from diverse origins

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Background

The limited treatment options for Carbapenem-Resistant *Acinetobacter baumannii* (CRAB)-invasive infections, including colistin, cefiderocol, and sulbactam-durlobactam, highlight the urgent need for new therapeutic agents. Zosurabalpin belongs to a novel class of tethered macrocyclic peptide antibiotics—the first new antibiotic class discovered in years. It specifically targets the $LptB_2FGC$ complex, essential for lipopolysaccharide transport in Gram-negative-bacteria. In this study, we evaluated the *in vitro* efficacy of zosurabalpin against 148 CRAB global clinical isolates and compared zosurabalpin's performance with currently available antibiotics, including rifabutin, which is also under investigation for CRAB treatment.

Methods

Hundred-forty-eight CRAB clinical strains isolated from the Institute of Medical Microbiology, University of Zurich, Switzerland; Ben-Gurion University of the Negev, Israel; and SBÜ Hamidiye Etfal Hospital, Turkey, were included in the study. All isolates carried plasmid-borne carbapenemases, including 64xOXA-23, 8xOXA-40, 11xOXA-72, 27xOXA-23/OXA-40, 2xOXA-23/OXA-72, 2xNDM-1, 10xNDM/OXA-23, 18xNDM/OXA-40, 4xNDM/OXA-58, 1xNDM/OXA-72, and 1xVIM/OXA-23/OXA-40. Zosurabalpin minimal inhibitory concentrations (MICs) were determined by broth microdilution according to CLSI guidelines, using Mueller-Hinton medium supplemented with 20% heat-inactivated horse serum. MICs of the remaining antibiotics were determined according to the EUCAST method: colistin and sulbactam-durlobactam with Mueller-Hinton medium (with a fixed durlobactam concentration of 4 mg/L), cefiderocol[4] with iron-depleted Mueller-Hinton medium, and rifabutin with RPMI.

Results

Zosurabalpin MICs ranged from ≤0.06 to 1 mg/L, with no CRAB isolates exhibiting elevated MICs beyond the distribution of the wild-type population, regardless of the carbapenemase type. The MIC_{90} values for cefiderocol, sulbactam-durlobactam, colistin, and rifabutin were 8, >128, 1 and >16 mg/L, respectively. Zosurabalpin demonstrated an MIC_{90} of 0.5 mg/L, indicating potent *in vitro* activity and no cross-resistance with any of the tested antimicrobials.

Conclusion

These independent results position zosurabalpin as a very promising therapeutic alternative for CRAB infections, including metallo-carbapenemase-producing-CRAB, with the potential to overcome current treatment limitations and contribute to combating the global rise of multidrug-resistant pathogens.

P-45* Identification and characterization of Legionella effectors targeting the mitochondrial network

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Intracellular bacteria manipulate and subvert cellular processes in their eukaryotic hosts in a sophisticated manner. While the facultative intracellular pathogen *Legionella pneumophila* naturally replicates in free-living amoeba, inhalation of this pathogen can lead to a severe pneumonia due to the ability of the bacterium to replicate in alveolar macrophages. During the intracellular state, *L. pneumophila* secrets over 300 "effector proteins" into their respective hosts. This enables the formation of a *Legionella*-containing vacuole (LCV) by subverting phagosome maturation and other cellular processes. While LCV formation is essential for the survival and replication of the pathogen, many other host cellular processes are targeted and subverted to further support the survival and replication of intracellular *L. pneumophila*.

Mitochondrial function and dynamics are essential for virtually all cellular processes. Not surprisingly, intracellular bacteria such as *L. pneumophila* specifically target and modulate mitochondrial components and their function. In this study, we identified several *L. pneumophila* effector proteins localizing to and modulating mitochondria. Some *L. pneumophila* deletion mutant strains lacking individual mitochondria-targeting effector proteins are impaired for intracellular replication and inhibit host cell respiration less efficiently compared to the parental strain.

Ongoing studies aim at a mechanistic characterization of mitochondria-targeting *L. pneumophila* effector proteins using cell-biological and biochemical approaches. Specifically, the work aims at characterizing a putative kinase targeting mitochondria. Overall, this project will provide insights into the virulence of *Legionella* and will potentially make novel bacterial probes available for specific activation or inhibition of mitochondrial components.

P-46*

Evaluating long-term stool preservation methods for maximizing the recovery of viable human fecal microbiota

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The gut microbiome plays a fundamental role in human health, prompting efforts to catalogue and preserve its diversity across human populations. While DNA sequencing dominates microbiome research, cultivation remains essential for mechanistic studies and therapeutic development. Yet, best practices for long-term stool preservation remain limited. Here, we compared eight cryopreservation treatments for maintaining viable stool microbiota over a 1-year storage period at -80°C (freezers) or at -196°C (liquid nitrogen), using samples from a total of 15 Swiss infants, children, and adults. Combining cultivation on six media with 16S rRNA sequencing, we show that ultra-low temperature cryopreservation has minimal impact on microbiome diversity compared to fresh cultures. However, there were small but notable shifts in the microbial community, which were consistent across all treatments, driven by the depletion of slow growing bacteria rather than freeze-thaw stress. Standard glycerol preservation and simple snap-freezing was shown to performed comparably to more complex and costly protocols. Machine-learning sample classification correctly predicted the culture to the correct donor and demonstrated that the cultured samples retained donor-specific microbial signature of each donor even after a year of storage. Taken together, our findings offer practical, low-cost strategies for stool biobanking.

P-47 Prevalent Chromosome Fusion in Vibrio cholerae O1

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Cholera is a waterborne infectious disease affecting millions of people yearly and frequently arising where sanitary infrastructure is inadequate. Cholera is caused by a pathogenic lineage of *V. cholerae* (*Vc*). *Vc* typically carries two chromosomes. Of thousands of sequenced genomes, only three *Vc* with fused chromosomes have been reported to date from natural environments. These have been considered rare exceptions to the bipartite genome structure. However, due in part to limitations of short-read sequencing, the prevalence of chromosome fusion in *Vc* remains unknown.

We long-read sequenced 467 *Vc* isolates, which have been isolated from 47 patients living in 21 households in Dhaka, Bangladesh. We assembled raw-reads using flye and confirmed chromosome fusion using Pulsed-Field Gel Electrophoresis. We compared strains with a fused and non-fused chromosomes in terms of growth, biofilm formation and expression of prominent virulence factors. We measured the stability of chromosome fusion under laboratory conditions.

We observed chromosome fusion in 58/467 clinical *Vc* isolates and identified multiple independent fusion events which appear to be stable enough to be transmitted within households. Fusions occurred in a 12 kilobase-pair homologous sequence shared between the two chromosomes and were stable for 200 generations under laboratory conditions. We found no effect of fusion on *Vc* growth, virulence factor expression, nor biofilm formation, nor did we find evidence for recombination deficiency of the fused chromosome strains. Next, we will compare the transcriptomes of fused and non-fused strains to elucidate other phenotypic changes in an untargeted manner. Further, we will passage fused-chromosome isolate under physiological conditions to identify factors which stabilize chromosome fusion within patients.

Together, our results show that chromosome fusion in *Vc* via homologous recombination is more prevalent and potentially more stable than previously thought. The clinical or phenotypic consequences of fusion appear to be minimal but remain to be comprehensively explored.

P-48 Rapid detection of Isoniazid and Fluoroquinolone resistance in Mycobacterium tuberculosis using the Xpert MTB/XDR assay in a low TB and low MDR-TB prevalence setting

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Isoniazid (INH) is a key antibiotic for tuberculosis (TB) treatment. The Xpert MTB/XDR assay is a rapid molecular test designed to detect mutations linked to resistance to INH and second line drugs. Initially developed for rifampicin-resistant (RIF-R) specimens, this study assesses its application for detecting INH resistance also in rifampicin-susceptible (RIF-S) isolates.

The performances of the Xpert MTB/XDR were evaluated on 100 *Mycobacterium tuberculosis* (MTB)-positive specimens (39 clinical samples and 61 culture isolates), from the region of the Lausanne University Hospital, corresponding to low TB and low MDR-TB prevalence. The study included 41 retrospective specimens and 59 consecutive specimens. Xpert MTB/XDR was compared to Sanger sequencing and phenotypic DST. Sensitivity of Xpert MTB/XDR was further analyzed by semiquantitative categories of Xpert MTB/Ultra.

The Xpert MTB/XDR demonstrated 96% of sensitivity and 100% of specificity for INH resistance detection compared to culture and 100% of sensitivity and specificity compared to Sanger sequencing across all specimens. For Rif-S specimens (n=91) the Xpert MTB/XDR achieved 100% of sensitivity and 100% of specificity against both culture and Sanger sequencing. Similarly, Xpert MTB/XDR showed 100% sensitivity and specificity for FLQ resistance detection compared to culture with no comparator available for Sanger sequencing. In MTB detection, Xpert MTB/XDR performed equal or better than Sanger sequencing across Xpert MTB/Ultra semiquantitative categories, achieving 100% of results for Xpert MTB/Ultra positive high and medium, 85,7% for low, 50% for very low; notably at traces categories the Xpert MTB/XDR could provide results in 40% of cases.

The Xpert MTB/XDR assay demonstrates high sensitivity and specificity for detecting INH resistance as well as quinolone resistance, including in RIF-S tuberculosis, confirming its utility in low TB, low MDR prevalence settings. These findings highlight the potential of Xpert MTB/XDR for rapid and accurate resistance determination even in paucibacillary “TRACE” specimens when compared to sequencing.

P-49 Synergistic inhibition of multidrug-resistant Staphylococcus aureus by commensal bacterial species from the human nose

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Staphylococcus aureus is an important human pathogen, and infections with methicillin-resistant *S. aureus* (MRSA) rely on last-resort antibiotics for treatment. *S. aureus* primarily resides in the nose, and nasal carriage is a risk factor for infections. With rising antimicrobial resistance, alternative strategies to prevent *S. aureus* and MRSA colonisation are needed. Notably, commensal bacterial species from the nose can restrict *S. aureus* colonisation, presenting a potential avenue for decolonisation. This is either evidenced in *in vitro* experiments focused on pairwise interactions, or in correlation analyses of observational data that include the complexity of interaction networks, but lacks a comprehensive understanding of the ecological interactions governing this inhibition. Here, we address this gap by conducting systematic *in vitro* experiments to identify combinations of commensal species that inhibit MRSA.

We assembled all possible combinations of commensals belonging to 7 species most commonly found in the human nose (*Cutibacterium acnes*, *Corynebacterium accolens, Staphylococcus epidermidis, Corynebacterium tuberculostearicum, Dolosigranulum pigrum, S. aureus* and apathogenic *Neisseria*) and assessed inhibition of MRSA strain JE2. MRSA inhibition was observed for all commensal species, to varying degrees. Combinations containing Staphylococcal commensal species reliably inhibited JE2 to the strongest degree. The same level of inhibition was achieved when combining Corynebacterial species with *D. pigrum*, showing strong synergistic inhibition. MRSA inhibition was sustained through serial passaging. Importantly, similar levels of inhibition were achieved for MRSA clinical isolates and strains resistant to last-resort antibiotics.

Our data highlight the potential of microbiota-based therapies to prevent *S. aureus* colonisation or facilitate its decolonisation from the human nose, ultimately reducing infection rates and our reliance on antibiotics.

P-50 Novel Temperate Staphylococcus epidermidis Phages from Wastewater Reveal Diverse Integration Sites and Host Interaction Patterns

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Temperate phages shape the evolution of coagulase-negative staphylococci yet remain understudied compared to *Staphylococcus aureus*. In particular, little is known about the diversity and host interactions of *S. epidermidis* phages in environments like wastewater. Here, we investigated four temperate *S. epidermidis* phages (PG_89, PG_90, PG_91, and PG_93) previously isolated from wastewater. Comparative genomic analyses with all staphylococcal phages support their assignment as four novel species within the *Rockefellervirus* genus, constituting a new family. Upon lysogenization of a prophage-free *S. epidermidis* host, PG_89 and _93 integrated into the dihydrolipoamide dehydrogenase gene (Se1int), whereas PG-2021_90 and _91 inserted into an intergenic region between *comK* and a mobilization gene (Se2int). Termini analysis indicated non-canonical or unresolved DNA packaging strategies.

Phage host range was assessed by spotting assay (10^{10}-10^{2} PFU/mL) against 43 strains, including *S. sciuri* (19), *S. epidermidis* (18), *S. saprophyticus* (3), *S. schleiferi* (2), and *S. nepalensis* (1). Twelve strains were susceptible to at least one phage, including three multidrug-resistant *S. epidermidis* strains. At the species level, PG_89, _90, and _93 infected 3 out of 5 tested species, while PG_91 infected 2 out of 5.

Lysis from without (LFW), a phenomenon in which phage attachment triggers cell lysis without productive replication, was observed in a couple of strains. PG_89, _91, _92, and _93 directed LFW in 2/43, 4/43, 4/43, and 3/43 strains, respectively. Notably, *S. epidermidis* C5855 exhibited LFW with all four phages. Adsorption assays revealed that phage binding to this strain occurred rapidly and with efficiency comparable to that of their original propagating hosts. Our findings reveal novel *S. epidermidis* phages with diverse integration sites, broad host interaction patterns, and evidence of LFW in multidrug-resistant strains. The high adsorption efficiency observed in non-permissive hosts suggests complex phage–host dynamics and underscores the need to further explore their ecological and functional roles.

P-51

Dual Roles of Mei2 Govern Distinct Stages of Sexual Development in Fission Yeast

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Compartmentalized regulation of RNAs is emerging as a key driver of developmental transitions, with RNA-binding proteins performing specialized functions in different subcellular components. Using RNA-sequencing we observed minimal changes in gene expression between gametes and early zygotes in *Schizosaccharomyces pombe*, indicating that regulation primarily occurs post-transcriptionally. Consistent with this, the RNA-binding protein Mei2 is a key regulator of sexual development in fission yeast. Mei2 is known to form a nuclear focus with the long non-coding RNA meiRNA. This meiotic condensate sequesters Mmi1 - a YTH family protein that represses meiotic-specific transcripts during mitosis. Inactivation of Mmi1 is essential for meiotic progression, and cells lacking this structure arrest at late stages of development.

Interestingly, the absence of Mei2 causes an earlier developmental arrest, suggesting that Mei2 also functions independently of the meiotic condensate. We found that Mei2 localizes to cytosolic foci identified as P-bodies. Cytosolic Mei2 activity supports early zygotic events such as re-fertilization blocks and pre-meiotic S-phase, likely through its role in P-bodies.

Thus, Mei2 cytosolic functions drive early developmental stages, while its nuclear role is critical later. Unexpectedly, we found that the meiotic condensate forms before fertilization, without leading to the protein expression of its target transcripts. Instead, we discovered that while transcript abundance is independent of the condensate, the condensate regulates nuclear export of these transcripts, thereby controlling their access to the translational machinery. As the condensate is already functional before fertilization, many late-meiosis transcripts are present in the cytosol of gametes. Yet their protein products remain undetectable, unraveling an additional layer of post-transcriptional control. Ongoing efforts aim to determine whether P-bodies contribute to this regulation and to clarify the role of Mei2 in modulating translation of these targets beyond its function in the meiotic condensate.

P-52* Insights into the Potential Roles of Nucleoside Diphosphate Kinase Paralogs in Waddlia chondrophila

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The *Chlamydiales* order consists of obligate intracellular bacteria, including well-known pathogens and emerging environmental species, with diverse host ranges and metabolic capabilities. Among these bacteria, the *ndk* gene, which encodes nucleoside diphosphate kinase, is present in variable copy number. While most chlamydial species carry a single copy of the *ndk* gene, some species have two copies. This variation in *ndk* copy number may influence their metabolic capabilities and interactions with the host cells. In *Waddlia chondrophila*, both Ndk paralogs retain conserved kinase motifs but differ in subcellular localization, suggesting divergent functional roles. WcNdk1 is confined to the inclusion and likely supports nucleotide metabolism, while WcNdk2 localizes to the host nucleus, perinuclear region, and Golgi apparatus, implying involvement in host interaction. Azidothymidine (AZT), a known Ndk inhibitor, was found to impair the growth of *W. chondrophila*, possibly by targeting WcNdk2 due to its role in host interaction. Our findings shed light on the potential functions of Ndk paralogs in *W. chondrophila* and support a role for WcNdk2 in host manipulation and pathogenesis. This research offers a starting point for deeper functional analyses as genetic manipulation techniques for this organism become more accessible.

P-53 Hyphae in focus: comparison of mold growth on agar with and without SpinAX sample preparation

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Background
In clinical tissue samples, molds typically appear in hyphal form, while spore formation (macro- and microconidia) is largely restricted to superficial growth. For culture-based diagnostics, mechanical homogenization is essential and traditionally done manually with a mortar. The SpinAX system (AxonLab) offers automated, standardized homogenization, but its effect on hyphal integrity and fungal viability remains unclear.

Objective
To assess whether processing with the SpinAX system affects hyphal structure or growth of moulds on agar.

Methods
Mold isolates are first embedded in still-liquid Sabouraud agar to suppress sporulation. After solidification, agar plates are incubated at 28 °C until visible fungal growth appears. The agar block is divided in two: one half is conventionally processed, the other homogenized using the SpinAX system. Both preparations are then plated onto fresh Sabouraud agar and incubated at 28 °C for five days. Fungal growth and colony morphology are assessed qualitatively and quantitatively.

Results
In initial tests using *Aspergillus niger* and *Penicillium* spp. as model organisms, growth was comparable between conventional and SpinAX-prepared samples. Microscopy showed intact hyphal structures. No growth inhibition was observed after SpinAX homogenization. Additional data are being collected.

Discussion
SpinAX offers a standardized alternative, potentially valuable for spore-deficient clinical samples. Further validation with real patient specimens and more sensitive fungal species is needed to determine its full diagnostic value. These data will be presented here.

P-54 Physical insights on bacterial HGT in complex porous structures

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Horizontal gene transfer (HGT) is an important mechanism in prokaryotic evolution that allows bacteria to share beneficial genes that improve population fitness in a given environment. Experiments involving bacterial HGT are often performed in liquid cultures or Petri dishes (in relatively homogeneous conditions), whereas HGT often takes place in heterogenous environments such as soil where a variable nutrient and resource distribution supports large, diverse communities of micro-organisms. Due to the heterogenous structure of the porous soil matrix, the distribution of both the micro-organisms and the nutrients required to sustain them is also highly heterogenous. More importantly, soil is a host for exchange between environmental species and common human pathogens, serving as a reservoir for antibiotic resistance genes and pathogenicity islands. Using microfluidics chips mimicking the soil structure, we study how a naturally competent soil species, *Acinetobacter baylyi*, uptakes DNA in a structurally complex environment. To do this we infect the chips with *A. baylyi*, after which we inject a nutrient solution mixed with a PCR product encoding constitutively expressed GFP and an antibiotic resistance gene and flanked by homology regions, to be directly incorporated into the genome. Uptake and expression of DNA is then monitored by time-lapse video-microscopy to determine the rate and spatial distribution of transformation.

P-55* Nanomotion Technology for Rapid Antimicrobial Susceptibility Testing of Mycobacterium tuberculosis: Evaluating Novel Benzothiazinone Derivatives.

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Background
Mycobacterium tuberculosis remains a major cause of morbidity and mortality worldwide, particularly in low- and middle-income countries. The emergence of multidrug-resistant and extensively drug-resistant strains presents significant challenges for treatment. Rapid and accurate antimicrobial susceptibility testing is essential for optimizing therapy and addressing antimicrobial resistance. This study investigates the use of nanomotion technology, a phenotypic AST method that measures bacterial viability, to evaluate the activity of benzothiazinone derivatives targeting MTB.

Methods
Nanomotion-based AST was performed using the Resistell Phenotech device in a BSL-3 laboratory. MTB H37Rv and a DprE1-resistant mutant were exposed to benzothiazinones, and nanoscale vibrations were monitored over seven hours. The variance in nanomotion signals was analyzed, and the rate of variance decrease (k) was calculated to assess bacterial viability. Minimum inhibitory concentrations (MICs) were determined using resazurin microtiter plate assays for comparison.

Results
All benzothiazinone derivatives exhibited significant activity against H37Rv, with PBTZ169 showing the lowest MIC at 0.3 ng/mL. Nanomotion signals displayed substantial reductions in bacterial activity within seven hours for susceptible strains, with median k values ranging from -0.43 h^{-1} (PBTZ169) to -0.25 h^{-1} (BTZ043). Resistant mutants demonstrated minimal variance reduction (median k = -0.03 to 0.05 h^{-1}). The method achieved 100% differentiation for PBTZ169 and H2-PBTZ169. Time-to-result was reduced to seven hours, nearing the performance of molecular diagnostics.

Conclusions
Nanomotion technology offers a rapid, reliable method for phenotypic AST of MTB, providing real-time viability assessments that distinguish between susceptible and resistant strains. This method bridges the gap between molecular and phenotypic diagnostics and could facilitate improved MDR and XDR tuberculosis management. Ongoing work focuses on adapting the technology for direct sputum testing to streamline diagnostic workflows. Additionally, we are working with *M. smegmatis* to establish phage production according to our GMP protocol accredited by SwissMedic, paving the way for standardized, high-quality phage therapies.

P-56 Monitoring of Single-Cell Bacterial Lysis by Phages Within Integrated Optical Traps

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Aims

Antibiotics have significantly reduced bacterial infection-related morbidity and mortality, but the rise of antibiotic-resistant strains threatens their effectiveness. Phage therapy, which employs bacteriophages to lyse bacteria, presents a promising alternative. However, its clinical implementation is hindered by the lack of a standardized, rapid phagogram for selecting effective bacteriophages. Here, we demonstrate an innovative use of photonic crystal cavities as on-chip optical nanotweezers for real-time, single-bacterium and virion analysis, as well as phage susceptibility test (PST).

Methods

On a silicon photonic chip, resonant photonic crystal cavities enable trapping of a single *Escherichia coli* B cell and phages and differentiation without labelling or specific surface bioreceptors. The tool also allowed monitoring of stress-induced changes via transmitted optical power.

Results

E. coli cells were exposed to T4 *Myoviridae* and T1 *Tunaviridae* phages before injection into the trapping device. We report direct optical observation of a bacterium-phage lytic event. Prior to lysis, morphological changes induced by phage activity alter the optical transmission, correlating with bacterial stress. Lysis causes a sudden refractive index reduction, resulting in an abrupt drop in transmitted power and reduced imaging contrast. Notably, lysis occurs within 40 ± 5 minutes, significantly faster than conventional culture-based phagograms requiring 16–24 hours.

Conclusion

This method offers a rapid and precise approach for ultrafast PST at the single-bacterium level, addressing a major bottleneck in phage therapy implementation and paving the way for real-time bacteriophage selection in clinical settings.

P-57* Ectopic colonization by oral bacteria in the small intestine of stunted children

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Stunting, characterized by impaired growth and development in children who are low height-for-age, stands as the most prevalent manifestation of childhood undernutrition worldwide. However, effective treatments for stunting remain elusive, with even the most promising strategies yielding limited success in averting growth retardation and associated pathophysiological disruptions. Several studies have suggested a potential link between Environmental Enteric Dysfunction (EED), characterized by chronic inflammation of the small intestine, and small intestinal oral bacterial overgrowth (SIOBO). This association, if substantiated, could offer a novel explanation for the aetiology of undernutrition.

Within the Afribiota project, a large collection of bacteria from the upper gastrointestinal tract of stunted children has been established. *Streptococcus salivarius* represents the largest proportion of the isolates, reflecting its high prevalence of *S. salivarius* in SIOBO.

In this study, we aim to assess the colonization factors that favour the colonization and overgrowth of *S. salivarius* in the small intestinal tract of mice using a CRISPRi screening approach and to compare the colonization potential of *S. salivarius* in between different strains as well as in the context of healthy or undernourished mice and mice suffering of EED. First analyses show a strain-dependent potential of *S. salivarius* to colonize the murine intestintal tract with overall stronger colonization in undernourished mice. CRISPRi libraries are currently constructed for several clinical *S. salivarius* strains. Furthermore, a first CRISPRi screen has been performed on the reference strain HSISS4.

We expect from this study a better understanding of the main genetic factors favouring the overgrowth of *S. salivarius* in the intestinal tract of stunted mice, thus paving the way for the development of treatments able to prevent SIOBO in stunted children in the future.

P-58 Optimized Detection of ESBL Producers, Carbapenemase Producers, and VRE from Rectal Swabs Using Enrichment Broth for Superior Screening Sensitivity

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This study evaluates enrichment broths for improving the detection of multidrug-resistant (MDR) bacteria—VRE, ESBL producers, and carbapenemase-producing organisms (CPOs)—from rectal swabs. Comparing direct plating to enrichment-based methods significantly increased detection sensitivity, especially for CPOs (from 17 to 27 cases, a 58.8% increase). Detection rates for ESBL producers and VRE also improved significantly (by 20%). Pre-enrichment is therefore a valuable addition to routine MDR bacteria screening protocols, which is particularly useful when high accuracy is needed for infection control practices and outbreak management.

P-59 Multi-Omics characterization of the ‘Mycoplasma mycoides cluster’ reveals host-specific virulence signatures and vaccine targets

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The ‘*Mycoplasma mycoides* cluster’ encompasses the five pathogens *Mycoplasma mycoides subsp. mycoides* (*Mmm*), *Mycoplasma mycoides subsp. capri* (*Mmc*), *Mycoplasma capricolum subsp. capripneumoniae* (*Mccp*), *Mycoplasma capricolum subsp. capricolum* (*Mcc*), and *Mycoplasma leachii (M. leachii)* which cause diferent diseases in ruminant species. *Mmm* and *Mccp* cause the deadly diseases contagious bovine pleuropneumoniae and contagious caprine pleuropneumonia, respectively. Despite their economic impact especially in Africa and Asia, mechanisms of pathogenicity remain poorly understood, and current vaccines are suboptimal, highlighting the need for improved control strategies.

Here, we employed a multi-omics approach to characterize the genomic diversity of the cluster, to identify candidate virulence traits and pathways that correlate with host adaptation mechanisms of the different members. Our analysis included 82 *Mycoplasma* strains represeting five members of the ‘*M. mycoides* cluster’. The pan-genome comprised 279 core, 1,450 accessory, and 387 unique genes. Functional annotation of the core genome revealed enrichment in translation and ribosomal structure, and biogenesis. We identified 181 lipoprotein-related genes (144 accessory, 37 unique), none of which were part of the cluster’s core genome. As surface-exposed molecules, lipoproteins are important in host-pathogen interactions. Lipoprotein profiles were specific for the individual closter members, with *Mmc* harboring the highest number of lipoproteins but lacking subspecies-specific core lipoproteins, in contrast to *M. leachii*, which showed the lowest lipoprotein count but the highest number of *M. leachii*-specific ones. Using ensemble machine learning, we prioritized candidate genes involved in host-adaptation, including ABC transporter, ATP-binding proteins and prolipoprotein diacylglyceryl transferases. While several were transcriptionally active, only a subset were translated, suggesting condition-specific translation or post-transcriptional regulation.

Our findings underscore lipoproteins and membrane transporters as critical factors for pathogenicity including host specificity. This study provides the most comprehensive genomic resource for the ‘*M. mycoides* cluster’ to date, identifying prioritized surface-exposed antigens for next-generation vaccine development.

P-60 Sulbactam-durlobactam as an effective alternative option against NDM-producing Escherichia coli including cefiderocol and aztreonam-avibactam-resistant isolates in Swizterland

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Objectives
The recent identification of NDM-producing *Escherichia coli* isolates being resistant to last-line antimicrobial agents such as aztreonam-avibactam or cefiderocol represents a major concern. This study aimed to evaluate the in-vitro activity of a novel combination, sulbactam-durlobactam, known to be efficient against MDR *Acinetobacter baumannii*, which is commercially available in the USA, against those isolates.

Methods
The study included all consecutive and non-duplicate NDM-producing *E. coli* clinical isolates collected at the Swiss National Reference Center for Emerging Antibiotic Resistance (NARA) from January 2023 to May 2024 and recovered across all Switzerland. MICs were determined by broth microdilution for aztreonam, aztreonam-avibactam, sulbactam-durlobactam, tigecycline, eravacycline and cefiderocol. The EUCAST susceptible breakpoints were employed for all antimicrobial agents with the exception of sulbactam-durlobactam, for which no breakpoint is available.

Results
When considering 110 NDM-producing *E. coli* clinical isolates, with corresponding infections supposed to be treated with the last-line recommended options aztreonam-avibactam or cefiderocol, the susceptibility rates to those latter antibiotics was found to be 80% and 34%, respectively. Noteworthy, susceptibility rates of 89% and 100% were respectively found for tigecycline and eravacycline. It is noteworthy that sulbactam-durlobactam demonstrated exceptionally low minimum inhibitory concentration values, reaching a maximum of 0.06 mg/L for all tested isolates.

Conclusion
This study demonstrated the efficacy of the sulbactam-durlobactam combination in-vitro, when compared with other last-resort options, including aztreonam-avibactam, cefiderocol, tigecycline and eravacycline, against a contemporary collection of NDM-producing *E. coli* clinical isolates. Noteworthy, sulbactam-durlobactam combination demonstrated high efficacy against all cefiderocol and/or aztreonam-avibactam-resistant isolates. In light of these findings, the newly developed combination may be considered as a potential therapeutic option in infections associated with the aforementioned isolates, particularly when very few alternative therapeutic options are available.

P-61 Rapid NP detection of Cefepime/Taniborbactam susceptibility/resistance in Enterobacterales

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Background

Metallo-β-lactamases (MBL) producing Enterobacterales are widely distributed globally and typically confer resistance to all β-lactams, including carbapenems. To date, no clinically-useful molecule can inhibit MBL activities. Recently, the MBL beta-lactamase inhibitor, taniborbactam (TAN), has been developed, that will be marketed in combination with cefepime (FEP). The Rapid Cefepime/Taniborbactam NP test, was developed for the identification of resistance/susceptibility among multidrug-resistant Enterobacterales including producers of MBL.

Methods

The Rapid Cefepime/Taniborbactam NP test is based on the detection of glucose metabolisation resulting from bacterial growth in the presence of cefepime at a concentration of 64 mg/L and Taniborbactam at a concentration of 4 mg/L. The growth of bacteria can be visually detected by a color change of red phenol, a pH indicator, which transitions from red to orange and yellow as a result of the acidification of the medium due to bacterial growth. A total of 101 Enterobacterales isolates were selected for evaluation of the performance of the Cefepime/Taniborbactam NP test. The strains were previously characterized by the Swiss National Reference Centre (NARA) and included resistant strains (n=33) and susceptible strains (n=68) to the FEP-TAN combination. In the absence of an EUCAST or CLSI breakpoint, the provisional breakpoints S ≤ 8 mg/L and R ≥ 16mg/L were employed for the interpretation.

Results

The test demonstrated a sensitivity and specificity of 94% and 96%, respectively. All results were obtained within a three-hour incubation period at 35°C, representing a significant time saving compared to current antimicrobial susceptibility testing methods, including broth microdilution.

Conclusions

The results of this study demonstrate that the Rapid Cefepime/Taniborbactam NP test is a highly accurate and time-efficient method for the detection of bacterial resistance. When Cefepime/Taniborbactam will become commercially available, this test could therefore be easily integrated into the standard procedures of clinical microbiology laboratories.

P-62 The NitroSpeed Taniborbactam NP test; easy detection of β-lactamase sensitivity to taniborbactam

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Background
Metallo-β-lactamases (MBL) encoding genes are highly transferable, and their fast pace for developing new variants is alarming in an era where there is a lack of clinically available MBL inhibitors. The MBL inhibitors in development include third generation boronate compounds taniborbactam (TAN) and xeruborbactam (XER). TAN is in phase 3 clinical trials and has a broad range of activity towards all β-lactamase classes, including MBLs that possess a carbapenemase activity. The NitroSpeed Taniborbactam NP test was designed to rapidly assess TAN's β-lactamase inhibition, especially against metallo-β-lactamases (MBLs).

Materials
The NitroSpeed Taniborbactam NP test is based on the hydrolysis of (i) nitrocefin (absence/presence of β-lactamase), (ii) ertapenem (absence/presence of carbapenemase), (iii) and TAN (presence of MBL inhibited or not by TAN). A collection of 134 clinical isolates, 103 Enterobacterales and 31 Pseudomonas aeruginosa genetically characterized was included in the study for evaluating the performance of the test.

Results
The NitroSpeed Taniborbactam NP test showed 100% sensitivity, specificity, and accuracy when testing a large set of β-lactamases including NDMs, VIMs, IMPs, SPM, SIM, KPC, OXA-48, and others. No major errors or very major errors were observed. And the test presents an optimal turnaround time of ≤ 15 minutes.

Conclusions
The NitroSpeed Taniborbactam NP test is accurate and robust to identify the presence of MBLs inhibited or not by taniborbactam. Given the flexibility of the test, it could be used for screening any new compound supposed to be a potential β-lactamase and/or MBL inhibitor by simply replacing the taniborbactam by such molecule. This work represents first phase of evaluation of the test, and the second phase will aim in undersanding if the results can be or not extrapolated to detect either susceptibility or resistance to TAN when combined with cefepime.

P-63* Deciphering bacteriophage pharmacokinetics: clearance follows second-order kinetics in rats

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Background and aims
Dosing strategies in phage therapy (PT) remain empirical due to the absence of systematic pharmacokinetic (PK) studies, particularly regarding phage elimination kinetics and routes of administration. However, a comprehensive understanding of phage PK is critical for rational dose optimization, which may help overcome treatment failures, a persistent challenge highlighted by the negative outcomes of randomized controlled trials. We aimed to characterize phage PK in rats as basis for evidence-based dosing strategies in PT.

Methods
Wistar rats were surgically implanted with two subcutaneous inert tissue cages and a totally implantable venous access port. Purified phages with different morphologies and hosts were administered via different routes at doses of $5{\cdot}10^9$ PFU (systemic), or $5{\cdot}10^7$ PFU (local). Both single and repeated administrations were evaluated. The primary outcome was phage titers over time in blood (systemic) and tissue cage fluid (local).

Results
Phage clearance from the bloodstream was dose-dependent and followed second-order kinetics, with a peak concentration (C_{max}) of $2.1{\cdot}10^7$ PFU/ml five minutes after a single intravenous dose. Repeated administration improved exposure over time (24h-AUC: 10^6 versus 10^3 PFU·h/ml) but not peak concentrations (C_{max}: $1.7{\cdot}10^7$ PFU/ml). Both intravenous and local injections achieved therapeutically sufficient local concentrations (C_{max}: $2.9{\cdot}10^5$ and $5.5{\cdot}10^7$ PFU/ml, respectively), whereas intraperitoneal and subcutaneous applications failed to do so (C_{max}: $9.5{\cdot}10^2$ and <20 PFU/ml).

Conclusions
In rats, phage clearance appears to follow second-order kinetics, a dose-dependent pattern not yet described in classical models of drug elimination. Intravenous and local administration seem to be the more suitable routes. These findings enable improved prediction of phage titers over time and have significant implications for dosing strategies in therapy. Further studies are needed to explore pharmacokinetic/pharmacodynamic (PK/PD) relationships in PT.

P-64* Chlamydia vaughanii: the biology and pathogenic role of a newly discovered intracellular bacteria

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Background and aims

Bacteria belonging to the *Chlamydiales* order are strict intracellular, Gram-negative organisms which are characterized by a two-stage developmental cycle.
Several new species of *Chlamydiales,* such as *Piscichlamydia salmonis* or *Clavochlamydia salmonicida,* were recently discovered in fish farms. These fish pathogens can cause epitheliocystis, a respiratory system disease which drives substantial economic loss.
Recently, our group isolated a new species, from a tropical fish, which belongs to the *Chlamydia* genus, *Chlamydia vaughanii*. This discovery followed an event where 95% of fish in a tropical aquarium died suddenly, but the pathogenic role of *C. vaughanii* in these deaths has not yet been determined.

Methods

Using a specific, real-time PCR which targets the *mutS* gene together with immunofluorescence and confocal microscopy, we investigated the permissivity of different host cells to *C. vaughanii*. The lifecycle was also characterized by electron microscopy to visualize the developmental forms and by assessing the number of infectious particles along the life cycle. The intracellular trafficking of *C. vaughanii* in Hela cells was also investigated using immunofluorescent staining of organelles and evaluating co-localization.

Results

C. vaughanii were able to successfully replicate inside McCoy, Vero, and four different human cells including monocyte-derived macrophages. Surprisingly, it could not multiply in a fish cell line (EPC175) at 25°C. However, growth could be detected in the same cell line at 30°C.
Like other species of the *Chlamydia* genus, amoebae and insect cells were not viable hosts.
Furthermore, electron microscopy showed that it exhibits the expected biphasic life cycle typical of *Chlamydiales*.

Conclusion and future directions

C. vaughanii is the first *Chlamydia* species to be isolated from fish and successfully cultured in mammalian cells. To better understand its biology and assess its potential pathogenicity towards fish and humans, we are currently developing a zebrafish model of infection by *C. vaughanii*.

P-65* Microbiome-Based Biomarkers in Alzheimer's Disease: A Multi-Layered Approach to Cognitive Impairment

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Alzheimer's disease (AD) is a neurodegenerative disorder characterised by amyloid-β (Aβ) accumulation and cognitive decline, with growing evidence implicating the gut and oral microbiota in its pathophysiology. We applied shotgun metagenomic sequencing and multi-layered statistical modeling to characterise microbial signatures associated with cognitive impairment in a well-defined clinical cohort (n=61) spanning from unimpaired individuals to those with mild cognitive impairment and AD.

Using our in-house bioinformatics pipeline (https://github.com/metagenlab/zshoman) and statistical workflow, we uncovered distinct taxonomic and functional alterations in the stool microbiome of cognitively impaired individuals, aligning with hypothesised microbial mechanisms in AD.

Key findings revealed a notable increase in phages, particularly a Siphoviridae phage and a phage capsid protein, both of which exhibited significant correlations with Aβ load. Additionally, a reduction in Streptococcus sanguinis, a bacterium implicated in choline metabolism, was observed, suggesting a potential link between choline metabolism dysregulation and Aβ accumulation. Furthermore, the enrichment of oral commensals Streptococcus salivarius and Bifidobacterium dentium in the impaired group supports the hypothesis that oral microbial translocation may contribute to neuroinflammatory processes through the oral-gut-brain axis.

Our findings highlight a convergent microbial signature involving bacterial, viral, and metabolic components in early cognitive decline. This work underscores the utility of multi-omic microbiome profiling to uncover potential biomarkers and mechanistic pathways in AD, opening avenues for microbiome-informed diagnostics and interventions.

P-66 Spatiotemporal growth of biofilm controls intrinsic permeability of complex porous systems

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Soils, aquifers and filtration systems are porous structures where biofilm control bio-chemical activities and also influence the transport and consumption of substances. Yet, a detailed description of how microbial growth clog individual pores and controls medium permeability remains elusive. With experiments based on microfluidics and time-lapse video-microscopy, we dynamically measure the medium intrinsic permeability while a bacterial population grows under the constant injection of sterile resources. We repeated our flow experiments with motile and with non-motile strains (Pseudomonas putida sp. KT2440 WT and its mutant ΔfliC that has no flagellum). It results that the overall biomass does not control the medium permeability, but its spatial organisation. In fact, non-motile mutant colonize the entire medium, while the WT and motile strain only part of it even though the overall biomass is the same. We successfully predict the measured permeability with a mechanical model for the entire porous medium permeability, that relies on the biofilm coating the walls of a series of pipes (pores) of distributed size.

P-67 “Beat it!”: Tissue homogenization by vibratory mill provides high bacteriological culture yield in routine tissue samples

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Background
Homogenization of tissue samples is a critical step in the pre-analytical preparation of tissue samples for bacterial culture which lacks standardization. Vibratory mills are utilizing impact and friction with grinding beads at high frequencies to achieve homogenization across diverse sample matrices. The aim of this study was to evaluate the performance of a vibrating mill by determining the effectiveness of its microbial recovery compared to two commercial homogenization systems.

Methods
Thirty-eight soft tissue samples with confirmed bacterial growth were analyzed. The tissue specimens were divided into three 5 mm aliquots. One aliquot was homogenized using the vibratory Mill Mixer MM 400 (Retsch), another with the ULTRA-TURRAX® (IKA), and the third with the newly introduced SpinAX (Swissmeca SA) homogenization system. The protocol for the Mill Mixer was selected based on preliminary testing while ULTRA-TURRAX® and SpinAX were performed according to manufacturer's instructions. The homogenates (100 µl) were cultured on standard bacteriological plates and the resulting colony-forming units (CFU) were counted.

Results
Among the three methods compared, the Mill Mixer produced the highest CFU counts in 29 samples (76%) and the second highest in 8 samples (21%). SpinAX achieved the highest CFU counts in 13 samples (34%) and the second highest in 16 samples (42%). In contrast, ULTRA-TURRAX® generated the highest CFU counts in only 6 samples (16%). The CFU_{50} and CFU_{90} for the Mill Mixer, SpinAX, and ULTRA-TURRAX® were 200, 20, 7 and 220, 220, 200 CFU, respectively.

Conclusions
The homogenization of tissue samples with vibratory mill provides high-yield bacteriological cultures, outperforming both commercial systems SpinAX and ULTRA-TURRAX®. As the disposable mixing cartridges of commercially available devices are not required, the costs of homogenization with the vibratory mill are significantly lower. In addition, the efficient liquefaction of the samples suggests that this method could be well-suited for automated laboratory workflows.

P-68 Validation of DNA extraction parameters for the analysis of the urinary microbiome

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The urinary microbiome remains understudied compared to other body sites. Variability in DNA extraction protocols, particularly in sample volume and lack of replicates, can significantly affect downstream microbiome analysis. In this study, we investigated the impact of urine input volume using patient samples on DNA yield and 16S rRNA gene profiles. We established a urine processing protocol to optimize DNA quantity and minimize variability. Additionally, the ability of a buffer to preserve the urinary microbiome over time was evaluated to allow for longer urine storage before processing.

Urine from catheterized patients was processed in triplicate from volumes of 4 to 30 mL. Urine pellets were concentrated and resuspended in 200 µL eNAT buffer (Copan). DNA extraction followed using the Qiagen DNeasy Blood & Tissue Kit with mechanical lysis. Separately, patient urine was stored in Urine Conditioning Buffer™ (UCB) (Zymo) for 72 h before processing. For all samples, 16S rRNA V4 PCR was performed and sequenced on Illumina MiSeq. Analysis was done using an in-house R pipeline.

Significant differences in the urine microbiome were revealed between patients ($p<0.05$), but no differences were observed between volumes from the same patient ($p>0.05$). However, larger volumes of urine were significantly less variable than smaller volumes ($p<0.001$), and larger volumes significantly decreased the risk of contamination ($p=0.018$). UCB revealed no significant change in microbiome composition after 72h of storage at RT ($p>0.05$).

The results of this study highlight the significant impact starting volumes have on the reproducibility and therefore accuracy of urinary microbiome analyses. Collecting larger urine volumes, adjusting processing parameters, and sequence decontamination can significantly increase DNA yields and reduce spurious reporting for urinary microbiomes. Additionally, the possibilities of longer storage before processing or for home sampling can be explored to increase feasibility for microbiome studies.

P-69 Evaluation of a Borrelia burgdorferi IgG/IgM immunochromatography test as a rapid first step in the diagnostic of neuroborreliosis.

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In endemic region neuroborreliosis (NB) is an invasive severe disease that should be diagnosed rapidly in the first line together with other infectious etiology of neurological infections. Rapid and reliable Serology is needed to exclude neuroborreliosis. We aimed to evaluate an IgG/IgM Immunochromatography test (ICT) to look for *Borrelia burgdorferi* s.l. (Bbsl) global antibodies in serum and CSF.

Prospectively we tested in parallel all samples sent to our Reference Laboratory for NB diagnostic. Our procedure included antibodies detection in sera and CSF with Liaison XL (Diasorin) IgG and IgM as screening assays.

All reactive CSF will then be confirmed on Neuroborreliosis kit (Dako, Oxoid, Termo-Fisher) to determine intrathecal antibody synthesis. The chemokine CXCL13 is dosed in CSF with the CXCL13 ELISA (Euroimmun). ReaScan+ LYME IgM/IgG Combo (Reagena.) was evaluated to assess its capacity to screen neuroborreliosis cases. To increase the number we added 68 serum from our NB collection.

Between 2023 and 2024 we tested 328 Serum-CSF pairs; NB was diagnosed in 22 cases (6.7%). Sera and CSF were positive for the 22 NB cases with Reagena Combo ICT showing maximum sensitivity to detect the cases. From the non-NB patient (n=306) 87 samples were positive in the serum and 25 samples in the CSF giving specificities of 69.9% and 91.8% respectively. Screening with Diasorin IgG/IgM we obtained sensitivities of 90.9% for serum and 100% for CSF and specificities of 71.2% and 82.7% respectively.

Completing with more NB sera (n=68) we could obtained a higher sensitivity to 97.1% for Diasorin IgG/IgM combination CSF is the best sample to screen for NB patient with higher sensitivity and specificity as serum cannot exclude totally a NB.

This assays detecting IgG/IgM together is a potential screening test to detect most neuroborreliosis cases in the routine microbiological diagnostic.

P-70 Impact of the new WHO critical concentration for rifampicin for diagnosis of rifampicin resistance of Mycobacterium tuberculosis in Switzerland

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Aims
Infections with *Mycobacterium tuberculosis* (MTB) continue to be a major public health concern worldwide, particularly due to the increasing number of drug-resistant strains. Rifampicin (RIF) is a key drug in TB treatment and accurate detection of RIF resistance is crucial. In 2021, the World Health Organization (WHO) lowered the critical concentration (CC) for phenotypic RIF testing from 1mg/L to 0.5mg/L. The goal was to reduce potential misclassification of MTB as susceptible in cases of borderline resistance mutations. However, manufacturers continue to supply systems based on the outdated CC (e.g. BD BACTEC SIRE Kit). The implementation of in-house testing requires cost-intensive validation. We have introduced the CC of 0.5mg/L RIF at the Swiss National Reference Laboratory for Mycobacteria and analyse its impact.

Methods
Phenotypic drug susceptibility testing (pDST) was performed for 394 clinical MTB isolates collected in our laboratory between 2021 and 2024 using the MGIT system (BD, Franklin Lakes, NJ), including the new (0.5mg/L) and the former (1.0mg/L) CC for RIF. Genotypic resistance analysis was performed by next generation sequencing using Illumina technology (San Diego, CA) in combination with the WHO catalogue of MTB resistance mutations.

Results
RIF resistance was detected in five percent of the isolates, among predominantly multi-drug resistant (MDR) TB. The results of genotypic and phenotypic DST strongly correlated for RIF. One MDR TB isolate with a borderline RIF resistance mutation (*rpoB*, D435Y) was resistant at 0.5mg/L but susceptible at 1.0mg/L. One RIF-mono-resistant isolate (*rpoB*, L430P, borderline RIF resistance mutation) was missed by pDST.

Conclusion
Rifampicin resistance is rare in Switzerland, an area with a low TB incidence. Borderline rifampicin resistance is even rarer. The introduction of in-house testing of RIF 0.5 mg/L is associated with high costs. The adaptation of commercial tests is important for a broad application of the new CC.

P-71

Legionella Detection and Enumeration in Water Samples by ISO 11731-2017: Which method is the most sensitive?

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Legionnaires' disease is a severe pneumonia caused by inhalation of *Legionella*-contaminated aerosols. Prevention of *Legionella* is relying on water disinfection and on accurate water testing to assess the effectiveness of these interventions. The norm ISO 11731:2017 provides multiple culture-based methods for detecting *Legionella* in water and recommends taking into account the most unfavorable result. This study evaluated six ISO-standardized techniques, including variations in media, concentration, and pre-treatments (heat or acid), applied to 276 hospital hot water samples. *Legionella* was detected in 120 samples, with substantial variability across methods. The most sensitive single technique (76%) was concentration and elution with membrane filter on GVPC medium after heat treatment. Combinations of three techniques yielded a higher detection rate (98%), while using all six techniques detected all positive samples. Techniques involving concentration and elution proved superior to direct plating or simple filtration. Heat treatment enhanced detection more effectively than acid treatment, especially in samples with interfering flora. GVPC medium consistently outperformed BCYE. The study supports using a strategic combination of methods, particularly those involving membrane filtration and GVPC medium with heat treatment, to optimize *Legionella* detection in healthcare water systems.

P-72 Mating preferences of wild fission yeast strains; how do fission yeasts select a partner?

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The choice of a mating partner is a critical life event, determining the course of evolution as well as genetic isolation and eventually speciation. To probe the basis of mate preference in fission yeast, we determined the in- and out-breeding ratios between twenty-four non-clonal wild *Schizosaccharomyces pombe* strains. We investigated breeding preferences of both homothallic (capable of mating-type switching) and heterothallic (mating-type locked) cells. Mutants predicted to alter partner choice, such as M-factor deletion mutants, only exhibited strong effects in heterothallic conditions. Remarkably, several wild strains showed altered mating preferences in both conditions.

The wild strain *S. pombe variant kambucha* (Sk) was the preferred partner in our mating assays. The lab strain (972) preferentially outcrossed Sk when provided with an option to mate a clonal lab strain cell of opposite mating-type or an Sk cell of opposite mating-type. Sk mating results in longer zygotes than lab strain on average[1]. We show this is caused by an increase shmoo response by subjecting heterothallic Sk strains to synthetic pheromone, showing shmoo lengths comparable to lab strain when exposed ten times less pheromone. Mating between lab strain 972 and Sk results in unviable spores, therefore 972/Sk hybrid strains retaining the breeding phenotype were used for outcrossing2. We are currently performing backcrosses, sequencing and QTL analysis to identify the genetic locus (or loci) responsible for the increased shmoo response. We will test causality of these genomic variations and aim to describe what molecular system(s) influence mate choice preferences in fission yeast.

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S-68*/P-73* nanomur: innovative nanomotion technology for antibiotic susceptibility testing - a game-changer for urinary tract infections?

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Background and study objective

Urinary tract infections (UTIs) are very prevalent and can be life-threatening in case of complication like sepsis. Currently, urine antibiotic susceptibility testing (AST) exceeds 30 hours, mainly due to culture, potentially leading to suboptimal antibiotic treatment. This complicates UTI management, especially amid rising antibiotic resistance.
The NANOMUR study aims to reduce the time-to-results for urine AST through innovative “nanomotion” technology and machine learning, bypassing the need for culture.

Methods

Turbid urine samples undergo the routine diagnostic workflow (gold standard) and nanomotion devices, testing for ceftriaxone and ciprofloxacin. Performance is evaluated using a confusion matrix. Time-to-results for both methods are also compared.

Results

204 experiments were conducted this far, 172 eligible after data cleaning. Nanomotion performance was calculated for monomicrobial samples (81 experiments). The accuracy was 82.72% (67/81), sensitivity was 85.92% (61/71), and specificity was 60% (6/10). Compared to monomicrobial samples, polymicrobial samples including one Gram-negative and one Gram-positive bacteria (55 experiments) showed no significant difference in nanomotion performance.
Nanomotion’s mean time-to-results was 4 hours 24 minutes, significantly lower ($p < 0.0001$) than the routine mean for monomicrobial or polymicrobial samples – respectively 37 hours 18 minutes and 56 hours 18 minutes.

Conclusion and perspectives

Nanomotion exhibited good accuracy and sensitivity but lacked specificity. However, the significant time gain of nanomotion could be a game-changer in patient management. The next phase of the study will include experiments at 37°C, allowing us to decrease the time-to-results even further and to assess if performance can be improved that way.

P-74

The prevalence of sexually transmitted infection pathogens in a Korean single-commercial laboratory over the last 3 years

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Background

A sexually transmitted infection (STI) is an infectious disease that spreads primarily through sexual contact. The clinical significance of STI lies in their potential to cause serious health complications if left untreated. Many STI can lead to chronic conditions such as pelvic inflammatory disease, infertility, and certain cancers. Early diagnosis and treatment are essential to prevent these long-term health consequences and reduce transmission rates within communities.

Therefore, we aimed to determine the prevalence of STI pathogens to provide important data for the development and evaluation of public health strategies

Methods

From January 2021 to December 2023, a total of 1,253,478 specimens ordered for multiplex real-time PCR tests detecting STI pathogens from medical institutions in all regions of Korea were included in this study. The specimens were urine, semen, and EPS (Expressed Prostatic Secretion) for male patients, and urine and vaginal swabs for female patients. Multiplex real-time PCR tests were performed using Anyplex™ II STI-12 Detection (Seegene, Korea).

Results

The prevalence of STI pathogens was 26.1% (326,952/1,253,478) over 3 years. The positive rates in males and females were 26.2% and 25.9%, respectively. The most frequently detected STI pathogen was *Ureaplasma urealyticum* (16.1%), followed by *Mycoplasma hominis* (9.1%), *Chlamydia trachomatis* (4.5%), *Mycoplasma genitalium* (3.0%), and *Neisseria gonorrhoeae* (1.0%). Among positive specimens, 23.0% of males and 25.7% of females showed detection of two or more pathogens (coinfection).

Conclusions

This study investigated that the prevalence of STI pathogens in a Korean single-commercial laboratory over the last 3 years. *U. urealyticum* is the most frequently detected pathogen. The positivity rate for *M. hominis* was higher in females compared to males. Over the past three years, the number of tests for STI-causing pathogens has steadily increased, but the positivity rate has decreased in Korea.

P-75 Evaluation of the MiSeq i100 for Whole Genome Sequencing in Medical Microbiology

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Background
Next-generation sequencing (NGS) has transformed medical microbiology by enabling whole genome sequencing (WGS) for pathogen characterization, outbreak investigations, and resistance determination. Adoption of NGS remains limited due to high cost, workflow complexity, and long turnaround times. Illumina's new MiSeq i100 is designed for improved efficiency, cost-effectiveness, and ease of use. This study is one of the first worldwide to evaluate the MiSeq i100's performance in the context of clinical outbreaks.

Methods
The MiSeq i100 was tested using two sets of libraries pools, i) a diverse pool of 11 bacterial species (G+C content: 27–74%, genome size: 1.7–8.5 Mb) and b) strains from 2 clinical outbreaks. Performance was assessed based on ease of setup, sequencing accuracy, and turnaround times. For the diverse genomes a single nucleotide polymorphism (SNP) analysis was carried out while the outbreaks strains were compared with core-genome multi-locus sequence typing (cgMLST). The results were compared to data generated with three other Illumina platforms.

Results
The MiSeq i100 demonstrated a streamlined workflow with easy run setup. Turnaround time for paired-end 150 bp sequencing was reduced to 7.5 hours (vs. 19 hours on MiSeq), potentially allowing to start two sequencing runs per day and a potential 24-hour workflow from culture to report. Quality assessment revealed very high concordance with existing platforms, with a maximum of two SNPs observed between technical replicates sequenced on different machines. cgMLST analysis confirmed stable outbreak cluster assignment with minimal allele differences (max 1 allele difference). Additionally, reagent handling was simplified, requiring no cold storage and enabling flexible experiment scheduling.

Conclusions
The MiSeq i100 offers a significant improvement in sequencing speed and usability, addressing key barriers to WGS adoption in clinical microbiology. Its reduced turnaround time, cost-efficient reagent system, and intuitive interface make it a promising tool for rapid and simple routine diagnostics and outbreak surveillance.

P-76 Exploring a nanomotion-based approach for rapid detection of antimicrobial resistance

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Infections caused by antimicrobial resistant (AMR) pathogens remain a significant global health problem. Current gold standard diagnostic protocols are time-consuming and often require 24-48 hours, forcing clinicians to initiate empirical treatment before susceptibility profiles are available. This delay in determining the causative pathogen and its susceptibility profile is critical, as it can lead to inappropriate initial therapy, compromising patient survival.

To address this issue, in this study we investigated the nanomotion-based technique, measuring the mechanical fluctuations of bacteria immobilized on a cantilever in response to specific antibiotic concentrations: 2 µg/ml for ceftriaxone (CRO) and 0.5 µg/ml for ciprofloxacin (CIP), based on EUCAST breakpoints. A total of 109 clinical isolates (51 *E. coli* and 58 *K. pneumoniae*) were provided by different members of the ERADIAMR consortium, each contributing isolates from their own collections. These isolates were selected based on their resistance profiles, specifically to cephalosporins and fluoroquinolones. Each strain was tested in triplicate for each antibiotic, resulting in a total of 654 experiments.

The nanomotion experiment consisted of three phases: an initial “blank phase” using a bare cantilever for quality control, a 30-minute bacterial adaptation phase (“bac phase”), and a final “drug phase” lasting 90 minutes, during which the antibiotic was introduced to the measurement chamber. Machine learning algorithms were applied to the nanomotion data, analyzing changes in bacterial movement during the drug phase, to classify bacterial phenotypes and predict resistance or susceptibility.

This study demonstrates that nanomotion-based technology offers a promising alternative to traditional AST methods, enabling rapid and accurate detection of AMR. Such advancements could significantly enhance clinical decision-making and reduce reliance on empirical treatments.

S-69*/P-77* A journey to antimicrobial discovery: roadblocks and interesting side quests

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The remarkable outer membrane of Gram-negative bacteria presents a significant barrier to antibiotic action. To overcome this, we aimed to develop antimicrobial nanobodies targeting the essential outer membrane protein BamA. Exploiting the β-barrel assembly machinery's crucial role in bacterial survival, we immunized alpacas to raise BamA-specific nanobodies. Using NestLink technology, we identified a small panel of high-affinity cell-surface binders. However, these initial nanobodies lacked antibacterial activity.

To elicit inhibitory nanobodies targeting a vulnerable BamA conformation, a subsequent immunization campaign yielded a next generation of high-affinity binders, again devoid of direct antibacterial activity. Intriguingly, one candidate synergized with the sensitizer PMBN, affecting bacterial fitness. Further investigation revealed a novel, previously undescribed effect of PMBN, potentially indicating a vulnerability in the BamA target and offering insights into outer membrane dynamics. This unexpected finding provides a rational foundation for future antimicrobial development.

Concurrently, we are intensifying our search for direct antimicrobial nanobodies by implementing a functional assay within our screening pipeline. This dual approach – understanding target vulnerabilities and directly screening for activity – represents our ongoing strategy to circumvent the outer membrane barrier and discover novel antibacterial agents.

S-46/P78 Tracing the tropism: How seasonal and avian influenza A viruses target epithelial cells across the human airway

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Influenza A virus (IAV) enters the human host via the respiratory tract, with the airway epithelium serving as the primary site of infection. While previous studies have demonstrated that human (hIAV) and avian (aIAV) strains exhibit distinct cellular tropism within the human epithelium, contradictory results have been described and the underlying determinants of cell type specificity remain poorly understood. Moreover, it is unclear how these patterns vary along different regions of the respiratory tract.
To address these questions, we used differentiated primary human airway epithelial cell cultures derived from both the upper (nasal) and lower (bronchial) respiratory tract to model the complexity and heterogeneity of the airway epithelium in vitro. The cultures were infected with either seasonal hIAV or aIAV strains. The infections were assessed at the level of viral mRNA expression through single-cell RNA sequencing and at the protein level via flow cytometry. Our analysis revealed that ciliated and secretory cells are the primary targets of infection. Strikingly, in bronchial cells, hIAV infects both cell types without a strong preference for either, whereas aIAV displays a significant tropism for ciliated cells. Further, we found that preferential targeting is established during the viral binding stage, despite both cell types displaying similar levels of the viral attachment receptor sialic acid in both α2,3- and α2,6-linkages. To further probe the glycan-mediated mechanisms of viral binding, we performed N-glycomics using mass spectrometry on sorted ciliated and secretory cells. This analysis revealed preliminary differences in surface sialylated glycan complexity and structures between the cell types, which might explain the observed tropism.
Our findings provide new insights into the cell-type specificity of seasonal and avian IAV across the human airway. Understanding these glycan-dependent interactions is key to elucidating the molecular barriers that limit aIAV infection in humans.

S-28*/P-79* Staphylococcal Phage Diversity in a Single Wastewater Treatment Ecosystem

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Antimicrobial resistance (AMR) is a global health threat, with *Staphylococcus aureus* recognized as a major contributor. Bacteriophages, potent bacterial predators, have re-emerged as promising alternatives to traditional antibiotics. However, some phages can also facilitate horizontal gene transfer of AMR genes via transduction. This duality underscores the need to reconcile their therapeutic potential with their role in bacterial evolution, and to develop informed strategies to address the AMR pandemic. In a previous study (Göller et al., 2021), we isolated and preliminarily characterized 94 Staphylococcal phages from a wastewater treatment plant in Zürich. Of these, 40 underwent whole-genome sequencing (WGS), and the morphology of 56 was analyzed by transmission electron microscopy (TEM). Here, we present a comprehensive characterization of this diverse phage collection. Comparative genomic analyses of the initial 40 phages against all Staphylococcal phages from the public domain (Virus-host database) support the assignment of two new viral families, three new genera, and 21 putative species to the International Committee on Taxonomy of Viruses. We achieved >90% (88/94) phage recovery and propagation of the >5-year-old phage stocks from 4°C and/or -80°C. Following optimization of phage propagation (solid lysis) and DNA extraction protocols, we performed WGS on an additional 49 phages using Illumina technology. Genomes were assembled with SPAdes and annotated using Pharokka, using Prodigal for gene prediction. Intriguingly, several genomes contain predicted introns truncating the large terminase subunit gene (terL), suggesting potential regulatory or splicing functions. Morphology for 35 phages was determined via TEM after polyethylene glycol precipitation and cesium chloride gradient purification. All phages showed icosahedral capsids and varied tail lengths, corresponding to myovirus- and siphovirus like morphotypes. Altogether, this work lays the foundation for further analyses on phage mediated bacterial evolution and for a rational selection of phage candidates for therapeutic and biocontrol purposes against a range of Staphylococcal species.

S-49*/P-80* **differential transcriptional analysis of chlamydia trachomatis following iron chelation**

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The life cycle of *Chlamydia trachomatis* (CT) includes an intracellular, biphasic developmental sequence. Additionally, in response to stressors such as IFN-γ, temperature changes or iron deprivation, the bacteria can survive, within the host, in a reversible persistent state. However, the precise mechanisms leading to the transition from replicative, reticulate bodies (RBs) to aberrant bodies (ABs) have not been characterized.

By comparing the transcriptomes of RBs and ABs, we have identified differentially expressed genes predicted to be part of a two-component regulatory system (TCS). TCSs are used by bacteria to sense and respond to their environment. AB formation was induced in CT-infected cells by depleting iron using the chelator 2,2'-bipyridyl (BPDL). RT-qPCRs were performed to quantify RNA levels and to assess gene expression during normal growth and persistence. In parallel, immunofluorescence microscopy was carried out to compare gene expression with morphological changes associated with AB. Results show that TCS genes were strongly downregulated in ABs compared to RBs. When BPDL stress was removed from ABs, expression levels of TCS genes returned to those found in control infections. Downregulation in gene expression varied based on the stressor applied, indicating a relationship between different stress stimuli and TCS gene regulation.

In parallel, we also characterized the expression of genes upregulated during persistence, namely the three genes of the *trpRBA* operon and genes encoding IncD, IncE, IncF, and IncG proteins. Incs are required for biosynthesis and establishment of the bacterial inclusion. Unexpectedly, preliminary results showed that expression of Incs was dependent on the host cell line. To further explore the role of the TCS during persistence, we will overexpress the TCS proteins and assess their impact on chlamydial morphology during persistence.

Uncovering the biological mechanisms that trigger the development of persistent bacteria may provide key insights into the processes underlying chronic chlamydial infections.

S-06*/P-81* Influence of conserved Cys-residues in a transcription factor on ICEclc activation in Pseudomonas putida

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Horizontal gene transfer (HGT) is major mechanism for producing bacterial genome variation. Different HGT mechanisms are known in great detail, but less is known about the underlying regulatory system(s) and their response to environmental signals.
Here, we use the Integrative and Conjugative Element ICE*clc* of *Pseudomonas putida* as a model system. ICE*clc* encodes genes allowing the degradation of 3-chlorobenzoate (3CBA). Its activation is tightly regulated, involving a combination of classical regulators—MfsR and TciR (a TetR- and LysR-type regulator, respectively)—as well as the recently discovered BisR and BisDC families [1]. Activation and excision of ICE*clc* is constrained to a small subset of cells, named 'transfer competent' (tc) cells, and is highest when cells have been grown to stationary phase with 3CBA as unique carbon source (2-5% of cells) [2]. Recent work also showed that 3CBA metabolism induces a strong oxidative stress, suggesting that cells experiencing above-average oxidative stress are more likely to activate the tc program.
To investigate the potential link of oxidative stress to ICE*clc* activation, we focused on the TciR transcription activator. TciR has weak homology to OxyR, the oxidative stress regulator of *Escherichia coli*. In silico analysis identified four cysteines in TciR, including one (C199) perfectly aligned with one of OxyR reactive cysteines [3]. Based on this, we constructed four mutants in *P. putida* ICE*clc tciR* carrying an individual cysteine-to-serine substitution, and monitored their effects on ICE*clc* activation using a fluorescent transcriptional reporter and on ICE conjugation. Replacement of 3 out of 4 Cys-residues by Ser decreased ICE*clc* activation by more than tenfold, whereas the fourth (C203S) reduced activation by two-fold. This supports the hypothesis of implication of oxidative stress in ICE*clc* activation, possibly involving intra-molecular disulfide bond formation, modulating TciR oligomerization that affect its activation of the *bisR* promoter.

S-66*/P-82* batch effects and data representation in maldi-tof ms data for machine learning driven identification of microorganisms

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Introduction

MALDI-TOF MS is a cornerstone of rapid microbial identification. Machine learning (ML) models applied to MS data promise to improve specificity, antimicrobial resistance (AMR) and virulence prediction, which potentially reduces turnaround times and optimized treatments. However, these ML models struggle with generalizability, as their performance declines when applied on different machines or over extended periods. Two factors contribute: (i) batch effects and (ii) inconsistent spectral quality. Considering that data representation enhances ML model effectiveness, optimizing data representation strategies may offer a needed solution.
We aimed to explore batch effects in MALDI-TOF MS data and evaluate current data representation strategies to improve model generalizability.

Methods

We analysed fifty clinical isolates of the closely related *Enterobacter cloacae* group on four different MALDI-TOF MS machines, with four technical replicates per machine and three different operators, generating 2400 spectra. Species identity and genetic diversity were confirmed using whole genome sequencing (WGS). Spectral differences across machines and replicates were assessed in relation to phylogenetic strain relationships, and spectral clustering was used to explore grouping patterns among spectra. We applied post-acquisition m/z calibration using genus-specific ribosomal protein markers to correct peak shifts and compared spectra.

Results

In the dataset, we had *E. hormaechei*, *E. ludwigii*, and *E. sichuanensis, E. kobei*, *E. cloacae* and *E. roggenkampii sensu stricto.* Preliminary results show that subspecies were more distinctly mapped in the t-SNE plot post-calibration. Spectra with fewer than 15 detected ribosomal markers mapped together despite being generated from different strains, operators, and machines; this pattern, visible before calibration, formed a distinct cluster after the calibration step.

Conclusion

Our results highlight the value of both noise reduction and the application of quality control. Low-quality spectra can affect reproducibility and lead to misidentification. Establishing exclusion criteria based on spectral quality is essential for reliable species identification and accurate downstream analyses.

S-75*/ P-83* The role of ribosomal hibernation factors for Legionella pneumophila starvation, virulence, and intracellular persistence

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Ribosomal hibernation downregulates energy-intensive translational activity in many bacteria upon encounteringadverse conditions. However, little is known about this process in *Legionella* pneumophila, an amoeba-resistantbacterium found in technical and natural water systems. *L. pneumophila* replicates in amoebae and also infectshuman alveolar macrophages, thus causing a severepneumonia termed Legionnaires' disease.
L. pneumophila possesses two different putative hibernation promoting factors (HPFs), which likely compete for the same ribosomal binding site: a homolog of the "long-HPF", which promotes the formation ofinactive 100S dimers, and a homolog of RaiA, whichinactivates ribosomes as 70S monomers. We found that *L. pneumophila* missing the RaiA homolog indeed forms more inactivated 100S dimers. Furthermore, we identified a homolog of the ribosomal silencing factor RsfS, which binds the 30S subunit and thus inhibitsribosome assembly, as well as the conserved GTPase HflX, which rescues stalled 70S ribosomes and putatively splits 100S dimers and inactivated 70S monomers.
Ribosomal hibernation factors contribute to long-term starvation survival of *L. pneumophila*, and both their loss and overexpression significantly prolong the lag time during regrowth in rich media. Moreover, all *L. pneumophila* strains lacking one or more ribosomal hibernation factors are impaired for virulence, and the overexpression of HPFs intensifies the virulence phenotype immensely. All mutants are taken up less efficiently by their natural host *A. castellanii*, in agreement with an initial delay and/or reduction in intracellular replication.
The mutants also form fewer persisters (non-growers) during the infection cycle, indicating that ribosomal hibernation regulates intracellular phenotypic heterogeneity. Interestingly, *L. pneumophila* strains lacking individual ribosomal hibernation factors tend to form less motile, *flaA*-expressing bacteria at the late stages of infection, and conversely, the loss of both HPFs significantly increases the emergence of the transmissive, flagellated sub-population. Taken together, the ribosomal hibernation factors of *L. pneumophila*regulate starvation, virulence, and persistence of the pathogen.

P-84 Harnessing Synthetic Single-Domain Antibodies for Characterisation and Diagnosis of Staphylococcus aureus Infections

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Staphylococcus aureus is a significant pathogen responsible for invasive diseases such as endocarditis and osteomyelitis. The rise of antibiotic-resistant strains has made treatment increasingly challenging, necessitating advanced research tools to understand this pathogen better. Further, despite advancements in technologies, Gram staining remains the standard in most diagnostic laboratories due to its simplicity and reliability. Traditional methods suffer from long turnaround times, while newer technologies face cost and accessibility barriers. To address these challenges, we have developed synthetic single-domain antibodies that specifically target the *S. aureus* cell surface. Using fluorescent-tagged conjugates we have demonstrated that these binders hold promise for simple and efficient diagnostics. Additionally, we show that conjugation to magnetic beads enables direct pathogen isolation from complex samples, facilitating bacterial studies in native states. Our approach offers a competitive advantage over immunoglobulin-based binding, enhancing clinical applicability. Building on this foundation, we aim to utilise our capture method to refine host-mimic media compositions for translational research on *S. aureus*. Furthermore, we envision a pathogen-staining protocol for digital microscopy-based diagnostics, ensuring compatibility with existing Gram staining workflows. By bridging fundamental research with translational applications, this work advances diagnostic precision and accessibility. Our methods can be adapted for other bacterial pathogens, contributing broadly to infectious disease research and precision medicine. This project paves the way for enhanced bacterial diagnostics and personalized treatment strategies, ultimately improving patient outcomes.

P-85 Environmental factors influencing the persistence of overlooked respiratory viruses in human saliva droplets in indoor settings

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Respiratory viruses cause widespread illness each year and can result in severe disease, particularly in children and immunocompromised individuals. Virus-laden respiratory secretions serve as a primary environmental reservoir, and the persistence of infectious virus within these particles increases the risk of transmission to susceptible hosts. Environmental factors, including relative humidity (RH), play a critical role in shaping viral stability during transmission. While much research on environmental persistence focuses on influenza virus, coronavirus, or surrogate viruses like bacteriophages, other respiratory viruses, such as adenovirus and rhinovirus, continue to circulate and pose public health burden. A broader understanding of environmental stability across diverse viral pathogens is needed to inform effective mitigation strategies, particularly in high-risk indoor environments. Additionally, studies have often relied on non-physiological media, which may not accurately reflect virus behavior in natural secretions like human saliva.

To address these gaps, we examined the inactivation kinetics of three respiratory viruses—human adenovirus type 4 (HAdV4), influenza A virus (H1N1pdm09), and human rhinovirus 16 (HRV16)—in human saliva and water droplets across RH conditions representative of indoor environments. Our results demonstrate that HAdV4, a nonenveloped DNA virus approximately 90 nm in diameter, remains infectious in human saliva over 24 hours, with less than 1.3 $\log_{10}$ decay within the first 4 hours across all tested RH levels (20%, 50%, 80%). In contrast, rhinovirus, a nonenveloped ssRNA virus around 30 nm in size, exhibits significantly greater decay at mid-range RH (50%).

These findings emphasize the virus-specific nature of environmental persistence and highlight the limitations of relying on data from a single virus for designing public health strategies. Ongoing work focuses on how viral characteristics, such as capsid structure and host cell entry mechanisms, influence environmental stability. Ultimately, these insights will contribute to engineering control strategies that mitigate airborne and fomite-based transmission of respiratory pathogens.

S-29*/ P-86* **A dual approach to discover and isolate staphylococcal temperate bacteriophages**

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Bacteriophages lead bacterial evolution and are re-emerging as promising tools to target resistant pathogens. Little is known about temperate phages in members of Staphylococcaceae family other than *Staphylococcus aureus*. These prophages may contribute to gene transfer, adaptation, and interactions with therapeutic phages—factors that need to be better understood to fully assess the risks and potential of phage-based interventions.

We developed a scoring system to identify optimal lysogens (strains carrying prophages) of the Staphylococcaceae family to explore their temperate phage landscape and to assess their bacterial adaptation properties. For this, an *in silico* pipeline was established to analyze 70 genomes from our institute. A score weight was defined for each trait of interest and manual curation was conducted. The ideal candidate harbors at least one intact prophage (Phastest), multiple plasmids (PlasmidFinder), virulence (VFDB) and resistance factors (CARD, ResFinder) or phage satellites (SatelliteFinder). 74% of the strains analyzed contain predicted intact prophages, carrying either one (34%), two (29%) or three (11%). Coagulase positive staphylococci (CoPS) were enriched in prophages (95%), followed by coagulase negative staphylococci (CoNS) (81%) and *Macrococcus* (72%). Overall, 21% of predicted lysogens additionally carry plasmid/s, 50% carry AMR gene/s and 13% carry virulence gene/s. Five lysogens (7.1% of all strains) co-carry plasmid/s, AMR/s and virulence gene/s. Concurrently, we established a robust UV induction protocol to induce CoNS prophages from selected strains. The protocol was successfully tested on selected diverse lysogens. Ongoing studies focus on the molecular characterization and taxonomic classification of these inducible CoNS phages along with their involvement in shaping host bacterial traits.

We highlight high predicted prophage content and additional adaptive elements among staphylococci. This dual approach combining bioinformatic screening and experimental induction offers a scalable strategy to discover and harness novel functional temperate staphylococcal phages, enabling to appraise their contribution to generate staphylococcal networks.

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