SGM·SSM

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ABSTRACT BOOK

Organization

Congress organization

Prof. Gilbert Greub, Institute of Microbiology, CHUV, Lausanne

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Clinical Microbiolgy
Adrian Egli, Basel ; Jacques Schrenzel, Lausanne ; Peter Keller, Bern

Early career
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Environmental Microbiology
Cara Magnabosco, Zurich ; Jürg Enkerli, Zurich

Lay Communication
Gilbert Greub, Lausanne

Molecular Microbiology
Hubert Hilbi, Zurich ; Jan-Willem Veening, Lausanne

Mycology
Alix Coste, Lausanne ; Jürg Enkerli, Zurich

Microbiomes
Pascale Vonaesch, Lausanne

Virology
Volker Thiel, Bern ; Valeria Cagno, Lausanne

Set-up of the Abstract book

SSM Congress Office Management, meeting.com congress organisation

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KEYNOTE TALK

K-01 MOLECULAR secrets of 7th pandemic *Vibrio cholerae*

Prof. Melanie Blokesch

Laboratory of Molecular Microbiology, Global Health Institute, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

The diarrheal disease cholera still sickens millions of people each year. Despite incredible progress in our understanding of *Vibrio cholerae*'s virulence mechanisms, studies on molecular processes that are not directly linked to the host remain limited for this pathogen.
In my talk, I will address this knowledge gap and present new insights into how the bacterium defends itself against mobile genetic elements such as plasmids and bacteriophages. Indeed, we recently identified two dedicated DNA defence modules (Ddm) in *V. cholerae* O1 El Tor strains, the latter being responsible for the ongoing 7th cholera pandemic. These systems are encoded on two major pathogenicity islands that are hallmarks of current pandemic strains[1]. We show that both modules cooperate to rapidly eliminate small multi-copy plasmids by degradation. Moreover, one system can defend against bacteriophage infection by triggering cellular suicide (abortive infection; Abi). We go on to show that this Abi-like mechanism also increases the burden of large conjugative plasmids creating a fitness disadvantage that counter-selects against plasmid-carrying cells. These two plasmid elimination strategies therefore answer the long-standing question of why plasmids, although abundant in environmental strains, are conspicuously absent from 7th pandemic *V. cholerae* patient isolates. Moreover, together with recent studies from other groups, our work highlights the importance of studying unknown genes and gene clusters that are located on prominent pathogenicity islands. Indeed, bacterial immune systems such as the ones described by our work, might have contributed to the success of the 7th pandemic clade of *V. cholerae,* which is considered the longest-enduring pandemic lineage in history.

[1] Jaskólska M.*, Adams D.W.*, Blokesch M. (2022) Two defence systems eliminate plasmids from seventh-pandemic *Vibrio cholerae. Nature,* 604:323-329.

KEYNOTE TALK

K-02 Combating Multidrug-Resistant Superbugs by Targeting the Host-Pathogen Interaction

Victor Nizet, MD

University of California, San Diego, US

Antibiotic resistance presents an ever increasing challenge to the public health with a dearth of new drugs in the development pipeline. A single-minded focus on direct antimicrobial activities overlooks the fact that significant infections are really a disease of the host-pathogen interaction. Indeed, before the patient has even seen a doctor, their infection is already being treated by multiple antimicrobials - namely the cellular and molecular components of the innate immune system. We see value in exploring potential novel therapeutic approaches for drug-resistant bacteria that aim to tip the host-pathogen interaction back in favor of the host. This talk will illustrate novel therapeutics that mitigate bacterial virulence, re-sensitize the pathogen to innate immune killing or directly boost the antibacterial killing capacity of host cells. Current MIC testing can be misleading, and overlook potent antibiotic activities that are recognized only the context of the normal innate immune system. In this new discovery and treatment framework, drugs used in medicine for other indications, antibiotics otherwise deemed ineffective, or natural host cell membranes can be “rediscovered” or “repurposed” for treatment of multi-drug resistant pathogens such as methicillin-resistant Staphylococcus aureus (MRSA) multi-drug Gram-negative pathogens including *Acinetobacter baumannii, Klebsiella pneumonia,* and mcr-1+ *Escherichia coli.*

KEYNOTE TALK

K-03 Into the post-meta’omics era of uncultivated microbes: Integrating omics and microscopy for ecophysiological studies

Prof. Alexander J. Probst

Research Center One Health Ruhr, University Alliance Ruhr, Environmental Metagenomics, Faculty of Chemistry, University of Duisburg-Essen, Germany

It has been known for decades that most prokaryotes cannot be cultivated and isolated under defined laboratory conditions. With the SeqCode at hand, scientists can now validly name bacteria and archaea based on their genomic information as type material. However, understanding and studying the physiology of the uncultivated majority *in situ* is still in its infancy. In this talk, I will provide examples of well-studied uncultivated archaea from the deep biosphere and demonstrate how far scientist can take microscopy methods in understanding microbes that we cannot cultivate in the lab. I will talk about their genomic information, their ultrastructure, and their infection with uncultivated viruses. I will show links between genomics and microscopy to resolve archaeal ecophysiology at high resolution and decipher interactions between microbes. Lastly, I will provide one example of heavily studied and very abundant archaea on our planet, which have already been cultivated *in situ* almost two decades ago but have only been assigned a *Candidatus* name based on their genomes from metagenomes in recent years. The post-meta’omics era is now.

MAIN TALKS – SHORT TALKS

M-01 Disease modelling and its contribution to decision making

Narimane Nekkab PhD, MPH on behalf of Professor Melissa Penny, PhD

Swiss Tropical and Public Heath Institute, Swiss TPH, Allschwil, Switzerland

Infectious disease modelling increasingly generates quantitative evidence to provide biological insights and support decision-making. The construction of this evidence requires analyses of various scenarios in which models can support understanding the biology of the disease at the individual level through to population-level public health implications. The robustness of the model predictions depends on the quality and mechanistic assumptions of disease and transmission dynamics described by the model and the data used to inform model parameters. In this presentation, I will discuss the extensive history of malaria modelling, highlighting how modelling can inform and fills gaps in evidence used to support policy decisions. I will show examples of how mathematical models of malaria have been used to inform global policy decisions for the first human malaria vaccine, how models inform characteristics of novel malaria interventions, and how modelling supports understanding malaria parasite resistance evolution informing genomic monitoring in clinical trials and implementation.

SESSION 1 – SCIENCE AND POLICY

S-01 Reorientation of the Coordinated Medical Service – a Journey Has Begun

S-02 IVDR: applications of the In Vitro Diagnostic Ordinance in Switzerland and possible Impacts

Dr. Alix Coste[1], Prof. Gilbert Greub[1]

Institute of Microbiology, CHUV, Lausanne, Switzerland

IVDR regulation represents a major challenge for diagnostic microbiology laboratories. Yet, not all of us are very clear about what this will mean in our day-to-day clinical microbiology practice. In this presentation, we'd like to give an overview of these new regulations, and to highlight their potentially deleterious aspects. In particular, the negative impact it may have on the quality of innovation and R&D, workload and costs. We will also propose limitations to reduce these negative impacts.

M-02 Early detection of sepsis using machine learning

Prof. Catherine Jutzeler

ETH Zurich, Zurich, Switzerland

Sepsis is a life-threatening condition that occurs when the body's response to infection causes organ dysfunction and tissue damage. Early detection and treatment of sepsis are crucial for improving patient outcomes. This talk will provide an overview of how machine learning can be used to recognize sepsis earlier and predict its course more precisely than currently possible for an individual patient. Specifically, the preliminary results from the Personalized Swiss Sepsis Study (PSSS) will be presented. The PSSS is a nation-wide initiative that collects and integrates continuous monitoring data, molecular data from bacterial pathogens and the host, and clinical data from sepsis patients admitted to the intensive care units of the Swiss university hospitals. Lastly, the challenges and opportunities of using machine learning for sepsis research and practice, such as data quality and availability, sepsis definition and labeling, as well as legal issues, will also be discussed.

S-03 MICROBIOTA analysis by cell-type recognition to enable fast and reliable medical diagnosis

Dr. Birge Ozel-Duygan [1], **Prof. Claire Bertelli** [2], **Prof. Gilbert Greub** [2]

[1] CHUV, Lausanne, Switzerland [2] Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

In recent years, several studies demonstrated that numerous diseases such as inflammatory bowel disease, obesity, type 2 diabetes mellitus and many more are linked to disorder in gut microbiota. As examples, various forms of inflammatory bowel disease including Crohn's disease and ulcerative colitis show depletion of enteric bacterial diversity and development of specific bacterial clades such as *Enterobacteriaceae*. Next generation sequencing technologies expanded our knowledge on taxonomic diversity of microbiota such that dysfunctional and healthy microbiota can be differentiated. However, despite these successes, requirements of specialized equipment and expertise as well as analysis time limit the use of next generation sequencing approaches to research settings; thus, preclude rapid analysis or treatment in clinical settings. We investigate the potential of flow cytometry to perform rapid analysis of human gut microbiome-associated diseases. The overall aim is to meet the crucial demand for a rapid, simple, affordable yet reliable alternative method for human gut microbiota status (i.e. healthy vs dysbiosis), and identification of disease state as well as providing real time quantitative and physiological information of dynamic microbiome composition.

We deployed supervised machine-learning algorithms to train representative human gut microbiota bacterial strain discrimination and for producing the classifiers. Our study showed an overall recall of 90% for a combined set of 29 bacterial strains. As a case study, we applied our pipeline on an open-source flow cytometric dataset of disease cohort. We found that patients with Crohn's disease (n=29) have a distinct flow cytometric profile compared to healthy controls (n=65). CellCognize can distinguish patients with Crohn's disease with >90% sensitivity and 95% specificity. We concluded that flow cytometry-based analysis of stained faecal microbiota coupled to machine learning provides dynamic, quantitative, and physiological information of microbiome composition, and thus holds great promise as a rapid diagnostic tool.

S-04 Genome validation and expansion of the family of Borg archaeal extrachromosomal elements reveals a syntenous core genetic repertoire

Dr. Marie Schoelmerich [1], **Ms. Lynn Ly** [2], **Dr. Rohan Sachdeva** [1], **Dr. David Dai** [2], **Dr. Christopher Mozsary** [2], **Dr. Scott Hickey** [2], **Dr. Christine He** [2], **Dr. John Beaulaurier** [2], **Dr. Sissel Juul** [2], **Prof. Jill Banfield** [1]

[1] UC Berkeley, USA [2] Oxford Nanopore Technologies Incorporated, USA

Borg linear extrachromosomal elements of up to 1.1 Mbp in length are associated with anaerobic methane-oxidizing archaea of the genus "*Candidatus* Methanoperedens" [1]. The first objective of this work was to validate the overall Borg genome topology with long-read nanopore sequencing. The second objective was to discover new Borgs, recover a complete Borg host genome, and shed light on the DNA methylation patterns. Thus, nanopore sequencing was performed on a subset of DNA samples from deep soil of a natural wetland where the majority of previously reported Borg genomes originate from. Methylation motifs and types of Borg DNA and host DNA were predicted using Nanodisco and Megalodon and Rerio models. Comparison of the nanopore assembled sequences to five published manually curated, Illumina-based Borg genomes validated their overall topologies. Nanopore sequences also provided a roadmap for the reconstruction of seven new Borg genomes. We find that all 17 Borg genomes have a largely syntenous core genome structure that comprises genes likely involved in replication, nucleotide processing, cell decoration and signaling. We also circularized and completed a 4 Mbp *Methanoperedens* genome inferred to be a Borg host and detect it has six distinct methylation motifs. Borg genomes generally have very distinct and pervasive methylation motifs composed of just two nucleotides. This work demonstrates that Borgs possess a core set of genes that likely derived from a common ancestor. Phylogenomic analysis further uncovers a consistent separation of Borgs into two main clades. The DNA modifications observed in Borg and *Methanoperedens* DNA suggest that the replication and gene expression processes of each entity are distinctly regulated.

[1] Al-Shayeb, B., Schoelmerich, M.C., West-Roberts, J. *et al.* Borgs are giant genetic elements with potential to expand metabolic capacity. *Nature* 610, 731–736 (2022).

M-03 New vaccines against emerging zoonotic infections: From animal models to clinical evaluation

Prof. Asisa Volz

Institute of Virology, University of Veterinary Medicine Hannover, Germany

Safety tested Modified Vaccinia virus Ankara (MVA) is licensed as third-generation vaccine against smallpox and serves as a potent vector system for development of new candidate vaccines against infectious diseases and cancer. Historically, MVA was developed by serial tissue culture passage in primary chicken cells of vaccinia virus strain Ankara, and clinically used to avoid the undesirable side effects of conventional smallpox vaccination. Adapted to growth in avian cells MVA lost the ability to replicate in mammalian hosts and lacks many of the genes orthopoxviruses use to conquer their host (cell) environment. At present, MVA serves as an advanced vaccine technology platform for developing new vector vaccines against infectious disease and cancer including emerging viruses. Previous work addressed the development of an MVA candidate vaccine against MERS with immunizations in animal models demonstrating the safety, immunogenicity and protective efficacy of MVA-induced MERS-CoV S-antigen specific immunity. Clinical safety and immunogenicity of the MVA-MERS-S candidate vaccine was established in a first-in-man phase I clinical study. Currently, MVA-MERS-S is being tested in an European-level collaboration for phase 1b/2a clinical evaluation. Recent work addressed the preclinical development of MVA vector vaccines against COVID-19. A recombinant modified vaccinia virus Ankara (MVA) expressing native, full-length S protein (MVA-SARS-2-S) has been tested as candidate vaccine in phase I clinical studies. Initial results from immunogenicity monitoring revealed induction of S-specific antibodies binding to S2, but low-level antibody responses to the S1 domain. Follow-up investigations of native S antigen synthesis in MVA-SARS-2-S infected cells revealed limited levels of S1 protein on the cell surface. In contrast, we found superior S1 cell surface presentation upon infection with a recombinant MVA expressing a stabilized version of SARS-CoV-2 S protein with an inactivated S1/2 cleavage site and K986→P and V987→P mutations (MVA-SARS-2-ST). When comparing immunogenicity of MVA vector vaccines, mice vaccinated with MVA-SARS-2-ST mounted significant levels of S broadly reactive antibodies that effectively neutralized different SARS-CoV-2 variants. Importantly, intramuscular MVA-SARS-2-ST immunization of hamsters and mice resulted in potent immune responses upon challenge infection and protected from disease and severe lung pathology. Our results suggest that MVA-SARS-2-ST represents an improved clinical candidate vaccine and that the presence of plasma membrane-bound S1 is highly beneficial to induce protective antibody levels.

S-05 Antimicrobial Cationic Coating for Rapid Self-Disinfecting Surfaces

Dr. Jules Valentin [1], **Dr. Samuel Watts** [1], **Dr. Mark Gontsarik** [1], **Dr. Aline Wolfensberger** [2], **Prof. Silvio Brugger** [2], **Dr. Mahsa Zabara** [3], **Prof. Stefan Salentinig** [1]

[1] Department of Chemistry, University of Fribourg, Fribourg, Switzerland [2] University Hospital Zürich, Zürich, Switzerland [3] T-LVG Technologies, Switzerland

The prevention of microbial infections is a global challenge. Efficient antimicrobial coatings that rapidly kill microorganisms upon contact can help minimize their transmission and biofilm formation. This work demonstrates the scalable synthesis and characterization of self-disinfecting nanofilms for the post-modification of hospital-relevant surfaces. Photoinitiated bulk polymerization of an air-dried [2-(methacryloyloxy)ethyl] trimethylammonium chloride (METAC) film on cotton (gowns), nitrile rubber (protective gloves), and glass surfaces (tables, screens) is used for their supercharging. A 6 nm thick coating dominated by cationic quarternary amine groups was shown by a combination of spectroscopic imaging ellipsometry and X-ray photoelectron spectroscopy. Surface chemistry analysis with XPS showed the covalent attachment of the coating to the surface. Further, all the coated surfaces demonstrated a significant increase in surface charge after coating. Antimicrobial *in vitro* evaluation of the coated surfaces demonstrated >3 log reductions of *Staphylococcus aureus* and *Escherichia coli* populations within 5 min of contact. The antibacterial activity is attributed to the strong surface charge leading to bacterial membrane disruption via the strong electrostatic interaction with the surface. The coating's range of compatible materials and its rapid bactericidal activity can combat the surface transmission of bacteria and may help to contain the spread of infectious diseases. Its synthesis in environmental conditions is promising for integration into industrial processes.

S-06*/P-142* Novel attachment inhibitors against human parainfluenza 3 virus with broad-spectrum activity

Mr. Gregory Mathez [1], **Dr. Paulo Jacob Silva** [2], **Prof. Francesco Stellacci** [2], **Dr. Valeria Cagno** [1]

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Respiratory viruses can cause severe infections leading to bronchiolitis or pneumonia. Human parainfluenza viruses are human respiratory viruses causing severe infections, especially in young children, elderly people, and immunocompromised. Human parainfluenza 3 virus (PIV3) is the most common type and can cause lower respiratory infections leading to hospitalization. Unfortunately, we are lacking vaccines and antivirals against this virus. With the aim to develop new antivirals, we designed attachment inhibitors endowed with virucidal activity and broad-spectrum activity against respiratory viruses.
To identify the natural attachment receptors used by PIV3, we performed a glycan array with two clinical isolates from university hospitals and with a laboratory strain. LS tetrasaccharide d (LSTd), an α2,3 sialic acid-based glycan present in the human respiratory tract, and heparin octasacharide were the top common hits. We synthesized modified cyclodextrins harboring LSTd (CD-LSTd) or sulfonates (CD-MUS). The structure of the macromolecule was chosen from previous work in which it was shown to confer not only antiviral activity but also virucidal activity. Both molecules showed antiviral and virucidal activity in vitro, although the sulfonated had higher potency. Additionally, they retained activity in human-derived respiratory tissues. Combination experiments suggest different proximal binding sites. Therefore we designed and synthesized a unique modified cyclodextrin mimicking both attachment receptors (CD-LSTd/SO_3) with higher potency compared to CD-LSTd. The modified cyclodextrin mimicking sialic acid (CD-LSTd) showed as well antiviral activity against different subtypes of Influenza A virus such as H1N1 or H5N1 and Influenza B virus showing its broad-spectrum activity.
Current work is dedicated to the optimization of the synthesis to achieve better potency and virucidal activity for optimal therapeutical administration as well as *in silico* modeling of the mechanism of action and resistance studies. Altogether, we designed promising new antivirals active against clinical PIV3 with a broad-spectrum activity against other respiratory viruses.

*Student presentation

M-04 Discovering patterns in growth and division with advanced microscopy

Prof. Suliana Manley

Laboratory of Experimental Biophysics, School of Basic Sciences, Institute of Physics, Interfaculty Institute of Bioengineering, EPFL, Lausanne, Switzerland

Discovering patterns in growth and division with advanced microscopy
We develop automated and super-resolution based methods that allow us to reveal patterns within the processes of growth and division, from bacterial and mammalian cells to mitochondria. Mitochondria are heterogeneous organelles that divide asynchronously from their host cell cycle, yet we discovered patterns underlying their division and genome organization. In bacteria, we developed methods to reveal cell wall remodeling patterns that underlie their cell cycle. The intermittent dynamics of these processes imply that a constant imaging speed may miss important features. Thus, we developed event-driven acquisitions, an adaptive microscope control that uses neural networks to enrich datasets for events of interest.

S-07* ***Staphylococcus aureus*: localization and responses to antibiotics in human patients**

***Ms. Vishwachi Tripathi*[1], Mr. Benedict Morin[2], Dr. Richard Kühl[3], Ms. Aline Loriol[1], Ms. Kristiina Kurg[4], Dr. Sébastien Herbert[5], Dr. Alexia Ferrand[5], Mr. Krittapas Jantarug[6], Prof. Pablo Rivera-Fuentes[6], Dr. Mario Morgenstern[3], Dr. Martin Clauss[3], Prof. Nina Khanna[3], Prof. Dirk Bumann[1]**

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Staphylococcal infections vary from superficial skin infections to life threatening bacteremia and are associated with high morbidity and mortality. Deep-seated and blood stream infections are difficult to eradicate with antimicrobial chemotherapy even if the causative *S. aureus* strain is susceptible in laboratory assays. Various mechanisms enable survival of antibiotic-exposed *S. aureus in vitro* and in mouse models, but evidence for human tissues is scarce. Here, we localized ~8,000 *S. aureus* in 20 biopsies from deep-seated infections of patients with or without antibiotic therapy using fluorescent vancomycin, immuno-staining and 3D microscopy. ~60-70% of *S. aureus* cells resided alone or in clusters of up to 4 cells. Large clusters were rare and typically undetectable in the tissues. The majority of *S. aureus* resided intracellularly in monocytes/macrophages but ~30% were extracellular. Antibiotic therapy for up to 7 days did not cause detectable shifts to larger bacterial clusters, general intracellular compartments, or specific host cells, compared to non-treated patients. These data fail to support biofilms, intracellular localization, or specific stressful niches as crucial permissive sites for antibiotic survival. Instead, antibiotic survival seemed to be a *S. aureus* population-wide property *in vivo*. To test this idea, we modified currently used *in vitro* assays to more physiological conditions and observed effective survival or even growth of *S. aureus*, even when exposing them to antibiotics that are commonly categorized as bactericidal. Thus, *in-vitro* conditions approximating in-patient conditions might predict treatment outcome better and provide a basis for identifying more effective control strategies.

*Student presentation

S-08/P-143 **Towards Label-Free Recognition of Single Cell Bacterial Species Based on Phase Contrast Timelapses**

***Ms. Bouke Bentvelsen*[1], Ms. Tania Miguel Trabajo[2], Dr. Helena Todorov[1]**

[1] Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland [2] University of Lausanne, Lausanne, Switzerland

Understanding the spatial-temporal dynamics of single-cell bacteria is crucial for unravelling colony formations and bacterial interactions, making it a fundamental aspect of microbiome research. However, traditional approaches like bacterial labelling are laborious, time-consuming, and challenging to scale up, particularly in complex microbial ecosystems. In this study, we propose a machine-learning approach that leverages the morphology of single cells from phase-contrast images, added with information autofluorescence across the spectrum measured in bins.
Building upon the work of Helena Todorov, who developed Dimalis—a pipeline capable of identifying and tracking cells in timelapse images while enabling feature extraction of cell masks and their fluorescence—we apply this methodology to a subset of a synthetic soil microcosm. The community consists of seven distinct bacterial species (Burkholderia, Curtobacterium, Microbacterium, Mucigalinibacter, P. *putida*(GFP), and P. *veronii*(mCherry)) that are seeded onto agarose patches prior to imaging. Using a random forest model trained on data from a single timepoint [2 hours after seeding], we achieved a classification accuracy of 94% when applied to in silico mixed single-cell observations of monocultures. Our ongoing work involves adapting the model to incorporate temporal data. Furthermore, we are testing the model on triple cultures, where the labelled P. *putida* and P. *veronii* serve as ground truth, enabling the identification of a single remaining species.
The proposed label-free recognition approach holds significant potential for advancing our understanding of bacterial dynamics in diverse environments, overcoming the limitations of traditional methods and paving the way for broader applications in microbiome research.

M-05 FROM zoonotic to pandemic – animal influenza viruses as the looming danger?

Prof. Martin Beer

Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

Seasonal epidemics and five pandemics in the last 105 years demonstrate the importance of influenza A viruses (IAVs) to humankind. All IAVs originate from wild waterfowl and are able to adapt to new hosts. The transition from birds to mammals plays a crucial role in further adaptation to humans and ultimately in continuous human-to-human transmission. So far, only the H1, H2 and H3 subtypes have been observed in humans, and only H1 and H3 viruses in pigs. However, the avian reservoir contains the full range of subtypes from H1 to H16. The segmented 8-component genome of IAV allows for numerous reassortments and new admixtures, which can lead to large jumps in addition to continuous genetic evolution.
This is important because IAVs, which are so abundant in birds, have to overcome major adaptation hurdles to establish infection cycles in humans and pigs. Reassortment can rapidly exploit already adapted genetic elements, allowing pigs to be infected with human viruses (anthropozoonoses) without further adaptation steps.
At present, zoonotic H5 viruses of clade 2.3.4.4b are spreading worldwide and have led to countless infections in wild birds and poultry, and from there to isolated spillover infections in carnivores such as foxes or even human infections are reported. In addition, reassortants of swine influenza viruses with the 2009 H1N1 pandemic virus are increasingly being observed in pig populations worldwide. Some of these viruses are already classified as “pre-pandemic”.
Finally, in recent years, another reservoir of influenza A viruses has been discovered: the bat. The virus strains found there (H17N10, H18N11 and H9N2) partly use different receptors and show characteristics suggesting a special adaptation to mammalian hosts. Their zoonotic potential will also be evaluated and discussed.
Using these examples, the hurdles to human adaptation are presented and discussed, and the risk of future influenza virus pandemics is assessed.

S-09 2022 European outbreak of Corynebacterium diphtheriae among migrants illuminated through whole genome sequencing

Dr. Helena Seth-Smith [1], **Dr. Luca Freschi** [2], **Dr. Federica Palma** [3], **Dr. Anja Berger** [4], **Dr. Stefanie Schindler** [5], **Dr. Daniel Palm** [2], **Dr. David Litt** [6], **Prof. Andreas Sing** [4], **Prof. Sylvain Brisse** [3], **Dr. Andreas Hoefer** [2], **Prof. Adrian Egli** [1], **Dr. European Diphtheria Consortium Diphtheria Consortium** [2]

[1] Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland [2] ECDC, Sweden [3] Institut Pasteur, France [4] Bavarian Health and Food Safety Authority, Germany [5] AGES, Austria [6] UKHSA

Increased numbers of cases of *Corynebacterium diphtheriae* were observed in Europe in the latter half of 2022, predominantly among migrants.. The majority of cases reported were of cutaneous diphtheria only, but respiratory cases were also reported. We investigated the outbreak at the genomic level.
Wound and nasopharyngeal swabs samples were taken from patients and contacts, predominantly migrants in reception centers across Europe. Isolates underwent whole genome sequencing (WGS). Genomic analysis used core genes to identify clusters and SNP analysis and time trees within clusters. DIPHTOSCAN identified *tox* encoding diphtheria toxin, and antimicrobial resistance (AMR) determinants.
We analysed *tox*-positive isolates of *C. diphtheriae* from Jan-Nov 2022 from Switzerland (52), Germany (118), UK (59), Austria (66), France (30), Belgium (21), Norway (8), The Netherlands (5), Italy (3), and Spain (1). cgMLST analysis identified four genomic clusters (GC) containing >15 genomes: GC795 (ST574, n=134), GC817 (ST377, n=114), GC671 (ST377, n=19), and GC217 (ST384, n=79). GC671 (ST377) stands out as carrying *ermX* (phenotypically resistant to erythromycin) and *bla*OXA-2 (phenotypically susceptible to all beta-lactams). Detailed SNP analysis within the four clusters showed root-to-tip SNP distances of up to 14 SNPs. The multi-country distribution of each main cluster suggests transmission prior to arrival in the countries. Time tree analysis suggested common ancestors of each cluster between 2020 and 2021.
Increasing *C. diphtheriae* case numbers among migrants in the second half of 2022 is a cause for concern, particularly considering the presence of genes encoding antimicrobial resistance. This is a multiclonal outbreak with evident transmission among migrant people. The proximal origin of this event remains to be determined but might be linked to lack of proper vaccination coverage in countries with disturbed public health systems. No forward transmission in the EU population was observed and novel cases have largely ceased to be reported in 2023.

S-10 First evidence of cyanobacteria from the Phorimidium genus producing cyanopeptoline: a study case in a Swiss lake

Mr. Sami Zhioua [1], **Dr. Diego Gonzalez** [1], **Dr. Guillaume Cailleau** [1], **Dr. Simon Sieber** [2], **Prof. Pilar Junier** [1]

[1] Laboratory of Microbiology, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland, [2] University of Zurich, Zurich, Switzerland

The sudden death of a dog on the shores of the lake of Gruyère in the canton of Fribourg prompted the search for possible toxin-producing cyanobacteria blooms. The investigations took place near the shore of the lake near La Roche. In this area, large mats of cyanobacteria accumulated at the bottom of the river, sometimes resurfacing and forming floating mats with the appearance of toad skin. A filamentous cyanobacterium was observed in several samples associated with the area. Identification of this cyanobacterium via molecular biology tools revealed the dominance of cyanobacteria related to the genus *Phromidium* spp. a group of benthic cyanobacteria known to produce toxins. This cyanobacterium represented more than 98% of the cyanobacterial community in the collected samples, which is very rare for a natural environment. This confirms that the conditions of the system favour the excessive development of this specific group of cyanobacteria. Toxicological analyses suggest that, contrary to the cases recorded in Canton Neuchâtel, it is not anatoxin-a that is produced but rather a type of cyanopeptolines, which is currently not known to be one of the toxins produced by *Phormidium* spp. Moreover, genomic and toxicologic analyses support the fact that this dominant species is capable of producing this specific toxin. Thus, considering that *Phormidium* spp. are found in floating mats, it is essential to target not only the usual cyanotoxins (anatoxin-a, microcystin, saxitoxin) but also cyanopeptolines.

S-11* Geographical Patterns and Genomic Analysis of SARS-CoV-2 Transmission Clusters during the Alpha, Delta, and Omicron Variants epidemic waves

Mrs. Yangji Choi [1], **Dr. David De Ridder** [2], **Mrs. Anaïs Ladoy** [3], **Prof. Claire Bertelli** [4], **Dr. Damien Jacot** [1], **Prof. Idris Guessous** [2], **Prof. Séverine Vuilleumier** [5], **Prof. Stéphane Joost** [3], **Prof. Gilbert Greub** [4]

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The COVID-19 pandemic caused by SARS-CoV-2 highlighted the importance of a comprehensive understanding of epidemiology. Genomic sequencing allows investigation of transmission chains and virus evolution. During the pandemic, the alpha, delta, and omicron variants displayed unique characteristics and clinical impacts. Here, we investigate geographical characteristics and contributions using genomics to explore transmission clusters and geographic patterns across SARS-CoV-2 variants.
We analyzed the SARS-CoV-2 genomes sequenced between October 2020 and April 2022, involving 4099 cases geocoded to exact residential locations in the canton of Vaud, Switzerland. First, genomic clusters were defined through the identification of cases possessing identical viral genomes based on nucleotide mutations. Second, we examined the number and size of genomic clusters throughout epidemic waves in relation to the implicated variants.
From the alpha to the omicron variant, we observed a consistent decrease in the number of genetic clusters, even after adjustment for sequencing efforts. Notably, the size of genomic clusters drastically decreased during the transition from the alpha to the delta variant and we measured a significantly reduced number of large clusters for delta variant compared to the others. A clear peak in the number of large clusters was apparent during the initial spread phase of the first two variants, but was absent for omicron. Using geographical distance, we also identified geographic-genetic clusters when the mean distance between cases within each genomic cluster was less than 1 km. The number of geographic-genetic clusters was also significantly higher for the alpha variant, but no difference was discernible in cluster sizes.
Our study highlights the importance of integrating genomic and geographic approaches to capture characteristics of epidemic waves. To better understand spreading behaviour, future investigations would benefit of including information on vaccination, socio-demographic aspects and living environments as well as sequencing bias, genetic divergence over time and cluster duration.

*Student presentation

S-12 Preparations for the first autochthonous transmission of West Nil Virus in Switzerland – a One Health approach

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West Nile virus (WNV) has a complex zoonotic life cycle including local and invasive mosquito species and their main hosts birds. WNV can infect mammals, including humans and horses. Infections in these two end hosts are most often asymptomatic or mild, while a minority develops neurological problems with associated high mortality.
Up to date no autochthonous infection in mammals has been reported in Switzerland. However, in 2022 WNV has been detected in local mosquitoes for the first time, suggesting an increased risk of transmission to humans and horses. Due to the broad mosquito vector and host spectrum, surveillance and control of WNV needs to be addressed with a “One Health” approach. At the national level, cooperation between the federal offices involved in WNV detection, prevention and control is facilitated through the One Health sub-body anchored in the Epidemic Act. The cantons are responsible for the control measures and coordinate with different sectors, including professionals and the general population. This collaborative, multisectoral and transdisciplinary approach especially in terms of communication is the key for an efficient public health response.
The first finding of WNV in mosquitoes is a proof of concept for a successful implementation of the One Health approach. Starting in 2000, mosquitoes have been monitored in Ticino. In 2013, the mosquito surveillance was extended to the main traffic routes from South to North. Finally, in 2017 the Swiss Mosquito Network was founded to combat invasive mosquito species. When WNV was detected in local mosquito species for the first time, the network of stakeholders at the national and cantonal level was already established. This allowed smooth coordination of measures and will further increase the preparedness for future possible autochthonous WNV transmissions to humans and horses.

M-06 Microscale strategies that govern nutrient flux in polysaccharide degrading Communities

Dr. Samuel Pontrelli

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Several ecological factors influence dynamics and structure of polysaccharide degrading microbial communities, including metabolic crossfeeding where primary degraders break down polysaccharides into monomers that can be metabolized by many species, and secretion of metabolic by-products to sustain metabolically diverse populations. However, deriving causal links between metabolite crossfeeding and community behavior is challenging due to other factors such as biological warfare, metabolic competition, environmental fluctuations, and spatial aggregation. In this study, we investigate a model chitin degrading community to explore how these factors determine the distribution of hydrolyzed, monomeric N-acetyl glucosamine (GlcNAc) among two competing species types: degraders, who produce extracellular chitinases to hydrolyze chitin, and exploiters, who consume GlcNAc without investing in chitinase production. We found that in a pairwise screen of 20 cocultures of degraders and exploiters, 13 of the cocultures delayed chitin degradation, and all exploiters inhibit at least one degrader. By examining a subset of these inhibitory relationships, we found that at least 10 are due to secretion of a toxin or for competition for GlcNAc based on substrate uptake kinetics. However, we also show that behavioral tradeoffs, such as particle binding, can be used to offset competitive disadvantages, and the complexity of species-specific inhibition phenotypes cannot be solely attributed to a single trait. This research highlights the complex interplay of physiological and environmental factors in polysaccharide degrading communities that influence nutrient flux, species dynamics, and particle degradation rates.

S-13 A unifying theory of environmentally-mediated interaction dependencies

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Ecological interactions, the impact of one organism on the growth and death of another, underpin our understanding of the long-term composition and the functional properties of communities. Interactions from classical ecology are typically understood to be fixed values, representing for example the per-capita rate of consumption of a prey organism by a predator. Yet in microbial ecology, such fixed descriptions are inadequate: recent work has shown how measured interactions can depend on environmental context, the time at which they are measured, and the sampled position in a spatially-structured community. In this work, we develop a theoretical framework that provides a unifying explanation for all three of these dependencies based on models in which the feedback between organisms and their chemical environment is explicitly represented. At the heart of our theory is the 'instantaneous interaction', a quantity that describes whether a given organism is changing the local environment in a way that helps or hinders another at a given moment in time. This context-dependent quantity then gives rise to time and spatial dependencies as the environment changes over time and/or space. We demonstrate the power of this framework by predicting a time-dependent switch in the measured sign of the intra-specific interaction of an antibiotic degrading bacterium, which we verify experimentally. We then show how the time and spatial dependencies in small crossfeeding communities recently described in separate studies are reflections of the same fundamental phenomena. Our framework helps to clarify the meaning of the term `interaction' in the microbial context, and provides the groundwork for ecological microbiome models in which these dependencies are taken into account.

S-14*/P-144* co-immobilisation of a defined gut consortium for continuous cultivation – a demonstration study

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Bacteria in the gut are found to grow in both planktonic as well as sessile lifestyle. Food particles and the mucosal layer provide a spatial dimension to which certain microbes can adhere. Recent studies have shown that these planktonic and sessile communities can widely differ from one another.
This study aimed to investigate the possibility to immobilise a defined gut consortium. We hypothesised that immobilisation can be used to mimic both growth lifestyles to enable stability and can result in different communities in one bioreactor allowing for microbial ecology studies.
We co-immobilised a previously established gut consortium, consisting of 9 species, into porous polymer beads that were used to inoculate bioreactors. After two consecutive batch fermentations for bacterial bead colonization, the bioreactors were operated further under continuous mode for 14-18 days. Operation parameters (retention time and CO_2-headspace gassing) were altered throughout the continuous fermentation to investigate changes in the sessile and planktonic bacterial communities. Bacterial communities were assessed by 16S amplicon sequencing. Effluent metabolites were quantified by HPLC-RI.
All 9 species established in both bioreactors. Main end metabolites and abundant taxa stabilised. However, species presence and abundance strongly depended on the community type (sessile or planktonic) and the fermentation operation. Three species (*L. rhamnosus*, *E. limosum* and *P. faecium*) were most abundant in the bead (sessile) community, while these were generally subdominant in the effluent (planktonic) community. Conversely, species that dominated the effluent community were either low in abundance or absent in the bead communities. Retention time switch and CO_2-limitation did only affect the effluent community composition and metabolites.
Taken together, we demonstrate that co-immobilisation of a defined gut consortium offers a novel tool to investigate biotic or abiotic interactions in both, sessile and planktonic defined gut communities in parallel.

*Student presentation

S-15*/P-145* Habitat fragmentation and single-cell heterogeneities create ecological opportunities for reversal of bacterial competition

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Far from growing in well-mixed suspended cultures, bacteria in nature often proliferate in highly structured and fragmented habitats (*e.g.* soil pores, plant roots, animal guts). Fragmentation leads to bacteria being constrained to smaller habitats, shared with potentially fewer other species. Microbial communities in such fragmented systems at a larger scale can therefore be considered as assemblies of micro-communities interacting within the boundaries of each microhabitat. The effects of micro-habitats on the emergence of bacterial interspecific interactions are poorly understood, but potentially important for the maintenance of diversity at a larger scale. To study this more precisely, we decided to cultivate species-pairs in picoliter droplets and study emerging interaction behaviour. We focus on four possible competition scenarios: imposed substrate competition, substrate indifference, tailocin killing and antibiotic inhibition. We used fluorescently tagged bacterial strains to track their growth in individual droplets with epifluorescence microscopy and flow cytometry, in contrast to their growth in liquid-suspended culture as non-fragmented habitat control. In contrast to liquid culturing, we observed that competition was partly reversed in a fragmented culture, which was a direct consequence of more variable founder cell physiologies and small founder population sizes. Mathematical simulations confirmed that a weaker competitor can take advantage of growth heterogeneity to outcompete a stronger competitor at a small population size, and suggested that a population which displays a wider range of growth kinetics among its cells will increase its chances at the micro-scale to establish in a fragmented habitat. Additionally, we observed that tailocin-producer bacteria could more efficiently kill tailocin-sensitive bacteria at small population sizes in micro-habitats, creating opportunities for a tailocin producer to dominate sensitive strains in rare cases. Our results thus demonstrate how habitat fragmentation can support alternative competitive outcomes and contribute to the maintenance of higher species diversity.

*Student presentation

S-16 Microorganisms as a benchmark: towards the remote detection of life beyond Earth

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One universal question is: “Is there life beyond Earth?”. The conditions on the early Earth supported the development of prokaryotes as a first form of life. Accordingly, microorganisms might be good candidates for an extra-terrestrial life. In order to detect traces of extra-terrestrial life, we developed an approach based on the homochirality phenomenon. Chirality defines molecules that exist in two conformations as left-handed (L-) and right-handed (D-) enantiomers. Carbon-based molecules such as amino acids and sugars are chiral and typically found in only one of the two forms within living organisms. This asymmetry is called homochirality and is considered a unique and universal feature of life. Homochirality can be detected by assessing the fractional circular polarization of light upon encountering asymmetric molecules. In this context, the FlyPol instrument, a sensitive spectropolarimeter, is proposed as a tool to detect circular polarization from space to assess the presence of life on the Earth surface and ultimately on other planetary bodies. Towards this goal, we will first explore the variety of spectropolarimetric signals obtained from existing natural systems both in laboratory and in natural environments. Therefore, we performed enrichment cultures of microorganisms present in natural terrestrial sulfur-rich environments, as this element can be electron acceptor during microbial respiration instead of oxygen. The cultures were incubated under varied temperatures (-20, 0 and 20°C) and in presence/absence of light. Our preliminary results illustrate how those conditions affect the microbial community composition and how the associated spectropolarimetric signals vary. Such results, in combination with a modelling work, provide a handle on expected signal in terms of magnitude and shape for relevant microorganisms. This combined approach paves the way to explore microbial life that thrives in a wide range of different environmental conditions and towards the remote detection of life beyond Earth using spectropolarimetry.

M-07 Pathogenesis of polymicrobial biofilm-associated infections

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The Gram-positive Enterococci are commensal inhabitants of the gastrointestinal tract, as well as opportunistic pathogens associated with endocarditis, urinary tract infections, and wound infection. Many Enterococcal infections are difficult to treat due to their multi-drug resistance, association with bacterial biofilms, and polymicrobial nature. The goal of our research is to understand the molecular mechanisms by which *Enterococcus faecalis* interacts with other bacterial species, such as *Staphylococcus aureus* and *Escherichia coli*, and the host in the context of these polymicrobial, biofilm-associated infections. In this talk, I will share our most recent mechanistic discoveries of synergy and antagonism between these frequently co-isolated bacterial species within the complex ecological environment of the host, and the impact of these interactions on biofilm-associated infection outcomes.

S-17 Live tracking of *Pseudomonas aeruginosa* infections in lung organoids reveals strong metabolic and biophysical fitness trade-offs

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Pseudomonas aeruginosa is a particularly challenging pathogen, often causing chronic lung infections in immunocompromised patients, which are virtually impossible to treat. We know surprisingly little about what physiological traits enable *P. aeruginosa*'s success in the lung environment, be it during colonization or when facing antibiotic treatment. This, in turn, limits our ability to develop novel therapies. To provide a new perspective on *P. aeruginosa*'s physiology in a realistic lung environment, we developed AirGels, a tissue-engineered human lung organoid that models airway infections and enables monitoring of pathogen physiology at high spatial and temporal resolution. To identify the genetic factors promoting its adaptation to the mucosal airway environment, we performed transposon-sequencing (Tn-seq) on *P. aeruginosa* colonizing *in vitro* airway epithelia at the air-liquid interface. As expected, we found that metabolic flexibility is a key factor enabling initial colonization. Using genome-scale metabolic models and *in silico* gene essentiality analysis, we determined the role of specific catabolic and anabolic pathways for mucosal colonization. Less expected was the observation that mutations commonly found in chronic *P. aeruginosa* strains, including those related to biofilm formation, resulted in severe fitness defects in colonization. Using time-lapse imaging in AirGels during infection, we quantitatively explored the mechanisms by which these mutations lead to changes in fitness at the mucosal surface. We found that chronic strains, i.e. mutants that preferentially form biofilms, pay a substantial fitness cost as aggregation limits their ability to explore the mucosal environment. While the biofilm lifestyle was detrimental early in colonization, Tn-seq under antibiotic selection highlighted that these same mutations dramatically increase fitness during treatment. Overall, our study demonstrates strong metabolic and biophysical trade-offs between colonization and tolerance in the airway. We expect our results to have relevant implications for treatment strategies during chronic lung infections.

S-18 CELL lysis caused by prophage induction leads to the formation of biofilm streamers in Burkholderia cenocepacia H111

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DNA is a component of biofilms, but the triggers of DNA release during biofilm formation and how DNA contributes to biofilm development are poorly investigated. One key mechanism involved in DNA release is explosive cell lysis, which is a consequence of prophage induction. In this study, the role of explosive cell lysis in biofilm formation was investigated in the opportunistic human pathogen *Burkholderia cenocepacia* H111 (H111). Biofilm streamers were used as a biofilm model in this study, as DNA is an essential component of their matrix. H111 carries three prophages on its large chromosome, and the involvement of each prophage in DNA and membrane vesicle (MV) release, as well as their contribution to streamer formation, were studied in the presence and absence of genotoxic stress. The results show that two of the three H111 prophages encode functional lytic bacteriophages that can be induced by genotoxic stress, and their activation causes DNA and MV release by explosive cell lysis. Furthermore, it is shown that the released DNA enables the strain to develop biofilm streamers, and streamer formation can be enhanced by genotoxic stress. In summary, this study demonstrates for the first time the importance of prophages in streamer formation and suggests that care must be taken in the choice of antibiotics for treating bacterial infections, as genotoxic stress may not only promote biofilm formation but also the formation of MVs, which are known to protect the bacterial cells from certain antibiotics.

S-19 Fluorescence-guided imaging for point-of-care visualization of bacterial biofilms on orthopedic material – An in vitro study

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Background
Bacterial biofilms formed on implants during periprosthetic joint infections (PJIs) are in-homogeneously distributed. This impairs both diagnosis and treatment success since it is unclear which parts of the prosthesis are contaminated. The wound imaging device Moleculight *i:X*™ (MolecuLight Inc., Canada) allows for detection of biofilms by certain bacterial species in chronic wounds. Here, we examined whether the device could be used to visualize bacterial biofilms formed on prosthetic materials.

Methods
We investigated the ability of the Moleculight *i:X*™ device to detect biofilms formed on prosthetic materials (polyethylene, titanium alloy and cobalt-chrome-molybdenum) *in vitro*. Discs of 1 cm diameter were incubated with a defined inoculum of clinical PJI isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* (2 and 7 days), or *Cutibacterium acnes* (8 and 11 days). The biofilms were supplemented with 5-aminolevulinic-acid 24h prior to imaging. The presence of red fluorescence, indicative of bacterial biofilm, its intensity (RFI), as well as the amount of colony forming units (CFUs) bacteria recovered from sonicated discs were determined.

Results
The Moleculight *i:X*™ device detected bacterial biofilm formation of all tested strains and on all evaluated materials, although detection on polyethylene was superior to detection on metal materials (3-6-fold), while CFU counts were similar. Mature biofilms showed increased RFI as compared to early biofilms (1.2-1.8-fold). RFI varied across the different strains with signal intensities hierarchy of *E. coli* > *S. aureus* > *S. epidermidis* = *C acnes*. CFUs and RFIs fold changes between early and mature biofilms were similar for all strains.

Conclusion
Our *in vitro* findings shows that Moleculight i:X™ can detect biofilms on various materials. Our work suggests that intraoperative use of the device during arthroplasty revision surgery might help to differentiate if an implant is infected or not. Further evaluations on explanted prostheses as well as intraoperative assessment are required.

S-20* The Legionella pneumophila nitric oxide receptors Hnox1, Hnox2 and NosP control biofilm architecture and virulence

Ms. Sarah Michaelis [1], **Mr. Tong Chen** [1], **Ms. Camille Schmid** [1], **Prof. Hubert Hilbi** [1]

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Legionella pneumophila is the causative agent of Legionnaires' disease. In the environment, the pathogen colonizes biofilms and infects amoebae. The facultative intracellular bacteria replicate in free-living amoebae as well as in mammalian macrophages within a unique compartment, the *Legionella*-containing vacuole (LCV). Macrophages and amoeba hosts of *L. pneumophila* produce NO, a gaseous signaling molecule, which is toxic at high concentrations. NO is a highly reactive free radical, which diffuses freely across membranes. At sublethal concentrations NO likely functions as a signaling molecule. *L. pneumophila* produces three NO sensors, NosP, Hnox1 and Hnox2. So far it is unknown, which physiological responses NO triggers in *L. pneumophila*.
In order to characterize the response of *L. pneumophila* to NO, the effects of NO on the bacteria alone were assessed. Nanomolar concentrations of NO caused a delay in the expression of transmissive traits, including the flagellin promoter, P*flaA*. To delineate the pathway, through which NO regulates P*flaA*, *L. pneumophila* mutant strains lacking the three NO receptors NosP, Hnox1 and Hnox2 were generated and characterized. Interestingly, compared to wild-type *L. pneumophila*, the NO-receptor mutants sedimented more slowly, exhibited a different biofilm architecture and replicated less efficiently in host cells. These phenotypes are currently further investigated by analyzing the expression of transmissive and replicative traits in infection experiments using fluorescence microscopy and flow cytometry.

*Student presentation

M-08 Induction and antagonism of an endogenous retroelement-based immune response by respiratory RNA viruses

Prof. Benjamin G. Hale

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Human host cell recognition of 'non-self' nucleic acids, such as viral double-stranded (ds) RNA, activates a powerful interferon-mediated antiviral defence. Thus, to ensure specificity, it is widely believed that immunostimulatory 'self' nucleic acids (such as viral-mimetic endogenous retroelement nucleic acids) must be strictly silenced by the host, thereby preventing aberrant immunopathology in the absence of infection. Intriguingly, we recently described a mechanism by which cells appear to have co-opted the regulated release of such endogenous retroelements to augment antiviral immunity. We found that infection with the respiratory RNA virus, influenza virus, triggers the loss of small ubiquitin-like (SUMO)-modified TRIM28, a host cell transcriptional repressor protein that normally plays a key role in endogenous retroelement silencing. By integrating knock-out and reconstitution cell models with system-wide RNA-Seq, we provide evidence that influenza virus triggered loss of SUMO-modified TRIM28 leads to expression of endogenous retroelements, some of which are bi-directionally transcribed and result in a cellular source of 'self' dsRNA in the cytosol. Consequently, loss of SUMO-modified TRIM28 potentiates canonical cytosolic dsRNA-activated interferon-mediated defenses. Notably, although wild-type influenza virus robustly triggers loss of SUMO-modified TRIM28, the induction of interferon-stimulated genes is limited unless expression of the viral dsRNA-binding protein, NS1, is abrogated. We are therefore currently investigating whether this implies an influenza virus strategy to antagonise such a host response by sequestration of immunostimulatory endogenous retroelement dsRNAs. Furthermore, we have identified a respiratory RNA virus virulence factor conserved in the *Paramyxoviridae* family that engages with TRIM28, and studies are ongoing to assess whether such viral proteins have evolved to directly target and inhibit infection-triggered loss of SUMO-modified TRIM28. Our studies in this area should uncover whether co-opted endogenous retroelement-based immunity is a broadly-acting antiviral mechanism against different exogenous respiratory RNA viruses, and could reveal a new aspect of 'self' versus 'non-self' virus-host interplay.

S-21*/P-146* CRISPRI-Seq identifies genes enhancing phage endolysin Cpl-1 susceptibility in *S. pneumoniae*

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Streptococcus pneumoniae is a human pathogen killing above 1.6 million people per year. Moreover, antibiotic resistance remains a significant issue, which justifies the development of complementary strategies. Cpl-1 is a bacteriophage lytic protein (lysin) that has been shown to have high potential for clinical use. In this study, we aimed at using the novel CRISPRi-seq method to potentially identify genes interfering with Cpl-1 mechanism of action. Accordingly, we used an IPTG-inducible pooled CRISPRi composed of a 1928 sgRNAs library. Strain D39V was shotgun transformed with the library before being challenged with Cpl-1. Then, we compared the fitness of the sgRNAs to identify genes potentially involved in an increase of Cpl-1 susceptibility or tolerance. Identified genes were then further investigated using single CRISPRi clones or knockout mutants by kinetic growth assay. Eight genes were initially found to be linked to an increase in Cpl-1 susceptibility (*rodA, uppP, pbp2b, mreD, mreC, tacL, cps* and *mvk* genes) and seven to an increase in lysin tolerance (*divIB*, *ftsE*, *ftsX, divIVA*, *pgm*, *guaB* and a gene coding for a hypothetical protein). Further experiments confirmed the involvement of *rodA*, *uppP*, *pbp2B*, *mreD*, *mreC* and *mvk* genes as Cpl-1 susceptibility factors. However, no significant increase in Cpl-1 tolerance was observed for the candidate genes, which was not surprising given that natural selection of resistant clones has neither been reported in vitro nor in vivo. Although supplementary experiments are needed, we obtained promising preliminary results guiding the development of potential synergistic strategies for the treatment of pneumococcal infections

*Student presentation

S-22* Mechanism and regulation of bacterial predation via ixotrophy

Ms. Yun-Wei Lien [1], **Dr. Gregor Weiss** [1], **Mr. Davide Amendola** [1], **Dr. Kang Soo Lee** [1], **Dr. Go Furusawa** [2], **Dr. Martin Pilhofer** [1]

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To ensure survival in the environment, bacteria evolved diverse strategies to compete with other organisms. Several members of the family Saprospiraceae are known to have a specialized and very efficient antibacterial behavior called ixotrophy. It is characterized by catching prey bacteria by an unknown mechanism followed by lysis of the prey cells. The underlying molecular mechanism of ixotrophy is poorly understood. Genome analysis revealed contractile injection system (CIS) gene clusters in ixotrophy-active bacteria. Bacterial CISs resemble a bacteriophage-tail and play a major role in bacterial cell-cell interactions. Hence, we hypothesized a potential role of CISs in the ixotrophy of Saprospiraceae.
Here we identify the macromolecular machinery involved in ixotrohpy of the filamentous multicellular gliding bacterium *Aureispira* sp. CCB-QB1. Cryo-electron tomography revealed that every *Aureispira* cell harbors multiple contractile injection systems (CISs), working in a type VI secretion system-like mode of action. These novel CISs were attached to the cytoplasmic membrane and connected to an extracellular antenna-shaped structure. Light microscopy and functional assays further showed its involvement in bacterial killing and revealed a so far unknown mode of regulation. We further show that grappling hook-like structures on the cell surface of *Aureispira* might be involved in catching flagellated prey bacteria. Single particle cryo-EM was used to achieved the high-resolution structure of grappling hooks which also gave us the identity.
In summary, we comprehensively characterized a new mechanism of a bacterial predator to shape its ecological niche by using a novel CIS.

*Student presentation

S-23* Elucidating the molecular determinants underlying the anti-Phytophthora activity of potato-associated Pseudomonas

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Several bacterial strains are known and used today as biocontrol agents, protecting crop plants from different pathogens like fungi, oomycetes or bacteria. These biocontrol strains use different mechanisms of action to protect the plant, such as secretion of antimicrobial compounds, competition for nutrients and/or space or induction of immunity in plants. A single bacterial strain can express several of those biocontrol traits, often depending on the environmental conditions, such as the presence of other microorganisms.
Pseudomonas putida strain R32 is known to inhibit the growth and development of *Phytophthora infestans*, the causing agent of potato late blight, which severely impacts potato production world wide. R32's antagonistic behavior can be attributed to different biocontrol traits such as the production of pyoverdine, a siderophore helping in iron competition, or synthesis of the strongly antimicrobial compound hydrogen cyanide.
In order to better understand biocontrol traits of R32, a Tn5 mutant bank with more than 8000 mutants was created. This tool is allowing us to examine which genes may be involved in the antagonistic behavior against *Phytophthora infestans*. In a first screening process of the mutant bank, we could already identify 32 promising mutants which lost the ability to inhibit the oomycete at different developmental stages. We are currently identifying which genes are affected in the mutants. This may reveal new important mechanisms of action, or help to identify important regulatory pathways involved in biocontrol properties.
In the end, the better understanding of the genes involved in antagonistic behavior of R32 and of how they are regulated will hopefully allow to improve the performance of biocontrol strains in the field.

*Student presentation

S-24*/P-147* Single-gene functional interrogation of bacterial secondary bile acid dehydroxylation in *Clostridioides difficile* colonization resistance

Mr. Luca Beldi [1], **Dr. Yuan Dong** [1], **Mr. Colin Volet** [2], **Prof. Rizlan Bernier-Latmani** [2], **Prof. Siegfried Hapfelmeier** [1]

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Clostridioides difficile is a common cause of nosocomial and antibiotic therapy associated intestinal infection. Research of the past ten years has identified strains belonging to the Lachnospiracea family that are able to 7α-dehydroxylate primary mammalian bile acids as major contributors to intestinal colonization resistance against *C. difficile.* A wealth of correlative data from *in vitro*, animal and human studies has since led to the hypothesis that bile acid 7α-dehydroxylation (7DH) is causally related to the protective effects of *Clostridium scindens* and related 7α-dehydroxylating organisms. Recent reports have begun to challenge this hypothesis, but unavailability of a genetic system in these organisms has so far precluded testing causality more specifically using isogenic 7DH deficient *bai* gene mutant strains. In the presented work, we have studied bile acid metabolism and *C. difficile* resistance functions of a newly available 7DH-deficient *baiH* mutant of *Faecalicatena contorta*, its 7DH-competent wild-type parent strain, and a well-characterized wild-type *C. scindens* strain in a gnotobiotic mouse *C. difficile* infection model. A comprehensive correlation analysis of 7DH-dependent bile acid metabolome and *C. difficile* infection resistance will be presented. To our knowledge, this is the first single-gene functional interrogation of bacterial 7DH in *C. difficile* infection resistance.

*Student presentation

M-09 MOLECULAR diagnostics – what, when and how

Prof. Hege Vangstein Aamot

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Molecular diagnostics is a rapidly growing field that applies molecular biology techniques to diagnose and monitor infections, and provide rapid, targeted treatment. It ranges from simple PCRs to metagenomic sequencing. This talk will present different applications, their strength and limitations exemplified through our work on orthopaedic implant-associated infections.

S-25 A HIGH THROUGHPUT TBEV SERONEUTRALIZATION ASSAY OVERCOMES ELISA LIMITATIONS

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Tick-borne encephalitis (TBE) virus (TBEV) is the most common tick transmitted Flavivirus transmitted in Europe. Typically, TBE manifests a biphasic course with an initial viremic phase with non-specific flu-like symptoms, followed by a phase with neurological involvement in about one third of patients. Due to the non-specific clinical manifestation, the diagnosis of TBE is based on the detection of specific antibodies. Enzyme-linked immunosorbent assays (ELISA) are most frequently used due to their simplicity and quick availability of test results. However, their interpretation can be challenging due to *Flavivirus* cross-reactive antibodies. Therefore, serum neutralization assays remain the gold standard in *Flavivirus* serology. However, specific and time-consuming protocols under biosafety level (BSL) 3 conditions are required for clinically relevant Flaviviruses. Furthermore, comparing seroneutralization test results of different viruses is hampered by different kinetics of infection and cell tropism. To overcome these limitations, we evaluated a reporter viral particle (RVP) based assay neutralization assay in which the infectivity is measured by luminescence and that can be performed in BSL-2 conditions. This RVP-based test system was established for TBEV, Yellow Fever Virus, Dengue Virus Type 2, Zika Virus, and West Nile Virus. The RVP-based and the wildtype seroneutralization assays for TBEV showed a good correlation. The parallel assessment of the sera's neutralizing capacity against different flaviviruses allows for a direct evaluation of antibody specificity. In approximately 20% of the sera tested positive for TBEV with ELISA, we were able to determine positivity for other flaviviruses, showing the possible clinical relevance of this test.

S-26* Nanobody-mediated capture of gram-negative pathogens – a journey through the sugar jungle

Ms. Michèle Sorgenfrei [1], **Dr. Lea Huber** [1], **Ms. Andrea Printz** [1], **Dr. Melissa Remy** [2], **Dr. Fanny Wegner** [1], **Mr. Fabian Ackle** [1], **Dr. Damien Morger** [3], **Prof. Adrian Egli** [1], **Dr. Peter Keller** [4], **Prof. Markus Seeger** [1]

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Sepsis caused by blood stream infections (BSIs) affects 18 million people world-wide every year and has unacceptably high mortality rates of 10-20% in Western countries. BSI diagnostics can take several days, which forces clinicians to treat sepsis patients with empiric broad-spectrum antibiotics. However, cephalosporin- and carbapenem-resistant Enterobacteriaceae, like *Escherichia coli*, and *Klebsiella pneumoniae* are often treated with inadequate antibiotics. To shorten BSI diagnostics, nanobodies were raised against highly conserved outer membrane proteins (OMPs) to capture these gram-negative pathogens from patient blood. Nanobody pools were first enriched against detergent-purified OMPs and then deep-mined via the mass-spectrometric detection of peptide barcodes called flycodes. The flycode technology proved particularly useful to identify rare nanobodies recognizing the targeted OMPs in the native context of clinical isolates. A set of nanobodies recognizing OmpA or OmpF of *E. coli* were thoroughly characterized using flow cytometry and exhibited a high species coverage of >79 %. When coupled to magnetic beads, the nanobodies enabled capturing live bacteria with K-12 origin with a sensitivity of <50 CFU/mL bacteria and an efficiency of >95 %. In case of clinical isolates, capture is less efficient and requires further optimization, but we are confident that our nanobodies are applicable for omitting time-consuming blood culturing in the future and thereby substantially accelerate antibiotic susceptibility testing.

*Student presentation

S-27* The implications of DNA extraction methods on Legionella quantification in drinking water: towards method standardization

Mr. Alessio Cavallaro [1], **Dr. William J. Rhoads** [1], **Dr. Frederik Hammes** [1]

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Legionella spp. are amongst the most concerning waterborne opportunistic pathogens in drinking water, causing Legionellosis, which is a notifiable disease in Switzerland. *Legionella* are often found in building plumbing systems, which are a typical point of contact with humans. Hence, monitoring their levels in drinking water is important to plan interventions to curtail their presence, and is required legislatively. Quantitative PCR is an established alternative to culture methods that can provide a much faster, high throughput and more precise enumeration of target bacteria in water samples. However, PCR-based methods are not routinely applied for Legionella monitoring in Switzerland, and methods differ notably between testing laboratories. Here, we studied the impact of different DNA extraction method on downstream molecular analysis. Through a community science campaign, we collected 50 shower hoses and related water samples, and compared two commonly used DNA extraction methodologies to the same biofilm and water phase samples. The two methods showed clearly different extraction efficacies, which was reflected in the both the quantity of DNA extracted and the concentrations of *Legionella*. Notably, one method resulted in higher enumeration nearly in all samples by a log unit and detected Legionella in 11 samples undetected by the alternative method. Moreover, the DNA extraction method affected results from the water phase more, which often is the only target for monitoring of environmental *Legionella*. We furthermore performed 16S rRNA amplicon sequencing on the samples: while there was no significant change in the community structure, individual taxa relative abundance, such as Legionella, varied depending on the extraction method employed. Given the implications of these findings, we advocate for the standardization of DNA extraction methods used in *Legionella* detection and quantification in drinking water. This will further enhance the reliability of quantitative PCR, promoting it as the preferred method for *Legionella* monitoring in Switzerland.

*Student presentation

S-28* A prospective method comparison of a novel PCR-based assay and conventional culture method for the identification of obligate pathogenic dermatophytes in clinical samples

Mr. Dominique Hilti [1], **Dr. Faina Wehrli** [1], **Ms. Azra Nuredini** [1], **Dr. Martin Risch** [1], **Prof. Lorenz Risch** [1], **Dr. Susanna Bigler** [1], **Dr. Nadia Wohlwend** [1]

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Introduction
Despite its lengthy turnaround time and labor-intensive workflow, conventional culture procedures are currently regarded as the gold standard diagnostic approach for dermatomycosis. However, novel molecular assays promise a rapid yet sensitive and specific dermatophyte identification. Therefore, we evaluated the performance of a EUROArray Dermatomycosis PCR-based procedure and compared it to current methods.

Methods
To compare the EUROArray Dermatomycosis procedure to the cultural approach in identifying pathogenic dermatophyte species in clinical samples, 562 clinical nail, skin and hair samples collected from May to November 2022 were prospectively tested with the PCR system and compared to cultural growth results as well as KOH classification.

Results
Among all 562 samples, 186 samples were classified as KOH positive, 47 cultures showed at least 1 obligate pathogenic dermatophyte (OPD) while PCR analysis revealed 230 samples with an OPD.
In 164 (88.2 %, $p < 0.0001$, Pearson's chi squared test) KOH positive samples an OPD was identified via PCR while only 35 (19 %) samples showed cultural growth. Among 364 culturally and KOH negative samples, an OPD was still identified in 54 (14.8 %) and facultative pathogenic fungi (FPF) were detected in 73 (20 %) samples by PCR. Agreement among all three methods was found in 61.4 % of samples (310 triple negative / 35 triple positive). There was no instance where an OPD was identified culturally while PCR was negative, although identification at the species level was discordant in 2 / 47 samples.
Among 149 FPF identified in 117 samples via PCR, 105 specimens of Fusarium sp. were identified, suggesting potential contamination.

Discussion
Although all culture grown dermatophytes were validated via PCR, false negative results still pose a major limitation just as potential contamination with Fusarium spp. does. Nevertheless, our data shows a clear benefit of PCR assays in the diagnostic management of dermatomycosis.

*Student presentation

M-10 Organic and conventional agriculture promote distinct soil microbiomes with contrasting metabolic potentials

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Soil microorganisms deliver numerous ecosystem functions essential for crop production. Changes in agricultural practices could potentially alter soil microbial diversity and the underlying metabolic potential encoded in their collective genomes. This offers opportunities to harness microbial resources for developing sustainable cropping systems that can counteract the negative impacts from agricultural intensification. Here, we explored how different organic and conventional farming systems shape diversity and metabolic potential of the soil microbiome by analyzing soils from the DOK long-term field trial that compares five different farming systems since 1978. The soil microbiome was characterized by DNA metabarcoding and shotgun metagenomics. Extensive auxiliary data on soil properties, greenhouse gas emissions, and crop performance from decades of research were integrated into a comprehensive system comparison.
Organic fertilization as an integral part of organic farming increased diversity and altered the taxonomic and functional structure of the soil microbiome compared to stockless systems. The plant protection regime appeared to be of subordinate importance. At the taxonomic level, organic farming promoted microbial guilds involved in the degradation of complex organic compounds and the regulation of pests, while stockless systems were characterized by oligotrophic communities adapted to carbon-limited environments and appeared to select against known biocontrol organisms. Functional annotation of the metagenomes based on universal (EC, EGGNOG, INTERPRO2GO, SEED) and specific (CAZy, NCyc) ontologies revealed gradual changes based on the type of fertilizer inputs. While conventional stockless farming systems were dominated by genes indicative of accelerated elemental cycles and molecule transport, the genetic capacity of organically managed soils were dominated by genes required for degradation of complex lignocellulolytic compounds and internal nutrient cycling. These results add to the emerging evidence that long-term organic and conventional management can promote soil microbiomes with unique genetic capacities that might ultimately alter key biogeochemical processes in agriculturally managed soils.

S-29 Improving the assessment of soil archaeal diversity: a comparison of universal prokaryote and domain-specific metabarcoding

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Diversity surveys of soil prokaryotes often rely on universal prokaryote PCR-primers that result in the detection of low numbers of archaeal taxa. The prevailing richness of soil bacterial taxa may lead to incomplete assessments of the less dominant soil archaea. Consequently, we compared soil archaeal diversity using the prokaryote PCR-primers 341F/806R and the archaea-specific PCR-primers Arch349F/SSU666ArR that amplify the variable region 3 of the gene encoding the small ribosomal RNA. In-silico analyses indicated that the prokaryote PCR-primers matched 88.3% of deposited archaeal and 97.7% of bacterial sequences, while the archaea-specific PCR-primers matched 88.8% of archaeal and 1.5% of bacterial sequences. Amplicon sequencing of DNA extracted from 54 soil samples from grassland, arable land, and forest, yielded 36 archaeal ASVs if prokaryote PCR-primers were used, which strongly contrasted with the 806 archaeal ASVs obtained using archaea-specific PCR-primers. The difference can be explained by the dominance and preferential amplification of bacteria, thereby resulting in only 0.5% archaeal sequences when using prokaryote PCR-primers. In comparison, the archaea-specific PCR-primers resulted in 99.7% archaeal sequences providing a highly increased coverage of soil archaeal communities. Alpha-diversities and community compositions were not or only weakly correlated between prokaryote- and archaea-specific assessments (r=-0.07 to 0.50). Community structures were more strongly correlated (r=0.75), which is due to dominant ASVs detected with both primer pairs. The prokaryote PCR-primers failed to detect a substantial portion of archaeal diversity, i.e., 779 archaeal ASVs, that included functionally important taxa such as 446 ASVs of the predominantly ammonia-oxidizing Nitrososphaeria, and 19 ASVs of potential methanogens of the phylum Halobacteriota. Therefore, surveys relying on universal prokaryote PCR-primers provided an incomplete picture of soil archaeal diversity. More specific assessments will be needed to cover the majority of archaeal taxa in soil ecosystems and to characterize their ecological preferences.

S-30 Functional diversity in microbial communities an 8,000-year lacustrine sedimentary sequence

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Lacustrine sediments represent an ideal natural setting to study the ecological and temporal evolution of microbial communities in the subsurface biosphere. However, there is a limited number of studies focused on understanding the functional and taxonomic variation of microbial assemblages in these environments. Here, we reconstructed the microbial functional and taxonomic composition of an 8000-year Holocene sedimentary succession from Lake Cadagno using shotgun metagenomics. We used the ribosomal protein RpS3 clustered at 90% identity ($RpS3_{90\%}$) as a single-copy marker gene for the taxonomic characterization. In sulfidic sediments deposited in the last ~220 years, Alpha, Gamma, and Deltaproteobacteria are the dominant members of the microbial community; however, methanogens from Euryarchaeota and microorganisms from the water column like Cyanobacteria, Chromatiales, and Chlorobi are also present. Within the Deltaproteobacteria, sulfate-reducers related to Desulforomonadales, Desulfobacterales, and Syntrophobacterales are abundant, which is likely explained by the high sulfate concentrations at these depths. Older sediments (>220 years) are characterized by low organic matter concentrations and a scarcity of electron acceptors. Here, archaeal populations related to Bathyarchaeota account for 18.8-39.34% of the microbial community. Furthermore, a comparative protein sequence analysis of $RpS3_{90\%}$ clusters from Bathyarchaeota revealed the presence of 39 distinct subgroups from this phylum differentially distributed by sample depth. Analyses of the metabolic potential of the microbial community revealed a uniform distribution in higher-order functional categories (e.g. amino acid metabolism, nucleotide metabolism, etc.) with sediment depth. However, functional profiles of marker genes involved in the biogeochemical cycling of sulfur and carbon indicate a potential variation in the distribution of preferred metabolic pathways with sediment depth, along with differences in the identity of the taxa potentially encoding these functions. Thus, our results suggest that functional redundancy is a scale-dependent phenomenon in microbial communities from Lake Cadagno sediments.

S-31 Finding an anatoxin-a producing strain in the haystack of benthic cyanobacterial communities

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In the last ten years, blooms of benthic cyanobacteria producing derivatives of anatoxin-a, a potent neurotoxin, have been repeatedly reported in rivers and lakes of temperate regions all over the world. For three series of events, in New Zealand, the USA, and Canada, a cohesive cluster of *Microcoleus* strains was found to be responsible for the toxin synthesis. Here, we document a similar toxigenic event that occurred at the mouth of the river Areuse in the lake of Neuchâtel (Switzerland) and caused the death of several dogs. Using 16S rRNA-based community analysis, we show that benthic and scum-associated communities in the river are dominated by Oscillatoriales and especially by strains within the *Microcoleus* genus. We correlate the detection of one specific sequence variant with the presence of anatoxin-a derivatives and use metagenomics to assemble a complete circular genome of the strain of interest. The strain is distinct from the ones isolated in New Zealand, the USA, and Canada, but belongs to the same species (>95% average nucleotide identity); it shares significant genomic traits with them, in particular a relatively small genome and incomplete vitamin biosynthetic pathways. Overall, our results suggest that the major anatoxin-a-associated benthic proliferations worldwide can be traced back to a single ubiquitous *Microcoleus* species, rather than to a diversity of cyanobacterial lineages. We recommend that this species be monitored internationally and studied in detail in order to help predict and mitigate similar cyanotoxic events.

S-32 Disentangling the role of the oral commensal *Streptococcus salivarius* in small intestinal inflammation in the context of childhood malnutrition

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Stunted child growth is an inflammatory syndrome arising from chronic undernutrition and is associated with severe long-term effects including cognitive delay and blunted immune responses. Globally, stunting affects 100,000,000 children under 5 years old, with the highest prevalence in low- and middle-income countries. We have recently analyzed the small intestine bacterial composition of stunted sub-Saharan African children revealing that stunted children display small intestinal bacterial overgrowth (SIBO) characterized by the overrepresentation of oral species, specifically *Streptoccocus salivarius.* We further showed that this overgrowth is associated with small intestinal inflammation. Thus, we hypothesized that contrary to the common perception that *S. salivarius* exhibit anti-inflammatory properties, it could drive dysbiosis and trigger inflammatory responses when ectopically colonizing the small intestine. We assessed for the inflammatory potential of a collection of 40 *S. salivarius* strains isolated from upper gastrointestinal samples of children suffering of SIBO. A screening in an IL-6 reporter cell line shows that there are pro-inflammatory *S. salivarius* strains and that this potential is strain-dependent. We further characterized the inflammatory properties of these isolates exposing small intestinal epithelial cells with different bacterial fractions and subsequently analyzing the expression of pro-inflammatory genes by qPCR. Our results suggest that the pro-inflammatory potential is based on a heat-labile cell wall component. To identify the specific bacterial traits involved in inflammation we are investigating cell surface structures by electronic microscopy, comparing the genomes, and performing bacterial transcriptomics of pro- and non-inflammatory strains. Further, we will assess for strain-level diversity in the small intestinal samples of children suffering or not of SIBO and stunted growth, and for interactions between different *S. salivarius* strains. The results of this project will provide new insights into the role of oral communities in gut microbiota dysbiosis and will help to unravel ways of preventing small intestinal inflammation and associated pathologies.

M-11 Antimicrobial resistance in a One Health perspective

Prof. Jesper Larsen

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Antimicrobial resistance in pathogenic bacteria poses one of the biggest threats to global health, food security, and development today. The presence of resistance genes in ancient soil samples and modern natural, agricultural, and human ecosystems demonstrates that the use of a One Health approach is essential for our understanding and management of antimicrobial resistance. In this talk, I will provide two examples that illustrate the usefulness of integrating surveillance, molecular epidemiology, bioinformatics, and microbiology. First, I will show how *Staphylococcus aureus* clonal complex 398 underwent a successful host jump from humans to livestock through a series of gene loss and acquisition events involving modulators of the human innate immune system and resistance genes, respectively, how it disseminated within livestock populations, and how it (re-)acquired the ability to spread to and cause serious illness and even death in elderly and immunocompromised persons in community and healthcare settings. Second, I will provide evidence that an ancient biological arms race between antibiotic-producing dermatophytes and skin bacteria has driven the selection and evolution of clinically relevant resistance genes.

S-33 Determining the impact of veterinary antibiotics sub-dosage on resistance transfer in animal gut microbiota - a One-Health approach

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Antimicrobials are an important global resource and have been widely used over the years. However, excessive usage of antibiotics has been driven by pressure and development of bacterial resistance worldwide. Resistance genes can be genetically transmitted and exchanged between pathogenic species co-habiting environments, human and animal communities. Our aim was to evaluate whether sub-dosage of widely administered antibiotics in veterinary medicine could enhance plasmid transfer, and consequently, resistance genes exchange in animal gut microbiota. *Escherichia coli* carrying IncL (bearing the bla_{OXA-48} gene) or IncI1 (bearing the $bla_{CTX-M-1}$ gene) plasmids used as models were submitted to sub-inhibitory concentrations of amoxicillin, apramycin ceftiofur, colistin, enrofloxacin, erythromycin, florfenicol, narasin, lincomycin, neomycin, oxytetracycline, sulfamethazine, and tiamulin. Additionally, treatments were performed with and without an antioxidant supplementation (edaravone, used for treating neurological disorders). Then strains were submitted to conjugation assays to determine conjugation frequency, RT-qPCR to evaluate the genomic expression of SOS response associated genes, and fluorescence ROS detection assay to evaluate the stress oxidative response. Increased conjugation frequencies were observed when treating both isolates with florfenicol (IncL, 13.7-; IncI1, 17.2-fold) and oxytetracycline (IncL, 97.2-; IncI1, 8.9-fold) compared to the control. Increased expression of the SOS-associated *recA* gene (IncL, florfenicol, 2,5- and oxytetracycline,1.6-fold; IncI1, florfenicol, 2.9- and oxytetracycline, 3-fold), and increased reactive oxygen species production (IncL, florfenicol, 3.9- and oxytetracycline, 3.2-fold; IncI1, florfenicol, 7.2- and oxytetracycline, 24.8-fold) were also observed. Edaravone reduced plasmid conjugation when increased, and reduced oxidative stress and the expression of *recA* gene for both plasmid scaffolds. Our data showed that antibiotic used in veterinary medicine, oxytetracycline and florfenicol, can induce transfer of plasmid-encoded resistance genes and therefore may really contribute to the worldwide spread of antibiotic resistance. In addition, antioxidant edavarone decreased plasmid exchange frequencies, and may play a role in controlling antibiotic resistance spread.

S-34 Development of a triplex digital PCR assay for monitoring opportunistic pathogens in water systems

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Cases caused by the pathogen *Legionella pneumophila* are rising in Switzerland and Europe. Other opportunistic pathogens, including *Mycobacterium avium* complex and *Pseudomonas aeruginosa, also pose risks, with increases in incidence.* Household water is a notable potential source of exposure to these pathogens. To mitigate risks associated with exposure to opportunistic pathogens, water quality monitoring, and treatment present prospects for interventions. To enhance water quality monitoring, we developed a new digital polymerase chain reaction (dPCR) assay. During dPCR, the sample is partitioned into nanoliter droplets, each droplet functioning as equivalent to a single PCR reaction. By multiplexing dPCR, we can quantify multiple targets simultaneously. Here, we combined three individual quantitative PCR assays targeting *L. pneumophila*, *M. avium* complex, and *P. aeruginosa* into a triplex digital droplet PCR (ddPCR) assay. Based on Poison distribution of positive and negative droplets for each fluorophore, the three targets are quantified. DPCR provides significant benefits compared to existing methods. Current culture-based methods are time and cost-intensive. In comparison to quantitative PCR, dPCR allows for direct quantitative measurement without the use of standard curves. This saves time and resources, particularly in the context of multiplexing, where standard curves for each target are required for quantification via qPCR. Additionally, the combination of endpoint PCR measurements and direct quantification reduces impact of inhibitory substances on measurement as well as potential biases due to shifts in standard curves. Here, we present monitoring data of treated and stored greywater, obtained over 5 months using our triplex dPCR assay. Preliminary results of 22 processed samples, showed 15 positive samples for *P. aeruginosa (*average of the log-transformed 3.1 +/- 0.52) and none positive for *L. pneumophila* or *M. avium* complex. This assay can assist to implement new measurement standards with quantitative and fast results, helping decision-makers to react fast and to control outbreaks.

S-35 Implementation and Validation of two Real-Time PCRs on an In-House Automated Molecular Diagnostic Platform for the Detection of Alongshan Virus (ALSV), a novel tick born pathogen associated with Tick-Borne Encephalitis Like Disease

Dr. Onya Opota [1], **Dr. Florian Tagini** [2], **Ms. Zahera Dance** [3], **Mr. Valentin Loup** [3], **Dr. Alix Coste** [3], **Dr. Rahel Ackermann-Gäumann** [4], **Mr. Reto Lienhard** [4], **Dr. Antony Croxatto** [5], **Dr. Valeria Cagno** [3], **Prof. Cornel Fraefel** [6], **Dr. Jakub kubacki** [6], **Prof. Gilbert Greub** [3]

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Background
Alongshan Virus (ALSV) is a tick-borne flavivirus reported to cause a disease similar to Tick-Borne Encephalitis (TBE). Ticks carrying ALSV have been identified in Switzerland at a higher prevalence than TBE virus (TBEV). We aimed to implement and validate a real-time PCR assay on our in-house automated molecular diagnostic platform for the accurate detection of ALSV for rapid and precise diagnosis of ALSV infections.

Methods
We focused on two previously described ALSV-specific Taqman RT-PCR assays targeting the NS3 gene and the NS5 gene. These assays were modified to accommodate the genetic diversity of ALSV sequences and optimized to match the technical characteristics of our platform. Positive controls included ALSV RNA from infected ticks from Switzerland and a synthetic plasmid containing the target amplicons of the RT-PCR.

Results
To determine the limit of detection of the ALSV PCR assay, we utilized serial dilutions of the synthetic plasmid. Amplification rates of 100%, 92%, and 20% were achieved for 100, 10, and 1 copies per reaction, respectively. The intra- and inter-run reproducibility, assessed through five independent runs using plasmid dilutions corresponding to 100 and 10 DNA copies per reaction, met the necessary requirements for diagnostic use. Specificity testing was performed against a panel of pathogens commonly found in cerebrospinal fluid, including bacteria, viruses, and fungi. Among the tested viruses, including members of the Flaviviridae family such as TBEV, no amplification was observed. We plan to extend the assessment of clinical specificity to specimens from our laboratory's biobank.

Conclusions
The PCR assay will enable rapid, sensitive, and specific detection of ALSV infections and syndromic testing alongside other encephalitis-associated viruses, such as TBEV on our in-house molecular diagnostic platform. This PCR assay will also enable the assessment of the medical significance of ALSV through retrospective and prospective clinical studies.

S-36 Survey on the presence and spread of West Nile Virus among potential avifaunal and entomological reservoir species in Canton Ticino

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West Nile Virus (WNV) is a flavivirus transmitted by mosquitoes and maintained in the environment through an enzootic cycle involving mosquitoes and birds. Birds are essential for virus amplification (amplifying hosts) since viremia can reach levels high enough for multiple transmissions, whereas mosquitoes (bridge vectors) are essential for virus spread between birds and mammals. In humans, infection with WNV remains mostly asymptomatic, but in a small percentage of cases, may cause encephalitis, meningitis, or acute flaccid paralysis with long-term neurological sequelae.
Since 2010, an active surveillance of WNV, and other flaviviruses, has been carried out in mosquito vectors collected in Canton Ticino (Switzerland). In 2022, WNV circulation was confirmed for the first time in Ticino by the presence of the pathogen on FTA cards and mosquito pools of *Culex pipiens*. This finding led us to extend the surveillance by analysing of the presence of WNV in birds during different seasonal periods to increase our knowledge on the dynamics of WNV spread via birds and vectors in our territory.
Our work describes the preliminary results of a pilot study on the presence of WNV in birds collected at the Ramsar site Bolle di Magadino, a peculiar wetland and marshy habitat playing an important role as a resting place for migratory birds, and areas in agricultural landscape of Piano di Magadino. Birds were captured during the migration period (mid-March to mid-May), the breeding season (mid-May to end of June), and the post-breeding period (end of June to end of September). Blood, cloacal and oral swabs were here collected to evaluate the presence and seroprevalence of WNV in birds. Moreover, Next Generation Sequencing is planned to provide a general overview of pathogens carried by birds.

M-12 Metabolic imprint of infection by *Chlamydia trachomatis*

Agathe Subtil PhD

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Metabolism refers to chemical transformations within living cells leading to the production of energy and building blocks, and disposal of waste. It is tightly regulated to meet cellular needs. Bacterial colonization of a tissue, either physiological (mucosae) or pathological (infection, dysbiosis), creates a unique environment as the result of reciprocal influence of the co-existing metabolisms. *Chlamydia trachomatis* is the leading cause of sexually transmitted disease of bacterial origin. What makes *C. trachomatis* particularly interesting regarding the metabolism of its host is that these bacteria grow exclusively inside cells, mainly epithelial cells of the genital tract. Many of *Chlamydia* biosynthetic pathways are incomplete, indicating that the bacteria use building blocks from the host. It has been assumed so far that the bacteria simply “steal” resource, such as glucose, from the cytoplasm of the cell. We have shown that infection also boosts hexosamine biosynthesis in the host, by activating a host enzyme called transglutaminase 2. Remarkably, rewiring of glucose fluxes in the host goes without affecting glycolysis nor oxidative phosphorylation. Nevertheless, the metabolic imprint of infection is not without consequences, and we will describe its effects on the epigenome of the host.

S-37 Structure-function analysis of the cyclic ß-1,2-glucan synthase

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The synthesis of complex sugars is a key aspect of microbial biology. Cyclic ß-1,2-glucan (CßG) is a circular polysaccharide critical for host interactions of many bacteria, including major pathogens of humans (*Brucella*) and plants (*Agrobacterium*). CßG is produced by the cyclic glucan synthase (Cgs), a massive multi-domain membrane protein. So far, its structure as well as the mechanisms underlining the synthesis have not been clarified. We used cryo-electron microscopy (cryo-EM) and functional approaches to study Cgs from *A. tumefaciens*. We were able to determine the structure of this complex protein machinery and clarify key aspects of CßG synthesis, revealing a unique mechanism that uses a tyrosine-linked oligosaccharide intermediate in cycles of polymerization and processing of the glucan chain. Our research opens new possibilities for combating pathogens that rely on polysaccharide virulence factors and may lead to new synthetic biology approaches for producing complex cyclic sugars.

S-38 Beyond the Core: Unveiling the Molecular Secrets of Bacterial RNA Helicases

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RNA helicases perform essential housekeeping and regulatory functions across diverse organisms, prokaryotes and eukaryotes, via their ability to bind hundreds of RNA molecules. These enzymes share a highly conserved catalytic core that use ATP hydrolysis to alter RNA structures, while each possesses unique domain(s) that are thought to confer specificity of action.
Phylogenetic analysis of bacterial RNA helicases reveals considerable diversity and rapid evolution in their C-terminal extensions (CTEs). Understanding the impact of this RNA helicase diversification, particularly in the C-terminal extensions (CTEs), on bacterial fitness remains an ongoing challenge. Our group has identified the DEAD-box RNA helicase RhlE2 as a new post-transcriptional regulator that is essential for *Pseudomonas aeruginosa* environmental adaptation and virulence [DOI: 10.1093/nar/gkab503]. Through a comprehensive characterization of RhlE2, we show that RhlE2 unique CTE significantly enhances the RNA unwinding activity of its catalytic core. Furthermore, the RhlE2 CTE is sufficient for the protein liquid-liquid phase separation, a process whereby proteins forms membrane-less microcompartments within the cytoplasm. It also mediates the RNA-dependent interactions with the RNase E endonuclease, a critical component of RNA degradation machinery in bacteria. By comparing RhlE2 activities with a second RhlE homolog present in *P. aeruginosa,* RhlE1, and constructing chimeras interchanging their CTEs, we provide evidence for the direct involvement of CTEs in RNA helicase sub-functionalization. Overall, this study enables the identification of RhlE2-specific enzymatic properties and unique interaction patterns. Such findings could lay the ground for the development of novel antivirulence compounds that target *P. aeruginosa* by specifically inhibiting RhlE2.

S-39* Subversion of IMPDH2 oncoprotein activity by Legionella effectors

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Legionella pneumophila is a facultative intracellular bacterium, which replicates in amoebae, but also in alveolar macrophages, and thus causes Legionnaires' disease. To establish its replicative niche, the "*Legionella*-containing vacuole" (LCV), *L. pneumophila* delivers ca. 300 different "effector" proteins to the host cell using the Icm/Dot type IV secretion system. These effectors subvert various cellular pathways by modulating host factors and thereby promote survival and growth of the pathogen. Inosine 5'-monophosphate dehydrogenase type 2 (IMPDH2) is an oncoprotein that catalyzes the conversion of inosine 5'-monophosphate (IMP) to xanthosine 5'-monophosphate (XMP), which represents the first and rate-limiting step of the pathway for de novo guanine nucleotide synthesis. The homotetrameric enzyme can form filaments under certain cellular conditions such as low GTP levels or treatment with inhibitors, affecting the regulation of IMPDH2 activity.
We observed that in cells treated with the IMPDH2 inhibitor mycophenolic acid (MPA), *L. pneumophila* reversed MPA-induced IMPDH2 filament formation. Intriguingly, the effect was dependent on a bacterial effector, which covalently modified IMPDH2 and caused disassembly of ATP-induced IMPDH2 filaments *in vitro*. By covalent modification, *L. pneumophila* might alter IMPDH2 activity or its distribution in the host cell and thereby modulate local GTP levels. In current experiments, we use activity assays to study the effects of the modification on IMPDH2 activity. Beyond, we aim to clarify the cellular localization of IMPDH2 and bacterial effectors during *L. pneumophila* infection by analysing isolated LCVs, mitochondria, and lipid droplets by confocal microscopy, western blot, and mass spectrometry. The goals of this project are to study the mechanism and consequences of IMPDH2 modification in the context of *L. pneumophila* infection as well as its implications for cell and cancer biology.

*Student presentation

S-40* Dissecting how galactosylated teichoic acids modulate the function of the Actin Assembly-Inducing Protein ActA in *Listeria monocytogenes* virulence

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Listeria monocytogenes, a Gram-positive foodborne pathogen, pose a significant threat to susceptible individuals, causing severe infections with high mortality rates. Teichoic acids (TAs) are cell surface-exposed glycopolymers that are further divided into membrane-tethered lipoteichoic acid (LTA), and wall-anchored teichoic acid (WTA). They have been found to play multiple roles in bacterial physiology, protection against host defense mechanisms and antibiotics, and host-cell interactions, specifically in virulence. Our previous studies showed that galactose (Gal)-deficient TAs mutants are severely attenuated in virulence, yet we could not attribute the virulence attenuation to the loss of the Internalin B retained by Gal-TA on the bacterial surface. Meanwhile, we observed that the ability to form actin tail was completely abolished in the Gal-TAs mutant, suggesting the malfunction of ActA, the actin assembly-inducing protein responsible for cell-to-cell spread in virulence. To dissect how Gal-TAs modulate the function of ActA, respective in-frame deletion mutants that feature Gal-deficient decoration on WTA, LTA, or both TAs were constructed and assessed by immunofluorescence. Our data showed that only Gal-WTA is responsible for the ActA dysfunction, yet Gal-LTA plays no role thereof. Interestingly, we found both surface-exposed WTA and ActA are sorted to the old pole, where F-actins are recruited for the formation of actin tails. To investigate the potential molecular interaction between WTA and ActA, we purified WTA polymers and recombinant ActA for surface plasmon resonance analysis. Our data confirmed the binding affinity in the micromolar range, suggesting the presence of an unknown carbohydrate-binding domain within ActA. Further investigation will be focused on the spatiotemporal distribution of ActA-WTA, which will shed light on the structure-function relationship of cell wall-associated macromolecules in Gram-positive pathogens, as well as the identification of novel targets for antibacterial development.

*Student presentation

M-13 Explaining new therapies to lay persons: the example of phage therapy

Round table discussion on lay communication in medical microbiology by experts panel.

M-21 Engaging citizens on AMR and behaviour changes on antibiotic consumption: the MAKEAWARE! Campaign

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Do citizens know how antibiotics work? Do people know how to consume them properly? How is the communication with the doctor managed during the therapy? Are citizens aware of antimicrobial resistance? To fill these gaps in, the MAKEAWARE! campaign, organised by the SPEARHEAD project - Innosuisse, aims at involving people, scientists, designers, artists, doctors and technologists to explore together the science and data on the use of antibiotics, and to collaboratively create an open repository of resources. During the presentation, workshop activities based on hands-on experiences spanning from the explorations of scientific tools to the creation of data visualizations will be introduced. Each workshop format invites people to actively take part in the creation of a map and a series of resources to understand the use of antibiotics. Participants are first introduced to the topic, then involved in learning-by-doing procedures, to finally contribute to increase data availability useful for the research, sharing their personal stories and experiences of antibiotic consumption and treatments and contributing to the research collectives.

S-41/P-148 Bacttles: a microbiology educational tool for general public and schools

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Serious games have gained special attention in the past years due to the increasing number of studies that report such strategies as beneficial among different levels of education. However, it is hard to find available educative tools that target very young audiences (primary school) in science-related topics. As part of my PhD thesis and motivated by my personal interest in scientific outreach, I present 'Bacttles', an educational tool for microbiology in the form of a strategic and easy-to-play card game that targets a wide range of ages thanks to its customizable degree of complexity. Bacttles addresses both the general public and teachers that wish to use it in their classrooms as a way of introducing microbiology concepts. Background information to teachers will be provided in form of an educator's booklet, which can also be used for informing interested lay people.
The lay out of the game and its mechanism evolved over time after multiple rounds of trial and re-design. The final version consists of a deck of cards, a 3D-printed board and tokens (with its paper-based alternative), and the educator's booklet, all digital content open source. The goal of the game is to be the most abundant species in a microbial community. Players start with a defined number of members in the community that may increase or decrease based on how they handle the environmental challenges that are presented.
In order to assess the impact of the game on the players' understanding of microbiological concepts, we targeted university open day fairs. Visitors playing the game were given a brief questionnaire before and after playing, in order to score differences in the acquisition of general concepts and concept extrapolation, and to score the general appreciation of the game. The game strategy and the results will be presented in this contribution.

S-42* Impact of the SARS-CoV-2 pandemic on media communication in French-speaking Switzerland

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For three years, the severity and scale of the Coronavirus pandemic continued to preoccupy the world's population. The epidemic began in China and gradually spread across Europe until it reached Switzerland, where a 1st wave of infections began in March 2020, followed by 5 others. Against this backdrop of crisis, Switzerland and the rest of the world had to take emergency health and political measures, and the media had to work around the clock to bring the public the latest news.
We have attempted to assess the impact of the Coronavirus on media communication in French-speaking Switzerland by carrying out both a quantitative and qualitative analysis, based on the number of Coronavirus-related publications recorded between 01/01/2020 and 07/31/2022 in the ARGUS DATA INSIGHT, a database listing over 650 media outlets broadcast in Switzerland.
In comparison with Swiss epidemiological data, we found that the pandemic generated Coronavirus-related media waves that aligned with waves of infections, but that the proportion of publications did not depend on the number of SARS-CoV-2 positive cases. Using a qualitative approach, we then analyzed the content of a selection of articles related to death and the Coronavirus using a Case Report Form. This analysis revealed that death was addressed along 6 different axes, showing a temporal evolution in the representation and perception of death by the Swiss population, independently of the kinetics of mortality curves linked to SARS-CoV-2. However, this showed us the limits of a purely quantitative analysis, and the importance of accompanying it with a qualitative analysis of the publications' content.
In conclusion, this study has confirmed that the Coronavirus pandemic has had a major impact on media communication in French-speaking Switzerland, relaying the fact that infection with an unknown and deadly virus has accompanied all aspects of daily life and the vision of death.

*Student presentation

S-43 Microbial interactions and soil functioning: a game to introduce key concepts in microbiology to biology students

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Microbiology is a difficult topic to teach given that the objects of study are for the most invisible to the learner. This is particularly true in the case of university students starting their training in biology and especially those interested in natural sciences which have thus a general interest in studying natural objects that can be seen with the naked eye. Nonetheless, microorganisms are key components of the biosphere and a thorough training in natural sciences must include a good microbiological background. This is significant when considering future societal challenges such as the climatic and resources crises for which microorganisms and their diversified metabolisms offer concrete sustainable solutions and a glimpse of hope.
As a result, using innovative teaching to increase student's interest for the invisible world is desirable. In the Academia, frontal teaching, which is a passive learning format, used to be the rule. However, such a format is no longer considered as the most adequate approach in teaching. Instead, alternative teaching formats such as tutorials, group activities, or problembased learning (PBL) are known to motivate students better. Moreover, these teaching approaches not only allow the students to passively acquire new knowledge, but they allow their consolidation in an active way. In addition to this, this allow training important skills such as effective communication, critical thinking, and time management. A similar engagement can be obtained by the use of games as part of the teaching experience. There are multiple examples of games associated to microbiology. However, all of those, perpetuate the association of microorganisms with their negative facets (e.g. diseases), instead of tapping on all the positive impacts of microbial life on ecosystem functioning. Accordingly, we designed a card game for the learning of key concepts in basic bacteriology and mycology for bachelor level students. Testing of the game was performed using two different approaches. In the first one, the students were given the task to complete and develop the design of a set of cards based on microbial species to be used as characters in predefined game dynamics. In the second one, the students were given the task of developing the game dynamics using the predesigned cards, with the additional constrain of connecting the game with practical hypothesis validation linking microbes to ecological functions. The first dynamic was useful for the teaching of basic concepts to second year biology students, while the second one was ideal for an advanced PBL module for third year biology students.

S-44 Explain microbiology using cartoons

Prof. Gilbert Greub

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Microbiology is facing new challenges such as increasing antimicrobial resistance and new emerging pathogens. But modern microbiology is also facing novel opportunities such as big data provided by omics approaches, as well as automation, digitalization, and artificial intelligence. This led diagnostic clinical microbiology to extend behind diagnostic of infectious diseases by providing modern tools to define dysbiosis and to get useful results for patients suffering from non-infectious diseases by studying their microbiota.
However, lay communication about these important topics are often impaired by the complexity of the technologies used and by the microscopic nature of microbes that is somehow limiting awareness and understanding of lay persons. Thus, as microbiologists, we have to innovate in communication and use new approaches.
After having developed several illustrations with Vincent Dutrait for the games Krobs & Mykrobs, we tried to illustrate some key concepts using cartoons, thanks to a tight collaboration with artists. We also tried to develop a unique creative process based on keywords and symbolic representation. For example, the clinical microbiologist is systematically represented wearing a white labcoat and having a microscope nearby, even when he already jumped into the future and use post-modern technologies. Also, combining several symbols in a single cartoon help to convey properly a complex message; thus, as an example, "to put a lab in a watch" nicely illustrates the fact that time to results is very important and that miniaturized automated product, such as the GeneXpert PCR allows such shorter time to results, while preserving all the technology present in modern laboratories.
In conclusion, cartoons are very useful in lay communication and might also be used to make aware politicians and decisions makers of (i) the major microbial threats, new emerging microbes & increasing antimicrobial resistance and of (ii) the new opportunities brought by modern tools.

M-14

Functional genomics by overexpression in the fungal pathogen *Candida albicans*: biofilms, genome plasticity and more

Prof. Christophe d'Enfert

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Candida albicans is a diploid yeast species responsible for life-threatening infections in hospitalized patients and the most frequent fungal commensal of the human gastro-intestinal and genital tracts. To elucidate gene function and advance our understanding of *C. albicans* pathogenesis, gene deletions have been widely used. However, this approach has some limitations due to the diploid nature of *C. albicans*. An alternative strategy is to create overexpression strain collections and screen for gain of functions or suppression of mutant phenotypes. To this aim, we have established resources for systematic gene overexpression in *C. albicans.* I will illustrate how these resources and innovative screening strategies have been leveraged to identify new players in biofilm formation and the control of genome plasticity that are intimately linked to *C. albicans* resistance to antifungals.

S-45

Pneumocystis jirovecii antigenic variation uses reassortment of subtelomeric genes' repertoires

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Surface antigenic variation is crucial for major pathogens that infect humans, e.g. *Plasmodium*, *Trypanosoma*, *Giardia*. In order to escape the immune system, they exploit various mechanisms in order to modify or exchange the protein that is exposed on the cell surface, at the genetic, expressional, and/or epigenetic level. Understanding these mechanisms is important to better prevent and fight the deadly diseases caused. However, those used by the fungus *Pneumocystis jirovecii* that causes life-threatening pneumonia in immunocompromised individuals remain poorly understood. Here, though this fungus is currently not cultivable, our detailed analysis of the subtelomeric sequence motifs and genes encoding six families of major surface glycoproteins suggest that the system involves homologous recombinations during meiosis. This leads to the reassortment of the repertoire of ca. 80 non-expressed genes of family I present in each strain, from which single genes are retrieved for mutually exclusive expression within subpopulations of cells. The recombinations generates also constantly new mosaic genes. Dispersion of the new alleles and repertoires, supposedly by immunocompetent carrier individuals, appears very efficient because identical alleles are observed in patients from all over the world. Our observations reveal a unique strategy of antigenic variation allowing colonization of the non-sterile niche corresponding to lungs of immunocompetent humans.

S-46 Identification of a core essentialome through genome-wide fitness profiling in phylogenetically diverse *Streptococcus pneumoniae*

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Streptococcus pneumoniae is an important human pathogen that causes over one million deaths annually. Although infections can be successfully treated, the rate of antibiotic-resistant *S. pneumoniae* strains has increased sharply in recent years. To successfully treat infections in the future, novel antibiotics are thus needed. The most effective targets for novel antibiotic targets are those that will infer the biggest fitness defect when blocked i.e. are essential for the bacterial cell to survive.
To identify the most essential *S. pneumoniae* genes, we developed a high-throughput method to construct pooled, genome-wide, inducible, CRISPR interference (CRISPRi) libraries. In CRISPRi, the binding of a catalytically dead Cas9 (dCas), targeted to a specific feature by a single guide RNA (sgRNA), results in a knockdown through blocking of transcription. High-throughput sequencing of the remaining sgRNAs (CRISPRi-Seq), can identify those genes that are essential by dropout of the sgRNA. We identify the most essential genes for *S. pneumoniae*, 7, 14, and 21 generations after the start of dCas9 induction.
We constructed CRISPRi libraries in six *S. pneumoniae* strains of diverse phylogenetic standings (D39V, TIGR4, EF3030, BHN418, PBCN0272, and PBCN0364). Within the core-genome of 1550 genes, we identify 193 universally essential genes that drop out of all libraries after 7 generations (core essentialome). Of these 193 genes, 77 (40%) function within translation, whilst 20 others (11%) have a function within nucleotide metabolism. Interestingly, we also identify 14 previously uncharacterized proteins at this timepoint. A further 227 genes are essential in all strains, but not necessarily at 7 generations, whilst 251 more are selectively essential in at least one strain.
Continued construction of CRISPRi libraries will further refine the core essentialome, and may yield new targets for the development of inhibitors. In addition, it may yield insights into specific essential genes in specific phylogenic clades of *S. pneumoniae*.

S-47* Investigating the genomic context of the *Chlamydia trachomatis* Lymphogranuloma venereum outbreak in Buenos Aires, since 2017 through international collaboration

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Background
Lymphogranuloma venereum (LGV) is a sexually transmitted disease caused by *Chlamydia trachomatis* (CT) serotypes L1 to L3. Since 2003, increasing LGV cases were observed in Europe, with L2b the predominant *ompA*-genotype. LGV has been detected in Buenos Aires since 2017 with a high prevalence of *ompA*-genotype L1. We will investigate the genomic diversity of LGV samples from Argentina, comparing them to international samples to further explore the global evolution of LGV.

Methods
We recruited 461 samples from patients with symptoms compatible with proctitis since 09/2017. We tested anorectal swabs for CT by real-time PCR targeting the cryptic plasmid. Next, we genotyped positive samples with PCR-RFLP of *omp*A. We performed whole genome sequencing (WGS) on 10 cultured samples (Illumina). Genomes were obtained from a further 40 samples using SureSelect (Agilent) target enrichment and Oxford Nanopore adaptive sampling. All genomes were compared phylogenetically with those from international databases.

Results
Between 31/08/2017 and 31/05/2022, CT infection was detected in 198/461 (43%) of the cases studied. The patients (190 Men-who-have-sex-with-men (MSM), one female, and seven trans-women, mostly with HIV co-infection) reported practicing unprotected receptive anal sex. PCR-RFLP genotyping of *omp*A revealed 163/198 (82.3%) LGVs, including 158 MSM and five trans-women. To explore the epidemiological context in Buenos Aires and expand the genomic investigation to unculturable clinical samples, we have developed target enrichment methods using SureSelect. Sensitivity data will be compared against the target load in clinical samples.

Conclusion
Through transcontinental collaboration between the two universities, and the technical development of STI genomics, we will be able to better understand the Argentinean strains in a genetic context. This will allow us to advance our knowledge of the global evolution of LGV.

*Student presentation

S-48 A reference-free consensus calling bioinformatic workflow for amplicon sequencing using Nanopore Rapid Barcoding Kit

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Sanger sequencing, the gold standard in clinical microbiology amplicon sequencing, offers high accuracy and crucial consensus sequences for downstream applications. However, it lacks flexibility and struggles with mixed samples. Oxford Nanopore Technologies (ONT) has revolutionized DNA sequencing, providing enhanced accuracy, versatility, faster turnaround time, and customizable read quantities. ONT's amplicon sequencing preserves amplicon length, generating high-quality reads. Nonetheless, it is time-consuming, taking over 3 hours for 96 samples when utilizing the ligation-based (LSK) method. ONT's Rapid Barcoding (RBK) library preparation offers a faster alternative, utilizing a transposase to randomly add barcodes and adapters without ligation as a single step. RBK significantly reduces the turnaround time to about 60 minutes for 96 samples, albeit with a lower read count. In addition, RBK delivers state-of-the-art results in SARS-CoV-2 sequencing with the ARTIC pipeline. However, analyzing RBK data without a reference sequence poses challenges due to fragmented reads, heterogeneity in amplicon sizes, and the inability of assemblers designed for longer reads to generate accurate consensus sequences. To overcome these challenges, we propose a novel bioinformatic workflow that utilizes the information from the longest and highest quality reads as a backbone for generating consensus sequences, providing a rapid and adaptable protocol applicable to various sequence types. Validation with previously analyzed SARS-CoV-2 samples using the ARTIC protocol demonstrates its effectiveness as an alternative to reference-based methods, producing consensus sequences up to 100% identical to reference-based approaches. Factors affecting consensus accuracy and computational time were also benchmarked. The new bioinformatic workflow will dramatically enhance the capacity of clinical microbiology laboratories to perform sequence-based diagnostics.

M-15 Characterization of the unique life-cycle and niche adaptations of the gut commensal SFB

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The gastrointestinal tract is inhabited by hundreds of bacterial species but the presence, or absence, of the commensal *Candidatus Arthromitus*, also known as segmented filamentous bacteria or SFB, can have a profound effect on the homeostatic immune status and reactivity of the host. SFB are anaerobic, *Clostridia*-related, spore-forming commensals found in the gut of many vertebrate species. Unlike other commensals, which normally reside in the lumen, SFB grows in close association with the host by intimately attaching to absorptive epithelial cells in the ileum and to cells overlying the Peyer's patches. Once attached, SFB undergoes a complex life-cycle that includes filamentation, division, differentiation and subsequent production of intracellular offsprings. Rapid expansion of SFB after weaning promotes the post-natal maturation of the mucosal immune system and a healthy state of physiological inflammation. The broad immunostimulatory properties of SFB protect the host from pathogens but can also influence disease severity in a number of disease models. Characterization of SFB in terms of its life-cycle and host attachment has, however, been hampered by a lack of tools beyond their mono-colonization of germfree mice. Through the development of an *in vitro* SFB host cell co-culturing system, we have gained insights into nutritional requirements of SFB and identified flagellation as a missing link in the SFB life-cycle. We are now interested in gaining greater insights into host-specific niche adaptations through genome analysis. Together, this work is providing a better understanding of the unique lifestyle of this still enigmatic bacterium.

S-49 Type 1 piliated uropathogenic *Escherichia coli* hijack the host immune response by binding to CD14

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Urinary tract infections (UTIs) affect 150-200 million people worldwide each year, and up to one-third of all UTI patients experience recurrences after antibiotic therapy, necessitating retreatment and promoting the development of antibiotic resistance. UTIs can be caused by different bacterial species, with uropathogenic *Escherichia coli* (UPEC) being the most common.
A key attribute of persistent or recurring bacterial infections is the ability of the pathogen to evade the host's immune response. Many Enterobacteriaceae causing recurrent UTIs, express type 1 pili, a pre-adapted virulence trait, to invade host epithelial cells and establish persistent infections. However, the molecular mechanisms and strategies by which pathogens actively circumvent the immune response of the host to establish persistent infections remain poorly understood.
We identified CD14, a major immune cell receptor and co-receptor for lipopolysaccharide detection, on innate immune cells as a binding partner of FimH, the protein located at the tip of the type 1 pilus. Interestingly, the FimH amino acids involved in CD14 binding are highly conserved across pathogenic and non-pathogenic strains of *E. coli*. However, only binding of UPEC to CD14 on dendritic cells reduced migration of those innate immune cells by overactivation of integrins and blunted expression of co-stimulatory molecules by overactivating the NFAT (nuclear factor of activated T-cells) pathway, both rate-limiting factors of activation of the adaptive immune system inside the lymph node. This response was binary at the single-cell level, but averaged in larger populations exposed to both piliated and non-piliated pathogens and *in-vivo*, presumably via the exchange of immunomodulatory cytokines.
While defining an active molecular mechanism of immune evasion by pathogens, the interaction between FimH and CD14 represents a potential target to interfere with persistent and recurrent bacterial infections caused by type 1 piliated pathogens.

S-50 "Stubborn" bacterial variants hooked on host cell phospholipid degradation

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The opportunistic pathogen *Pseudomonas aeruginosa* is a leading cause of life-threatening infections in humans. This bacterium is able to quickly adapt to a large range of environments including host tissues where it mobilizes a large arsenal of virulence factors. During chronic lung infections, small colony variants frequently emerge and correlate with disease progression. In this study, we show that these genetic variants, known for their enhanced capacity to form hard biofilms, are also prone to disrupt the lung tissue integrity. Using *in-vitro* evolution experiments in human serum, we could select for such genetic variants. We demonstrated that these mutants gained a selective advantage from their enhanced capacity to degrade human LDL trough the expression of a hemolytic phospholipase C (PlcH). PlcH is a secreted enzyme also known to contribute to *P. aeruginosa* virulence in the human lung where its substrate, phosphatidylcholine, is highly abundant. Numerous studies associated PlcH with lung surfactant and tissue membrane damage. However, very little is known regarding the molecular function and the regulation of PlcH during acute and chronic infections. Using a genetically encoded fluorescent reporter for live tracking of *plcH* expression, we showed that *plcH* transcription is enhanced in phosphatidylcholine-rich environments when high levels of the intracellular second messengers c-di-GMP and cAMP are achieved. Both signalling molecules play a crucial role in determining bacterial behaviour during acute and persistent lung infections thus allowing *P. aeruginosa* to finely adjust its virulence life-style. Consistently, we demonstrated that in a human lung organoid, PlcH contributes to the breaching of the epithelium in a c-di-GMP dependent manner. Our findings contribute to the understanding of the mechanisms by which *P. aeruginosa* regulates its virulence behaviour during disease progression and could therefore help for the development of novel anti-infective strategies.

S-51 Lymph node dendritic cells harbor inducible replication competent HIV despite years of suppressive ART

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Background
Recent studies have indicated that, in contrast to CD4 T cells, myeloid cells are relatively resistant to viral cytopathic effects, to the destruction by cytotoxic T-cells, and can store HIV in "virus-containing compartments". Together with their long half, potentially due to their self-renewing nature in tissues, HIV-infected myeloid cells could therefore represent a potential and yet unexploited tissue HIV reservoir.

Methods
To address this issue, we performed 1) an in-depth transcriptomic, phenotypic and functional characterization of lymph node (LN) DCs; 2) assessment of the susceptibility of LN DCs to HIV infection *in vitro*; 3) assessment of major virological parameters associated with HIV persistence in LN DCs isolated from viremic and aviremic ART treated HIV-infected subjects.

Results
We showed that the two major *i.e.* migratory ($Lin^-HLADR^+CD45^+CD11c^+CCR7^+$) and resident ($Lin^-HLA\text{-}DR^+CD45^+CD11c^+CCR7^-$) DCs were susceptible to HIV infection and supported cycles of *de novo* viral replication *in vitro*. We showed that LN resident and migratory DCs isolated from viremic individuals contained intact HIV provirus, were transcriptionally active directly *ex vivo* and were capable of producing HIV RNA/p24 upon TLR7/8 stimulation *in vitro* ($P<0.05$). Interestingly, both LN DC subpopulations isolatd from ART treated HIV-infected individuals contained HIV intact provirus and inducible replication competent HIV despite the expression of the anti-viral restriction factor SAMHD1. Of note, HIV-1 RNA was consistently detected in culture supernatants of LN migratory DCs from HIV-infected individuals who were treated for up to 14 years and detectable in culture supernatant of LN resident DCs in one individual treated for up to 19 years.

Conclusions
These findings indicate that LN DCs isolated from ART-treated aviremic HIV-infected individuals may represent a yet untapped reservoir of infectious HIV in LN tissues.

S-52* Spatial and temporal patterns in the population genetic structure of *Melolontha melolontha* are not reflected in those of its fungal pathogens

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The European cockchafer *Melolontha melolontha* is widespread throughout Central Europe, and a damage-causing insect in agricultural areas. The entomopathogenic fungus *Beauveria brongniartii* is its best characterized natural pathogen, and its presence is mainly confined to *M. melolontha* infested sites. Despite the availability of comprehensive historical and current records on cockchafer occurrence, the decades-long use of the fungus to control *M. melolontha* and the ecological knowledge of this host-pathogen system, in-depth population genetic analyses are missing for both organisms. Here, we established collections of *M. melolontha* and *Beauveria* spp. from 35 infested sites in the Alpine area including Switzerland, Italy and Austria and performed genotypic analyses on cockchafer individuals and fungal isolates based on double-digest Restriction site Associated DNA sequencing (ddRADseq). Phylogenetic analyses discriminated two-thirds of the fungal isolates as *B. pseudobassiana*, previously unrecognized as a significant pathogen of *M. melolontha*. A single mating type, i.e., MAT-1, was detected in *B. brongniartii*, suggesting a predominantly clonal reproductive mode. Individuals of both mating types, i.e., MAT-1 and MAT-2, were detected in *B. pseudobassiana*, for which a test of recombination also revealed a strong clonal pattern of reproduction across the area studied. Population genetic analyses of either *Beauveria* species confirmed the lack of spatial patterns, likely as a consequence of their fast dispersal through asexual spores. In contrast, populations of *M. melolontha*, originating from south or north of the Alpine Mountain Range, were genetically distinct, reflecting phylogeographic history and geographic barriers. Furthermore, the population genetic structures of *M. melolontha* and the two *Beauveria* spp. revealed no correlations, suggesting host independent dispersal of the pathogens. This study yielded the population genetics of an insect-pathogen interaction and provided new information on a second potentially important pathogen of *M. melolontha*, which could represent a basis for new *M. melolontha* biological control strategies complementing existing approaches.

*Student presentation

M-16 Engineering a symbiont as a biosensor for the honey bee gut environment

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The honey bee is a powerful model system to probe host-gut microbiota interactions, and an important pollinator species for natural ecosystems and for agriculture. While bacterial biosensors can provide critical insight into the complex interplay occurring between a host and its associated microbiota, the lack of methods to non-invasively sample the gut content, and the limited genetic tools to engineer symbionts, have so far hindered their development in honey bees. Here, we built a versatile molecular toolkit to genetically modify symbionts and reported for the first time in the honey bee a technique to sample their feces. We reprogrammed the native bee gut bacterium *Snodgrassella alvi* as a biosensor for IPTG, with engineered cells that stably colonize the gut of honey bees and report exposure to the molecules in a dose-dependent manner through the expression of a fluorescent protein. We showed that fluorescence readout can be measured in the gut tissues or non-invasively in the feces. These tools and techniques will enable rapid building of engineered bacteria to answer fundamental questions in host-gut microbiota research.

S-53 Engineering phagemid-based intercellular communication for distributed computing in *Escherichia coli* consortium

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Building large synthetic genetic circuits or combining multiple circuits in a single bacterial cell is a major challenge as they become too complex and inflict a high metabolic burden on the host cell. Distributing the circuit's function in a multicellular consortium can address this issue. This system will have further potential applications beyond biocomputing, for example, in biomedical therapy and bioprocess technology. In this project, we aim to engineer distributed computing in a consortium of Escherichia coli. Our system combines 1.) cascaded CRISPR-interference (CRISPRi) gene regulation to create single and multi-input logic gates and 2.) M13 phages for establishing intercellular communication in Escherichia coli. Intercellular communication is achieved by sending M13 phages carrying a single guide RNA (sgRNA) on a M13 phagemid between the donor and receiver cell populations. The donor cells will transmit this sgRNA constitutively or in the presence of chemical inducer. In combination with the catalytically dead Cas9 protein (dCas9), the transmitted sgRNA created transcriptional inhibition or activation of a reporter gene in the intended receiver cell population. Using this system, we constructed six orthogonal NOT gates. Upon receiving M13 phagemid encoding the corresponding sgRNA, the receiver cells successfully inhibit transcription 13- to 25-fold with negligible off-target interactions in more than 95% receiver cell population within 4 hours of co-incubation with the donor cells. Moreover, we have successfully layered NOT gates to build larger circuits, such as YES/buffer gates, NOR gates and OR gates, which also exhibit high degree of robustness and orthogonality.

S-54 Determination of universally essential *Staphylococcus* aureus genes by CRISPRi-Seq

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Staphylococcus aureus is the leading cause of a wide range of infections, from minor skin and soft tissue infections to life-threatening pneumonia and bacteremia. Deep-seated infections are becoming increasingly difficult to eradicate in part due to the spread of strains with resistance to various classes of antibiotics and the enormous variation in virulence phenotypes between strains. Successful treatment of these infections requires the development of new antimicrobial strategies targeting universally essential gene products. These are genes that are crucial under different environmental conditions and highly conserved within the species. Here, we developed a versatile CRISPR interference system (CRISPRi) for representative clinical *S. aureus* strains to conditionally downregulate genes on a genome-wide level. In our system, doxycycline-inducible dCas9 is integrated into the chromosome, allowing for tighter control than in previous studies. We performed multi-generation CRISPRi screenings, allowing us to identify genes with marginal and crucial influence on bacterial fitness. We defined 357 universally essential genes (always present and essential) and several strain-specific core genes (always present but not always essential). Among these, 105 genes are critical for survival and represents attractive antimicrobial targets. These genes are part of the replication and division machineries, central metabolism, and transcription/translation processes. The remaining 252 genes have marginal effects on growth and most of them belong to the same functional categories. Interestingly, 10% of these genes encode for unknown functions, suggesting new aspects of *S. aureus* biology that have yet to be characterized. Ongoing CRISPRi screens on different environmental conditions (*e.g., in vivo* models of infection, antibiotic stress) will further define the list of universally essential genes at different levels of complexity.

S-55* Prepare for landing: use of versatile double site-specific recombination systems to stably insert foreign DNA sequences in the chromosome of Mycoplasma *feriruminatoris*

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Mollicutes are the smallest cultivable free-living prokaryotes, bearing streamlined genomes resulting of reductive evolution. A limited number of Mollicutes have been used as models for the creation of the “minimal cell” and therefore have been amended for genome engineering, including genome transplantation. However, many species still cannot be genetically amended due to the lack of genetic modification tools. Since genome transplantation is technically challenging, overlong and often requires multiple modification cycles, we aimed at facilitating foreign gene insertion by engineering the genome of the fast-growing Mycoplasma feriruminatoris to create a site-specific insertion site by the use of a combined Cre-lox/Flp-FRT system. We introduced a tetracycline marker flanked by FRT and a modified loxP sequence into the genome of M. feriruminatoris, creating a selectable “landing pad”. In parallel, we developed a shuttle fosmid capable of integration into the M. feriruminatoris genome by expression of the Cre recombinase, allowing the insertion of any genes of interest in the chromosome of the bacterium. By using a synthetic cumate inducible promoter controlling the eFlp/FRT recombination system, the fosmid backbone together with all the resistance markers can be surgically excised and removed from the engineered mycoplasma chromosome, allowing only the gene(s) of interest to remain. This selective DNA integration/excision technology will increase the available synthetic biology tools for Mollicutes, facilitating the expression of recombinant antigens and settling the foundations for a future synthetic vaccine chassis based on M. feriruminatoris.

*Student presentation

S-56* Engineering of highly active multifunctional proline-rich antimicrobial peptide conjugates

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As we strive to expand our antibiotic arsenal, the threat of multi-drug resistant pathogens remains imminent. Host defense peptides, particularly proline-rich antimicrobial peptides (PrAMPs) like bactenecin 7 and apidaecin 1b, show great promise for antibiotic development. These peptides possess diverse mechanisms of action and demonstrate activity against a wide range of pathogenic bacteria, including those in the ESKAPE group. PrAMPs can translocate through bacterial membranes and target intracellular sites. Their high proline and arginine content allows them to adopt relatively rigid secondary structures involving polyproline type II- and pi-helices, providing recalcitrance against proteolytic degradation and selective binding to their targets. To harness the potential of PrAMPs for biomedical applications, we need to fine-tune them at the molecular level. This includes further enhancing proteolytic stability and reducing the likelihood of pathogen escape through intrinsic resistance or mutation. By designing a library of 97,216 AMP-AMP conjugates, consisting of pairs of 56 distinct PrAMPs coupled in different configurations bridged by short amino acid linkers, we aim to create peptides that can interact with two independent intracellular targets and enter bacterial cells through two different pathways. If successful, acquisition of resistance-attributing mutations is rendered extremely unlikely. Similarly, the linker is crucial, as its cleavage by bacterial proteases involved in AMP deactivation can release two free PrAMP molecules, potentially exhibiting even higher antimicrobial activity than the conjugate itself. Proteolytic cleavage may hence result in a deadly outcome rather than providing cell protection. NGS-supported in vivo activity screening (Mex) in recombinant Escherichia coli strains revealed over 10,000 hits, with some PrAMPs enriched in specific conjugate configurations. We anticipate that this set contains several multifunctional PrAMP conjugates with enhanced stability, low toxicity and reduced propensity for inducing resistance formation.

*Student presentation

M-17 AI in bacteriology: from growth detection to antibiotic susceptibility testing

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During the last decade, clinical bacteriology laboratories have experienced a revolution with the progressive introduction of fully automated systems. In addition, laboratory automation transformed plate reading, which could now be performed on digital images where microbiologists select microbial colonies for subsequent follow-up action. Intelligent algorithms for plates reading linked to expert systems might provide, in the future, a fully automated approach of the bacteriology workflow. Although presumptive identification of microbial colonies on complex specimen type remains a relative distant prospect other simpler tasks are and will be more easily automatized. Currently, the available imaging applications are mostly restricted to urine specimens or to some specific screens and validated on a restricted set of dedicated plates. This includes the selective detection of group B Streptococcus, vancomycin-resistant Enterococcus, methicillin-resistant Staphylococcus aureus or Streptococcus pyogenes pharyngitis. Here, we will discuss additional automation steps in the bacteriology workflow where automation and artificial intelligence could improve the overall process. We have developed and validated, in collaboration with Becton Dickinson, an AI for the detection of bacterial growth in the prospect of an automated release of sterile plates on a large variety of media and using any specimen type. In addition, we aimed to develop a biomass monitoring algorithm on subcultures. This monitoring application detects if sufficient biomass is present for follow-up actions such as MALDI-ToF identification or AST (Antibiotic Susceptibility Testing) preparation and could accelerate the laboratory workflow avoiding unnecessary overnight cultures. Finally, we will discuss the automated release of AST without purity check for wildtype profiles. Here, we postulated that if a profile is wildtype, the risk of contamination is unlikely and therefore can be automatically release. This significantly impacts the early availability of the AST results to the clinician.

S-57 Direct inoculation of MBT Sepsityper® blood culture pellets in Vitek2 system and early automated imaging of disk diffusion tests for Gram negative bacteria

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Background
Rapid bacterial identification and antibiotic susceptibility testing plays a crucial role in the management of blood cultures from patients. To take advantage of the Sepsityper (Bruker) blood culture pellet that is performed for MALDI-TOF identification, we assessed a direct standardized inoculation of Vitek2 systems (VT2) to decrease turnaround time for antibiotic susceptibility testing. In addition, we evaluated the impact of automated imaging of disk diffusion testing for the early detection of extended-spectrum beta-lactamases (ESBL) phenotypes.

Methods
Fifty strains of *Enterobacteriales* accounting for the most frequently encountered Gram negative species in blood cultures were spiked. More specifically, we selected 30 *Escherichia coli* (50% ESBL phenotype), 14 *Klebsiella pneumoniae* (50% ESBL phenotype) and 6 *K. oxytoca* (50% K1 hyperexpression phenotype). Blood culture pellets were performed using the MBT Sepsityper® IVD (Bruker). Pellets were resuspended in PBS, and McFarland 0.5 and 0.6 were used to inoculate Mueller-Hinton agar plates and VT2, respectively. Imaging was performed every two hours on BD Kiestra™ Total Laboratory Automation (Becton Dickinson).

Results
Mean difference for disc diffusion tests between direct and standard inoculation was -0.26mm +/-1.46 mm with a categorical agreement (CA) of 97.9%. Disc diffusion diameters were all interpretable after 6h of incubation. Mean time to detect synergism (ceftazidime – clavulanate or- ceftriaxone - clavulanate) was 6.9h +/-1.1h. CA between the VT2 direct versus standard workflow was 96.1%. Using this method, turnaround time is expected to be decreased by 6 to 24h for the release of antibiotic susceptibility testing results.

Conclusion
In this preliminary study, direct inoculation of Vitek2 and disc diffusion AST from blood culture pellets of *Enterobacteriales* demonstrated a high categorical agreement with standard methods. Furthermore, early detection of ESBL phenotypes was feasible. Further prospects include the development a software to automatically measure disc diameter and detect antibiotic synergism at early timepoints.

S-58*/P-149* Optical trapping of bacteria for ultrafast bacteriophage lysis detection at the single-cell level

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Regarding their high bacterial strain specificity, rapid and accurate selection of therapeutic bacteriophages is crucial in phage therapy clinical protocols. Here, we report the use of photonic crystal cavities as on-chip optical nanotweezers for ultrafast phage susceptibility testing (PST) at the single bacterium level. On a silicon photonic chip, resonant photonic crystal cavities allow the trapping of a single *Escherichia coli* B cell and sensing its stressed-induced modifications. This is achieved by monitoring the transmitted optical power through the photonic chip that carries information about the bacterium's characteristics. *E. coli* cells were put in contact with T4 *Myoviridae* phages before being injected in the trapping device. We report direct observation of a bacterium-phage lytic event in the optical cavity. The cell's morphological changes caused by the phage activity prior and after lysis are detected via the transmitted power as well through a microscope imaging system. The lytic event leads to a sudden refractive index reduction, which is attested by an abrupt drop in transmission and a reduced imaging contrast of the cell. Accordingly, only 40min ± 5min after mixing phages and bacteria (t = 0), we observe an abrupt decrease in transmission signal correlating with the bursts of the trapped bacterium. This detection of the lysis event is much faster than current culture-based phagograms usually requiring 16h-24h incubation times. This innovative phagogram approach paves the way to ultrafast PST at the single bacterium level.

*Student presentation

S-59 Evaluation of the 2 mL MWE ∑ Transwab ® Amies liquid specimen collection set for bacterial culture and Gram stain

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We evaluated the 2 mL MWE Sigma Transport specimen collection set (Cat.-No. MW176S 2ML) as alternative to the 1 mL BD and Copan E-swab sets commonly used to collect and transport swab samples for microbiology analyses. Due to the greater volume, the MWE tubes allow for PCR- and culture-based analytics in parallel, which is an advantage from the customer perspective. While all sets are virtually identical with regard to media formulation (modified Amies liquid medium), the material and microstructure of the swabs are markedly different between MWE (cellular foam) and BD/Copan (flocked nylon).
To assess whether the different swab and dilution factor has an impact on bacterial recovery, we first performed ATCC strain recovery experiments according to CLSI recommendations. We found that all specimen collection sets tested allowed for reliable detection of *S. aureus* and *B. fragilis* down to a concentration of 10-100 CFU/mL. Nevertheless, the higher dilution factor for MWE tubes was noticeable, as demonstrated by a 3- to 4-fold lower number of colonies per plate compared to BD/Copan. In a second step we evaluated bacterial culture and Gram stain performance using paired swab samples from oral mucosa, anterior nares and axilla. All transport media sets tested allowed for comparable recovery of the main classes of microorganisms without distortion of the relative abundance of specific morphotypes or strains, and regardless of whether inoculation and plating was performed manually or fully-automated on the BD KIESTRA FA InnoculA. Interestingly, the readability of automated swab culture results from oral mucosa was improved for MWE tubes. We speculate that - depending on the number of CFU plated - the higher dilution factor might allow for faster growth by lowering nutrient competition.
In conclusion, the 2 mL MWE transport medium set is an attractive alternative to the BD and Copan 1 mL E-swab tubes.

S-60 Ensuring Safety and Quality for Pandemic Response: The BioPreparedness Biobank

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In the face of emerging pandemic risks, rapid response is essential to minimize the impact of these events. The Multidisciplinary Center for Infectious Diseases (MCID) at the University of Bern established the BioPreparedness Biobank aiming to provide high quality and secure storage of high consequence pathogens, including bacteria, viruses, fungi, and parasites. The biobanks specificities are the capability to produce synthetic viral genomes in yeast hosts and the access to pathogens from the risk categories 3 and 4. MCID BioPreparedness biobank has achieved significant milestones, including compliance towards ethical and legal requirements, validated by the awarding of the VITA label by the Swiss Biobanking Platform, the establishment of automated storage in a BSL-3 facility, the implementation of a quality system including a quality manual, standard operating procedures, and a dedicated biobank information management system (BIMS). This biobank represents a crucial component in pandemic preparedness by ensuring access to relevant pathogens for research, enabling the rapid development of diagnostic and therapeutic solutions. The MCID BioPreparedness Biobank provides a key resource at the interface of clinic and research to help identify and counter emerging pandemic risks quickly and effectively.

M-18 Adaptation and immune escape of SARS-CoV-2

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Over the past 3.5 years, SARS-CoV-2 has repeatedly given rise to new more transmissible or immune evasive variants. This adaptive evolution of the virus has profoundly shaped how the pandemic unfolded and was followed in near real-time through large scale global genomic surveillance. I will give an overview of SARS-CoV-2 evolution and discuss how we can use the now abundant sequencing data to learn about the potential for future viral adaptations.

S-61 EVOLUTION of the highly diverse repertoire of type III secretion system effectors in the Chlamydiae phylum

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Known species of the phylum *Chlamydiae* infect a large range of eukaryotes including mammals, birds, fishes, arthropods and free-living amoebae. *Chlamydiae* use a type III secretion system (T3SS) to translocate effector proteins in the host cytosol. A T3SS is encoded in each genome of all known members of the *Chlamydiae* phylum and this molecular syringe is probably essential to their ability to parasite and manipulate eukaryotic cells. Secreted proteins evolve quickly and are much less conserved than genes coding for the T3SS apparatus. Nevertheless, effector proteins involved in bacteria-host interactions commonly mimic eukaryotic proteins and frequently share significant similarities with eukaryotic proteins.
To better understand the diversity and evolution of the chlamydial repertoire of secreted T3SS effector proteins, we analysed 51 genomes from the entire *Chlamydiae* phylum. Candidate effectors were identified using machine learning algorithms trained on different datasets of known effectors and based on similarity with eukaryotic proteins. Proteins harboring domain signatures that are only rarely found in prokaryotic proteins were identified by screening more than 16'000 representative bacterial and eukaryote genomes from RefSeq.
More than 5'000 candidates effectors were identified. Several effectors from *Waddlia chondrophila* were demonstrated to be secreted using *Yersinia enterocolitica* T3SS as an heterologous expression and secretion system. Many candidate effectors exhibit irregular distributions within the Chlamydiae phylum, supporting either multiple gene acquisitions, fast evolutionary rates or differential gene losses. The investigation of eukaryotic-like domains have shown that many domains such as RasGEF domains or F/U-box are frequently found in other eukaryote parasites such as *Legionellales*, *Rickettsiales*, *Piscirickettsia* or *Amoebophilus* spp. This might reflect convergent acquisition of similar host proteins or horizontal gene transfers between bacterial parasites. Altogether, this work highlight the major role of effector proteins on the adaptation of the members of the chlamydiae phylum to intracellular life.

S-62 Bioconvection in Lake Cadagno: the power of microorganisms

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The first form of life to appear on Earth more than 3.5 billion years ago was originated in a very different environment from the one we find now on our planet. Indeed, the oceans, as well as the atmosphere, were without oxygen, which did not appear until the "Great Oxidation Event" produced by the first microorganisms to have evolved photosynthesis from anaerobic to aerobic. Not far from us exists an ecosystem that encloses in its depths the characteristics like those found in the early oceans, the meromictic Lake Cadagno in Val Piora. Here we reported the eco-physiological role of a particular phenomenon, called bioconvection, which is produced by the "Purple Sulfur Bacterium" (PSB) *Chromatium okenii*, a primordial anaerobic photosynthetic microorganism. Regular monitoring of the chemical, physical and biological parameters of the water column, in combination with experiments, both in the laboratory (*in vitro*) and in the field (*in situ*), of the most important physiological parameters, such as CO_2 fixation, cell growth or metabolic activity, demonstrated for the first time the positive effect of bioconvection on *C. okenii* and negative effect on the other similar microorganisms in the bacterial layer. Furthermore, it was revealed that *C. okenii* needs precise light conditions in order to be able to set up the coordinated movement resulting in bioconvection.

S-63* Gene transfer agents in *Paracoccus* species

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Horizontal gene transfer (HGT) is an important driver of bacterial evolution. Besides conjugation, natural transformation and transduction, other lesser-known mechanisms exist such as transfer mediated by gene transfer agents (GTA). GTA are domesticated bacteriophages, which package bacterial DNA fragments and inject them into cells. Only a few GTA systems have been experimentally investigated besides the GTA of *Rhodobacter capsulatus* (RcGTA), discovered in 1973. In this study, we present a new bacterial model to study GTA-mediated HGT, *Paracoccus versutus*, which readily produces GTA particles (PvGTA) under standard laboratory conditions. While *Paracoccus* and *Rhodobacter* are quite closely related, their GTA differ in regulation and chromosome coverage. We find that the proportion of cells inducing PvGTA production is highest during the exponential phase and in the absence of physiological stress, while RcGTA expression is maximal in the stationary phase; consistent with this, the expression and transfer rate of PvGTA are not dependent on GafA, one of the main regulators of RcGTA. The content of PvGTA, unlike RcGTA, is strongly biased towards loci around the origin of replication, which suggests that PvGTA production could be synchronized with the initiation of DNA replication. Furthermore, PvGTA production and reception seem to be very specific, since none among three new strains of *P. versutus* and six other *Paracoccus* species demonstrated any ability for self-transfer or PvGTA reception. Thus, unlike other HGT mechanisms like natural transformation, GTA systems might have evolved to enforce gene exchange within clonal populations, either to spread beneficial alleles or to help counter the accumulation of deleterious mutations. In conclusion, our research emphasizes the importance of exploring alternative models of known systems to uncover both invariant and context-dependent phenotypes. Investigating GTA-mediated HGT provides valuable insights into the diverse mechanisms of bacterial evolution and gene transfer.

*Student presentation

S-64 Comparative Genomics of *Cronobacter sakazakii* strains from a Powdered Infant Formula Plant reveals evolving Populations

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Cronobacter sakazakii is an opportunistic pathogen causing life-threatening infections in newborns. *C. sakazakii* strains (N=26) isolated from a powdered infant formula (PIF) plant over a 15-years period were sequenced. Seventeen strains belonged to sequence type ST83 and nine to ST4. Core-genome MLST (cgMLST) revealed clonality of most ST83 and some ST4 strains, showing that these stains derive from persistent populations. A genome comparison between the ST83 and ST4 strains identified population-specific genes, of which some are potentially involved in persistence, such as aggregation and stress-resistance genes in ST83 and fimbrial genes in ST4 strains.
Using the oldest strain H1151-55 as reference, we detected 302 single nucleotide polymorphisms in the ST83 strain set, which shows that the strains evolve in the factory environment. Remarkably, many SNPs were identified in dairy-associated genes such as lactose utilization genes and proteases. Additional analysis showed that a transposon-based lactose operon is present in all PIF isolates, yet only in 171 of 449 public available *C. sakazakii* genomes, suggesting a role of lactose as evolutionary force. Further, the nucleotide identity between lactose transposons from different strains was frequently significantly higher than the average nucleotide iof the strains, pointing towards horizontal gene transfer (HGT).
Fifty-five homologous genes were present in fractions of the ST83 and ST4 strain sets, pointing again towards HGT. Further, different plasmids were present in strains that were clonal in cgMLST analysis, suggesting that plasmids play an important role for gene acquisition and horizontal gene transfer. Remarkably, four ST4 strains possessed a colistin resistance *mcr*6.1 gene, of which the one in strain FP-H363, isolated in 2007, is one of the earliest reported colistin resistances outside the species *E. coli*. *C. sakazakii* populations persist concomitantly in an industrial environment and adapt to it, which has a potential impact on public health.

M-19 Exudate-microbiome interactions on Maize roots

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Plant root exudates can have many functions including to act as semiochemicals for the recruitment, to serve as carbon substrates for microbial growth, or to structure the composition of the microbiome. Mechanistically however, relatively little is known how root microbes deal and cope with specialized plant exudates. We have built a culture collection of maize root bacteria to study toxicity, tolerance, and metabolization of plant-derived Benzoxazinoids, a group of bioactive and antimicrobial secondary metabolites of grasses including crops such as maize. Benzoxazinoids inhibited bacterial growth in a strain- and compound-dependent manner, which largely explained strain abundance on maize roots. We found a specific enrichment of bacteria that metabolise the major compound accumulating in the maize rhizosphere. Combining comparative genomics and transcriptomics, we identified an N-acyl homoserine lactonase in *Microbacteria* that mediates the key step in benzoxazinoid metabolization. Interestingly, we found the native root bacteria (isolated from maize) to tolerate and metabolise the benzoxazinoids better compared to non-host Arabidopsis bacteria, suggesting adaptation to the specialized metabolites of their host plant. Our work reveal that tolerance and metabolization of plant specialized metabolites are important competence determinants for root colonization.

S-65 Fighting aspergillosis using biocontrol bacteria

Mr. Aurélien Trompette [1], Ms. Apiha Shanmuganathan [1], Ms. Julie Pernot [1], Dr. Fabio Palmieri [2], Dr. Eric Bernasconi [1], Prof. Christophe von Garnier [1], Dr. Janick Stucki [3], Dr. Nina Hobi [3], Prof. Pilar Junier [2], Dr. Angela Koutsokera [1], *Dr. Niki Ubags* [1]

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Background
Development of alternative treatments for fungal infections is imperative with the rapid increase in antifungal resistance worldwide. *Aspergillus* spp. infection can modify the lung microenvironment and promote growth via oxalic acid release. *In vitro*, *Cupriavidus oxalaticus* was shown to degrade oxalic acid, thereby decreasing *Aspergillus* spp. growth. We aimed to optimize experimental models to demonstrate the potential of this biocontrol mechanism for clinical interventions.

Methods
BalbC/J mice were treated with cyclophosphamide and cortisone acetate followed by lung infection with *A. fumigatus* CEA10, colonization with *C. oxalaticus*, or a combination of both. Immune cell composition and hyphal development were evaluated in BALF and lung tissue after 72h. To investigate this process in a clinically relevant context, the same approach was applied on human-derived immortalized alveolar epithelial cells (^{AX}iAEC) were cultured on ^{AX}lung-on-chip. Transepithelial electrical resistance, cell morphology, and oxalic acid levels were assessed.

Results
We optimized an *A. fumigatus* infection dose leading to hyphal formation in the lungs but with minimal distress for the animals. Colonization with *C. oxalaticus* led to immune priming with negligible airway inflammation. *C.oxalaticus* exposure altered ^{AX}iAEC morphology and enhanced barrier integrity *in vitro*. Moreover, initial results indicate that *C. oxalaticus* colonization can control *A. fumigatus* infection development both *in vivo* and *in vitro*.

Conclusion
We successfully optimized clinically relevant experimental models to test the potential of biocontrol by environmental interference in the context of pulmonary aspergillosis. This is the first time that biocontrol bacteria are used to contain respiratory fungal infection.

S-66* Adaptations of pathogens in polymicrobial infections in pediatric cystic fibrosis patients

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Polymicrobial infections are frequently observed in individuals with cystic fibrosis (CF), a genetic disorder that primarily affects the airways. The accumulation of nutrient-rich mucus in the airways creates a favourable environment for bacterial pathogens. These infections often follow an ecological succession pattern, with initial colonization by pathogens like *Haemophilus influenzae* and *Staphylococcus aureus*, succeeded by pathogens such as *Pseudomonas aeruginosa*. The polymicrobial nature of these infections involves complex interactions among different pathogens, potentially leading to an increased risk of respiratory complications.
In this study, we examined the interactions between these three pathogens and investigated whether their interaction patterns change over time. Initially, we exposed clinical isolates (obtained from 22 children with CF aged between 0-8 years) to their own supernatant as well as the supernatant of the other pathogens to determine the impact of secreted compounds on isolate growth. Our findings revealed three key changes in the interaction network over time: (i) *P. aeruginosa*, considered the most virulent species, exhibited strong inhibition of *S. aureus* and *H. influenzae* at early time points but showed reduced inhibition at later time points; (ii) we observed a diversification in interaction patterns when *P. aeruginosa* was grown in either *S. aureus* or *H. influenzae* supernatants; (iii) *S. aureus* was benign to *H. influenzae* at the beginning but became inhibitory at later time points. Additionally, we screened the isolates for important virulence factors and found a decrease in protease production among late-stage *P. aeruginosa* strains, which was associated with reduced inhibition of *S. aureus* or *H. influenzae*.
Overall, we found that the virulent nature of *P. aeruginosa* against other bacteria was lower in older children, while the opposite was found for *S. aureus*. This ecological perspective indicates that pathogens adapt to their environment and competitors already at early stages of polymicrobial colonization in CF patients.

*Student presentation

S-67* Virulent Salmonella phages can overcome phenotypic resistance conferred by O-antigen phase variation in the gut

Ms. Anouk Bertola [1], **Dr. Nicolas Wenner** [1], **Ms. Nahimi Amare Bekele** [1], **Ms. Louise Larsson** [1], **Mr. Valentin Druelle** [1], **Dr. Andrea Rocker** [1], **Prof. Camilo Perez** [1], **Prof. Alexander Harms** [2], **Prof. Médéric Diard** [1]

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The enteropathogenic bacterium *Salmonella enterica* serovar Typhimurium (*S*.Tm) causes enteric disease in a wide range of hosts, including humans. Bacteriophages (phages) have been proposed as potent control agents to control *Salmonella*. Here, we address the role of phenotypic resistance in the interactions between *S*.Tm and phages, and its impact on their co-existence and co-evolution.
We have observed that the T5-like phage ϕ37 fails to eradicate and co-exists with *S*. Tm during multiple passages of *in vitro* culture and during intestinal colonization in mice. This co-existence is dependent on the presence of at least one of two epigenetically controlled O-antigen modifying systems (GtrABC and OpvAB), leading to the presence of a phage-resistant subpopulation of bacteria. This prevents the fixation of mutations in phage receptor, BtuB. Double inactivation of GtrABC and OpvAB prevents stable co-existence *in vitro* and *in vivo*.
During long-term infections in mice we observed the evolution of the ancestral phage ϕ37 and subsequent rise of *btuB* mutants in the bacterial population. We have found the phages to accumulate mutations in the L-shaped fiber, which allow the infection of previously resistant subpopulations of *opvAB* or *gtrABC*-expressing bacteria. This adaptation increased the selective pressure and drove the bacteria towards a rapid fixation of *btuB* mutations.
Taken together our data demonstrated that virulent phages can evolve to overcome phenotypic resistance conferred by O-antigen phase variation in the gut, leading to the rapid fixation of phage receptor mutations, potentially costly for *Salmonella*.

*Student presentation

S-68*/P-150* Modeling resource competition in bacterial co-cultures

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Microbial communities are complex heterogeneous systems composed of multitudes of different species. Interspecific interactions are assumed to be a driving factor for community assembly. To test this one would need good predictive models, requiring proper mathematical descriptions that connect interactions to growth kinetics and environment.
The main aim of this work was to formulate the mathematical framework to describe bacterial growth and interactions. We focused first on a binary system with two species, and from there, aim to extend to a community with multiple species.
The kinetic model we developed is based on chemical reaction networks and resource utilisation. We consider both well-mixed environments and surface growth with resource diffusion. Our hypothesis is that the major part of the observed growth behaviour can be explained from intrinsic kinetic parameters on the primary resource (e.g., mu-max, lag times), whereas a minor part is due to interactions originating from metabolite sharing.
In liquid cultures of mono- and co-cultures, the model predicts that some 75 percent of competitive growth behaviour is due to kinetic differences between species, whereas 25 percent is due to cross-feeding. This was in agreement with experimental observations of mono- and co-culture growth of Pseudomonas putida and Pseudomonas veronii on competing substrates. For surface growth, we find that differences in lag times of individual cells are a strong determinant for stationary phase biomass areas. This is the result of fast resource diffusion, leading to the fastest grower 'taking it all'. In contrast, intracellular distances play a less important role in micro-colony development. However, cross-feeding on metabolites leaking from cells leads to the creation of local new resources that influence interspecific interactions and reduce growth in comparison to mono-cultures.
In conclusion, we find that intrinsic growth kinetic parameters play a major role in community behaviour, but are modulated by interspecific interactions.

*Student presentation

M-20 Ecology and evolution of multidrug-resistant Mycobacterium tuberculosis

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Multidrug-resistant tuberculosis (MDR-TB) is among the main causes of death due to antimicrobial resistance. Experimental work has shown that drug-resistant bacteria often suffer a fitness defect in absence of drug, which can be mitigated by compensatory evolution. However, whether compensatory evolution plays any role in clinical settings remains unclear. I will present recent genomic epidemiological data from the country of Georgia and from South Africa, two epidemiologically distinct settings with a high burden of MDR-TB. Together, these data support a role for compensatory evolution in improving the fitness of MDR *M. tuberculosis* clinical strains, both within individual patients as well as between patients during transmission.

S-69 Convergence of hypervirulence and carbapenemase-mediated resistance in *Klebsiella pneumoniae* in Switzerland

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The growing crisis of carbapenem resistance in *Klebsiella pneumoniae* is being accelerated by plasmid-mediated carbapenemases dissemination. In addition to chromosomally encoded K1 and K2 capsule loci, plasmid-mediated dissemination of hypervirulence genes is a growing issue. The convergence of both phenomena, leading to carbapenem-resistant and hypervirulent superbugs, is a worrisome scenario already observed in several countries. In a Swiss institution, carbapenemase-producing *K. pneumoniae* were analyzed for plasmid-mediated virulence and resistance typing. Twenty-two non-repetitive clinical isolates, from one Swiss center in the 2020-2021 period, previously identified as carbapenemase-producers were sequenced using short- and long-read sequencing technology. Data was basecalled using superaccurate model and Flye assemblies were polished with Pilon or Medaka. Analyses were done with CGE tools, Kleborate and Ridom SeqSphere. The analysis revealed among the 22 *K. pneumoniae:* OXA-48 n=8, OXA-181 n=1, OXA-232 n=1, KPC-3 n=6, KPC-2, n=2, NDM-1 n=2, NDM-5 n=1, VIM-1 n=1. MLST showed CC258 (ST11, ST512, ST437), ST147, ST395, ST45, ST48, ST16, ST101 mainly polyclonal distribution. Two small possible clusters (<15 loci difference by cgMLST and same carbapenemase-plasmid) were detected: ST48 harboring an IncL-bla_{OXA-48} plasmid (63kb) (N=2) and ST395 harboring an IncFIIK-IncFIB-bla_{KPC-3} plasmid (187kb) (N=2). Virulence analysis showed that both ST395 KPC-3 clones harbored hypervirulent chromosomally encoded KL2 capsule in addition to aerobactin *iuc1-iutA* and *rmpA2* on a IncHI1B-IncFIB (297kb) plasmid. One NDM-1 ST16 isolate also harbored KL51 capsule, O3b O-antigen and plasmid-encoded *iuc1-iutA – rmpA2.* In this center two-year sampling of carbapenemase-positive *K. pneumoniae,* three of 22 (14%) presented genomic markers of hypervirulence, including the ST395 high-risk clone and the ST16 emerging clone, associated to high patient fatality rates in Brazil and Vietnam. Genomic monitoring of the convergence of hypervirulence to high-risk *K. pneumoniae* MDR clones is warranted. Importantly, long-read sequencing is essential for closure of full bacterial chromosomes and plasmids for a refined analysis.

S-70 Characterization of broad host range IncC plasmid bearing multiple carbapenemases: KPC-2, NDM-1, and VIM-24

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Antimicrobial resistance is threatening global health systems, and plasmids in Gram-negative bacteria are key drivers of its worldwide dissemination. *Klebsiella pneumoniae* has acquired multidrug resistance and is the causative agent of serious community- and hospital-acquired infections. This bacterium often harbors multiple plasmids and may bear multiple carbapenemases. Colombia is considered endemic for two variants of *K. pneumoniae* carbapenemase: KPC-2 and KPC-3. Recently, outbreaks of *K. pneumoniae* harboring other carbapenemases such as NDM and VIM metallo-β-lactamases, alone or in combination with KPC have been reported. *Klebsiella* strains previously identified as multiple carbapenemase-producers, from diverse regions of Colombia, were sequenced using Nanopore long-read sequencing technology. Data was basecalled using superaccurate model, Flye was used for assemblies and Medaka for polishing. The strains and their plasmids were characterized. We identified a *K. pneumoniae* ST987, with a KL64 capsular locus and O3b locus, that harbored a circular IncC-typed 178kb plasmid. It contained three carbapenemase genes: bla_{KPC-2}, bla_{NDM-1}, and bla_{VIM-24}, and showed a highly similar backbone to other reported IncC plasmids bacteria except for the antibiotic resistance islands. The bla_{KPC-2} gene was located in an NTE element (not Tn4401), bla_{VIM-24} on a class I integron, itself flanked by bla_{NDM-1} and an IS91 element. The conjugative transfer *tra* genes were also identified. The acquisition of multiple carbapenemases, often of different classes (serine-proteases and metallo-beta-lactamases) by *K. pneumoniae* is increasingly reported. Whether these carbapenemases confer an advantage, possibly to novel beta-lactamase inhibitors combinations, remains poorly understood. The identification of broad host range plasmids such as IncC plasmids with multiple carbapenemase genes represent a strong potential for horizontal antibiotic resistance spread among bacteria that may cause dire consequences for health institutions around the world. Long-read sequencing technology is allowing for refined analysis of plasmids, which is necessary to understand how antibiotic resistance spread is happening.

S-71* *Escherichia ruysiae* may serve as reservoir of antibiotic resistance genes across multiple settings and regions

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Escherichia ruysiae is a recently described species belonging to the former cryptic *Escherichia* clades III and IV. This species is mainly found in animals, but data regarding humans are scarce.
A stool sample from a healthy Swiss person living in India was screened for multidrug-resistant *Enterobacterales* by culture-based methods. Routine species identification by MALDI-TOF MS and phenotypic characterization by broth microdilution identified an extended-spectrum β-lactamase (ESBL)-producing *E. coli* (S1-IND-07-A). Illumina and Nanopore whole-genome sequencing confirmed that S1-IND-07-A was actually an *E. ruysiae* of clade IV phylogroup, sequence type (ST) 5792, and core genome (cg) ST89059. The serotype O13/O129-:H56-like and five virulence factors were identified. The $bla_{CTX-M-15}$ and other antibiotic resistance genes (ARGs) were detected in a conjugative IncB/O/K/Z plasmid. An international database search identified 70 additional *E. ruysiae* strains reported from 16 countries, most of which were isolated from animals (n=44), but also from the environment (n=15) and humans (n=11). A core-genome phylogeny analysis revealed five major STs (ST6467, ST8084, ST2371, ST9287, ST5792) and 3 other strains from humans, animals and the environment associated with CTX-M type ESBLs and the CMY-2 AmpC.
E. ruysiae is a species that can transfer dangerous ARGs to humans, threatening our antibiotic armamentarium. Therefore, improvement in routine identification and surveillance in all One Health settings is recommended.

*Student presentation

S-72

Development of an artificial activation system for studying zinc-finger transcriptional factors in *Candida auris*

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Candida auris has emerged as a multi-drug resistant yeast pathogen since 2009. The Centers for Disease Control and Prevention (CDC) considers *C. auris* as a public-health threatening pathogen due to its ability to cause nosocomial outbreaks with high mortality rates (up to 50%) and to develop resistance to all the current antifungal classes. Zinc-finger transcription factors (ZF-TFs), a family of regulators which are unique in the kingdom of fungi, play important roles in diverse cellular processes. We previously demonstrated that a ZF-TF Mrr1 is responsible for azole resistance in *C. auris*, by regulating the efflux pump Mdr1, which can export azoles out of the cells. Nevertheless, the function of the majority of ZF-TFs are still unknown in *C. auris*.
In this project, we created a plasmid to activate artificially ZF-TFs to study their function in *C. auris*. In this plasmid, the ZF-TF is hyperactivated by fusing 3XHa Tag sequence into the C-terminal of its nucleotide sequence. The plasmid with the hyperactivated ZF-TF was then transformed into the neutral intergenic locus *CauNi* of a wild-type *C. auris* strain combined with CRISPR-Cas9 system. We validated this system by hyperactivating the ZF-TF Mrr1, with which we showed that fusing the 3XHA Tag sequence into the C-terminal of *MRR1* significantly increased azole resistance by overexpressing the transporter gene *MDR1*.
ZF-TFs are the key regulators which control diverse biological facts in fungi. ZF-TFs play important roles in antifungal resistance, virulence, morphogenesis, interaction with host cells, sugar and amino acid metabolisms, meiosis and mitosis etc. We believe that this artificial activation system could be potential and powerful to explore ZF-TFs in diverse axes, and we hope that it will help us understand better how this novel pathogen *C. auris* emerges and threatens human beings

SPEED SCIENCE & POSTERS
P-001 – P-012

SP-01*/P-001* A reactor for the large-scale production of Legionella pneumophila-containing biofilms

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Building plumbing systems are often contaminated with the opportunistic pathogenic bacteria *L. pneumophila*. Growth of the pathogen in buildings is affected by environmental factors, such as temperature and the overall drinking water microbiome. The complexity and variability in real building plumbing systems makes it challenging to untangle and quantify the importance of individual factors on *L. pneumophila* growth. Moreover, the bacterium is able to establish in the biofilms that form inside the building plumbing system pipes. These biofilms can act as a reservoir of *Legionella* and are not easily accessible for microbial analysis.
To overcome this, we developed a reactor for large-scale production of *L. pneumophila*-containing biofilms on ethylene propylene diene monomer (EPDM) together with a natural drinking water community. Here, we report on biofilm formation and colonization with *L. pneumophila* over the course of the 12-week reactor commissioning. Culture-based quantification as well as ddPCR show that *L. pneumophila* established quickly in the biofilms reaching a concentration between 1 – 3 x 10^3 MPN/cm^2. The total cell concentration (TCC) on the coupons remained stable at 1 – 5 x 10^6 cells per cm^2 during the commissioning period, with *L. pneumophila* representing around 0.03% of the total cell count.16S amplicon sequencing analysis reveals microbial dynamics of biofilm drinking water community formation on the EPDM coupons and a diverse microbial community.
Together, our results indicate that the reactor produces biofilms with reproducible, high concentrations of *L. pneumophila* and the biofilms can be used for bench scale experiments.

*Student presentation

SP-02*/P-002* Dissecting recurrent UTIs using a 3D human mini bladder microtissue model

Ms. Gauri Paduthol [1], **Dr. Kunal Sharma** [1], **Dr. Mikhail Nikolaev** [2], **Dr. Kathrin Tomasek** [1], **Dr. Vivek Thacker** [1], **Prof. Matthias Lütolf** [2], **Prof. John McKinney** [3]

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Urinary tract infections (UTIs), primarily caused by uropathogenic *E. coli* (UPEC), affect approximately 150 million people annually worldwide. [1] Here we present an in-vitro model that can be used to study long-term, spatially localised host pathogen interactions of UPEC. The human mini-bladder model mimics the physiology of in-vivo bladder tissue, with flowing urine, stretching tissue and ability to study complex and chronic infection conditions.
We develop this microtissue model by culturing primary human bladder epithelial cells in a collagen scaffold, with nutritive media and urine. This stimulates multi-layer stratification, directional differentiation, with an apical layer of bi-nucleated cytokeratine-20 expressing umbrella cells and a tight tissue barrier. Micturition effects are established through induced urine flow and pressure-controlled tissue stretching. Upon infecting this microtissue with UPEC, firstly we observe the bacteria invade the tissue and grow in intracellular bacterial colonies. [2] Secondly, when the infected tissue is treated with Fosfomycin in an osmoprotected media, we observe rod-shaped UPEC convert into cell-wall deficient L-forms in the lumen. [3] We found that lumenal L-forms, upon cessation of Fosfomycin treatment, can reseed into the tissue and further propagate recurrent infections. And finally, we can distinctively simulate chronic untreated UPEC infections of over 24 hours without loss of microtissue integrity. In summary, the human mini-bladder provides an ideal in-vitro tissue model for studying the nature of intracellular niches of UPEC and identifying their survival and growth mechanisms.

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*Student presentation

SP-03*/P-003* A novel WYL domain containing protein is a transcriptional activator in response to genotoxic and oxidative stress

Ms. Lena Maria Keller [1], **Ms. Kim Flattich** [1], **Prof. Eilika Weber-Ban** [1]

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Transcriptional regulation allows all living organisms to adapt to changing environments as well as encountered stress conditions. Most transcription factors contain a signal sensor domain in addition to its DNA binding module. Such an example is the WYL domain that stands for a tryptophan-tyrosine-leucine motif and only exists in bacteria. The emerging class of WYL domain containing transcription factors is involved in the regulation of the DNA damage response and in the regulation of various phage defense mechanisms in bacteria. Previously, it has been suggested that the WYL domain is a nucleotide-sensing module which regulates the activity of the transcription factor. Interestingly, while some WYL domain containing transcription factors act as activators others act as repressors.
Here, we investigated another WYL domain containing transcription factor in Mycobacterium smegmatis, which we termed stress involved WYL domain containing regulator (SiwR). We found that the homodimer SiwR activates the transcription of its neighboring genes, that belong to the DinB superfamily, upon genotoxic and oxidative stress. We were able to identify ligands that modulate the activity of SiwR by binding to conserved residues in the WYL domain. In addition, we identified the SiwR promoter motif and were able to show that SiwR associates with the RNA polymerase complex in vitro. We used biochemical, structural and microbiological approaches to characterize a novel WYL domain containing transcriptional activator that helps to overcome genotoxic and oxidative stress.

*Student presentation

SP-04*/P-004* Investigation of the Min system in the division process of Acinetobacter baumannii

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Acinetobacter baumannii is a nosocomial pathogen associated with a high increase in antibiotic resistance. It is estimated that nearly 45% of all *A.baumannii* isolates are multidrug resistant. Being a public health threat and in association with poor clinical outcomes worldwide, the discovery of novel antimicrobial against *A.baumannii* is thus of critical importance.
Cell division is an essential step in the life cycle of the bacteria. Like *E.coli*, *A.baumannii* divides by binary fission to produce two identical daughter cells. Therefore, the cell has to ensure that the division site is correctly positioned at midcell. The first protein to localize to the division site is the conserved FtsZ protein, which forms a discontinuous Z-ring at midcell. FtsZ is responsible for the recruitment of the other cell division proteins that form the divisome. Not surprisingly, proteins that are known to impact division site positioning within bacterial cells, do so via influencing the placement of the Z-ring. In *E. coli*, two regulatory systems have been described: the Min system, encoded by the *minCDE* operon and the nucleoid occlusion system, encoded by *slmA*. The mutant lacking the *minCDE* operon is characterized by the formation of minicells that are not viable due to the absence of DNA.
In *A.baumannii*, homologs of the *minCDE* operon can be easily identified, whereas no SlmA homologs can be found. This project focuses on the characterization of the Min system in *A.baumannii*. The Min operon has been deleted. As expected, the mutant presents a growth defect and an aberrant morphology in liquid medium but shows significant differences from what was described for the *E.coli* mutant. Indeed, no minicells were observed. In addition, the mutants grew fine on agar plates. These observations suggest the presence of additional mechanisms involved in positioning of the division site or DNA segregation in *A. baumannii*.

*Student presentation

SP-05*/ P-005* Characterization of chlamydial ndk and its role in cell differentiation, from elementary bodies to reticulate bodies

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Chlamydia trachomatis and *Waddlia chondrophila* are pathogenic intracellular bacteria, which grow within an intracellular vacuole termed inclusion. Inside inclusions, the pathogenic bacteria alternate between two developmental forms, the infectious elementary body (EB) and the replicating reticulate body (RB). This transition is central to chlamydial biology; nevertheless, the intrinsic molecular triggers of transition are unknown.
This study focuses on characterizing the chlamydial *ndk* and investigating its role in chlamydial differentiation. The role of *ndk* genes family was suspected based on the results we obtained by a RNA sequencing screening aimed at identifying factors involved in the chlamydial RB to EB transition.
In *C. trachomatis*, the *ndk* gene (*Ctndk*) exists as a single copy, whereas *W. chondrophila* possesses two copies (*Wcndk1* and *Wcndk2*) organized in an operon. Notably, only WcNdk2 presents a N-terminal signal peptide. We demonstrated using immunofluorescence on cells ectopically expressing WcNdk2 that the signal peptide is responsible for the localization of the protein in the host cell nucleus. These findings were confirmed in cells infected with a *C. trachomatis* strain expressing WcNdk2. Additionally, we employed azidothymidine (AZT) to inhibit *ndk* function in *C. trachomatis or W. chondrophila*-infected cells. Since only *W. chondrophila* growth was affected by AZT treatment, we hypothesized that AZT inhibits the DNA binding ability of WcNdk2. Indeed, Ndk has been reported to bind and regulate *c-myc* in other organisms and our preliminary findings demonstrate a reduction in *c-myc* transcript levels upon treatment of *C. trachomatis* infected cells with AZT.
The role of NDK genes in development of *Chlamydiae* could be further investigated by the development of *ndk* null mutants. By disrupting Ndk function, it becomes conceivable to target *Chlamydia* differentiation, therefore opening new avenues for drug development aimed at combating *Chlamydia* infections.

*Student presentation

SP-06*/P-006* Predicting xenobiotic biotransformation by the urinary microbiota: Is paracetamol on the menu?

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Microbes are talented chemists capable of transforming xenobiotics within the human body. Most research on microbial xenobiotic metabolism has focused on the gut microbiota although the majority of xenobiotics are primarily excreted in urine. While microbial biomass and residence time in the gut is higher, xenobiotic biotransformations in the bladder have the potential to affect antibiotic resistance and urinary tract infections. However, we have limited knowledge of xenobiotic-transforming enzymes encoded in urinary microbiomes. We predicted the biotransformation potential of selected xenobiotics and used a comparative genome mining approach to profile the distribution of the candidate xenobiotic-degrading enzyme classes encoded in urinary tract bacteria. Our analysis revealed a discontinuous enzyme class distribution even among related species and a high conservation of paracetamol amidase homologs. We performed phylogenetic analysis and enzyme functional predictions for the paracetamol amidase homologs, and selected candidates for heterologous expression in *Escherichia coli*. Among our candidates, the amidase encoded in the genome of *Lactobacillus rhamnosus* UMB0004 was validated for paracetamol degradation activity and further biochemically characterized. Overall, this work provides new insights into urinary microbe-xenobiotic interactions as a foundation for understanding urinary microbial ecology.

*Student presentation

SP-07*/ P-007* **“It’s a trap!”: Toxin-antitoxin system Stheno prevents the spread of phage infection in bacterial populations**

Ms. Aisylu Shaidullina [1], **Mr. David Schwegler** [2], **Dr. Tetiana Brodiazhenko** [3], **Dr. Elisa Venturini** [2], **Dr. Elia Agustoni** [2], **Dr. Raphael Dias Teixeira** [2], **Dr. Bhanu Chouhan** [4], **Prof. Gemma Atkinson** [4], **Prof. Marek Basler** [2], **Prof. Vasili Hauryliuk** [4], **Prof. Sebastian Hiller** [2], **Prof. Alexander Harms** [1]

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Toxin-antitoxin (TA) systems are frequently encoded in the genomes of bacteria and their mobile genetic elements. They consist of two essential components: a toxin that significantly affects bacterial metabolism upon activation, and an antitoxin that inhibits toxin activity under normal physiological conditions. The importance of TA systems increased due to their potential involvement in the development of antibiotic resistance in bacterial pathogens. Additionally, recent findings have demonstrated that TA systems can serve as a defence system protecting bacteria against phage infection and thus acting as a natural barrier that limits the success of phage therapy.
Despite decades of intensive research, numerous questions remain unanswered regarding the activation of different TA systems and the impact of toxin activity on cellular functions. Here we present data on a novel tripartite TA system, named Stheno, discovered within a temperate *Escherichia coli* phage. The Stheno system effectively defends *E. coli* against multiple phages from the BASEL collection. Our genetic studies have revealed that Stheno specifically recognises a phage helicase that triggers toxin activation. Through metabolic labelling experiments and live microscopy, we have observed that activation of the Stheno toxin supresses phage replication by inhibiting ribosomal translation in the infected cells, leading to abortive infection.
Currently, we are investigating the molecular interplay among the three components of Stheno, which act together as toxin, antitoxin and transcriptional regulator controlling Stheno expression and activity. We believe that TA systems such as Stheno can become a valuable model for studying molecular interactions among TA components and consequences of toxin activation on the cellular level. Furthermore, studying the distribution of TA systems with mobile genetic elements can provide insights into their influence on controlling antibiotic-resistant bacteria by phage therapy.

*Student presentation

SP-08*/ P-008* **The insect pathogenic fungus Metarhizium in Swiss grassland soils**

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Entomopathogenic fungal strains of the genus *Metarhizium* are commercially available biocontrol agents (BCAs) used to control insect larvae in soil. The factors driving the establishment of applied BCA strains for effective insect control are only partially understood. The goal of this study is to examine correlations of biotic and abiotic factors with indigenous soil *Metarhizium* populations to identify potential key drivers for successful BCA establishment.
Presence of indigenous *Metarhizium* spp. was evaluated at 72 Swiss permanent grassland sites, representing different geographies and management intensities (see EU-project BIOINVENT). We assessed soil physicochemical parameters, weather conditions, vegetation composition, and soil bacterial and fungal microbiome data.
Taxonomic classification of fungal amplified sequence variants (ASVs) allowed assignment of 4 fungal ASVs to *Metarhizium* clade 1, which contains all currently commercialized BCA strains of this genus. These ASVs were representative for *M. robertsii* (1 ASV), *M. majus/M. guizhouense* (1 ASV), and *M. brunneum* (2 ASVs).
We detected different presence/absence patterns for the two *M. bunneum* ASVs at different sites and frequent absence of the *M. majus/M. guizhouense* ASV in mountain regions. Furthermore, we explored 14 abiotic factors, where specific *Metarhizium* ASVs were present or absent. Each ASV revealed associations with distinct factor compositions, while soil pH, phosphorous content and mean annual temperature are key drivers of the presence of at least one *Metarhizium* ASV. All 4 *Metarhizium* ASVs were associated with bacterial-, fungal- or plant community compositions e.g., bacterial communities and the *M. robertsii* ASV were both strongly correlated to soil pH. Moreover, we found correlations of distinct *Metarhizium* ASVs with individual plant species, and fungal and bacterial ASVs.
These results indicate that different species or strains of *Metarhizium* clade 1 occupy grassland that reveal different characteristic environmental factors. These findings may contribute to the selection of new BCA-strains, more adapted to specified environmental conditions.

*Student presentation

SP-09/ P-009 Chlamydia suis displays high transformation susceptibility with complete chromosomal integration into the intergenic rrn-nqrF plasticity zone

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The chlamydiae are a conserved bacterial phylum with limited evidence for horizontal gene transfer (HGT) beyond genus-level. Within the phylum, the *Chlamydiaceae* are the most studied family due to their importance for veterinary and human health. All *Chlamydia* are dependent on an intracellular niche, which serves as a barrier for natural genetic exchange, and further hampers the development and application of genetic tools. To date, the only example for recent inter-phylum HGT is tetracycline resistance in porcine *Chlamydia (C.) suis*, a potentially zoonotic pathogen and close phylogenetic relative of *C. trachomatis*, causative agent of bacterial sexually transmitted infection and ocular trachoma. Tetracycline resistance in *C. suis* has been detected in strains worldwide and is present as part of a genomic island that divides *invasin* (*inv*), located within a hypervariable chromosomal region of *Chlamydia* between the rRNA operon and the Na(+)-translocating NADH-quinone reductase subunit F gene (*rrn-nqrF* intergenic region). In this study, we aimed to expand the still limited number of available genetic manipulation systems for *Chlamydia* by generating chromosomal integration vectors for *C. suis*. These vectors, marked by an absence of the native chlamydial plasmid, contained only homologous *C. suis* sequences of the chromosomal region of interest merged with *E. coli* cloning vectors. In this study, we found that *C. suis* can not only be transformed with chromosomal integration vectors targeting the tryptophan (*trp*) operon of *C. suis*, but also integrate the homologous *trp* sequence and RSGFPCAT cassette into its genome. While *C. trachomatis* can be transformed with these shuttle vectors, integration of the plasmid sequence was not observed in the *C. trachomatis* chromosome. Moreover, by targeting the unique *C. suis inv* gene, we found that the *rrn-nqrF* intergenic region of *C. suis* is highly susceptible to transformation allowing integration of the complete vector without interruption of the targeted region.

SP-10/ P-010 The essential insertase BamA: an entry point for antimicrobial peptides in Gram-negative bacteria?

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Antimicrobial peptides are promising compounds to fight antibiotic-resistant pathogens. We recently showed the efficiency of TAT-RasGAP$_{317-326}$, an anticancer peptide, against a broad range of Gram-negative pathogens. To better understand the mode of action of this peptide, we selected *in vitro Escherichia coli* and *Acinetobacter baumannii* mutants resistant to it. Using whole genome sequencing, we repeatedly detected mutations in *bamA*, a gene coding for an essential component of the Bam complex, which is involved in the insertion of outer membrane proteins. We thus aimed at characterizing the role of this protein in the antimicrobial activity of TAT-RasGAP$_{317-326}$ and of other antimicrobial peptides. For this purpose, we used a CRISPR-Cas9 based technology to create point mutants of BamA and investigated their influence on antimicrobial peptides efficiency. We could observe that point mutations in a negatively charged region at the surface of BamA decreased bacterial sensitivity of *E. coli* to TAT-RasGAP$_{317-326}$, but not to other antimicrobial agents. In addition, *in silico* docking simulations predicted a binding affinity between TAT-RasGAP$_{317-326}$ and BamA of *E. coli*. In a further step we took advantage of an *E. coli* deletion mutant collection to investigate the role of other components of the Bam complex in the resistance towards antimicrobial peptides. Interestingly, deletion of some non-essential components of the Bam complex caused increased sensitivity to several antimicrobial peptides by a mechanism that still needs to be investigated. Taken together, our results indicate that Bam complex function is important for the activity of some antimicrobial peptides. We expect that these results will allow to apply Bam complex inhibitors to increase the efficiency of antimicrobial peptides towards Gram-negative pathogens.

SP-11/ P-011 ONE-step soft agar enrichment and isolation of human lung bacteria inhibiting the germination of Aspergillus fumigatus conidia

Dr. Fabio Palmieri [1], **Ms. Margo Magnin** [1], **Mr. Jérémy Diserens** [1], **Ms. Manon Gresse** [1], **Dr. Eric Bernasconi** [2], **Ms. Julie Pernot** [2], **Ms. Apiha Shanmuganathan** [2], **Mr. Aurélien Trompette** [2], **Prof. Christophe von Garnier** [2], **Dr. Thomas Junier** [3], **Dr. Samuel Neuenschwander** [3], **Dr. Saskia Bindschedler** [1], **Dr. Marco Pagni** [3], **Dr. Angela Koutsokera** [2], **Dr. Niki Ubags** [2], **Prof. Pilar Junier** [1]

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Fungi of the genus *Aspergillus* are widespread in the environment where they produce large quantities of airborne conidia. Inhalation of *Aspergillus* spp. conidia in immunocompromised individuals can cause a wide spectrum of diseases, ranging from hypersensitivity responses to lethal invasive infections. Upon deposition in the lung epithelial surface, conidia encounter and interact with complex microbial communities that constitute the lung microbiota. The lung microbiota has been suggested to influence the establishment and growth of *Aspergillus* spp. in the human airways. However, the mechanisms underlying this interaction have not yet been sufficiently investigated. In this study, we aimed to evaluate the presence of commensal bacteria antagonistic to *Aspergillus* in the lung. To this end, we enriched and isolated bacterial strains able to inhibit the germination of conidia from bronchoalveolar lavage fluid (BALF) samples of lung transplant recipients. We used a novel enrichment method based on a soft agar overlay plate assay in which bacteria are directly in contact with conidia and for which inhibition can be readily observed during enrichment. We isolated a total of five bacterial strains, identified as *Pseudomonas aeruginosa*, and able to inhibit the germination and growth of *Aspergillus fumigatus* in a soft agar confrontation assay, as well as in a high-throughput multiplate assay. Moreover, we also showed a strong inhibition of *A. fumigatus* growth on Calu-3 cell culture monolayers. However, the isolated *P. aeruginosa* strains were shown to cause significant damage to the cell monolayers. Overall, we validated this novel one-step enrichment approach for the isolation of bacterial strains antagonistic to *A. fumigatus* from BALF samples. This opens up a new venue for targeted enrichment of antagonistic bacterial strains against specific fungal pathogens.

SP-12/ P-012 Monitoring enteric viruses in Swiss wastewater

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Monitoring of enteric viruses in wastewater could inform about epidemiology of the associated disease within the population. Enteric viruses can infect the human gastrointestinal tract, causing a variety of symptoms and diseases. Norovirus is the most common cause of acute gastroenteritis around the world and can rapidly spread across individuals. Rotavirus causes severe watery diarrhea and vomiting, mainly affecting children <5 years old for whom subsequent dehydration could lead to hospitalization or even death. In Switzerland, Norovirus and Rotavirus infections occur mainly during the winter months. Both viruses are shed in high concentration in feces of infected individuals and are stable in aquatic environments, indicating the potential for monitoring these viruses in wastewater.
We are developing a multiplex digital PCR (dPCR) assay for the detection and quantification of Norovirus genogroups I and II and Rotavirus in Swiss wastewater samples. Twenty-four hour flow composite wastewater samples are routinely collected from 6 different wastewater treatment plants across the country. Wastewater is clarified through centrifugation, followed by viral RNA concentration and extraction using the Promega Wizard Enviro Total Nucleic Acid (TNA) Kit. Samples are further purified using the Zymo One-Step PCR Inhibitor Removal Kit, to reduce PCR inhibitors. Norovirus GI, GII and Rotavirus RNA concentrations will be measured using Stilla Technologies Crystal Digital PCR platform.
Routine monitoring of Norovirus and Rotavirus concentrations in the wastewater might reflect the emergence and decline of these viruses within the populations of the different catchment areas. Wastewater-based data on enteric pathogens may provide early insights into future waves of these viruses before patients with severe symptoms show up in the hospitals, which could ultimately support early decision-making of public health offices and clinicians.

MY THESIS IN 180 SECONDS & POSTERS P-013 – P 017

MT-01*/P-013* Understanding polymicrobial urinary tract infections: from retrospective data analysis, through catheter biofilms, to bladder microtissue models

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Urinary tract infections (UTIs) are one of the leading causes of bacterial morbidity, especially for women, with infections numbering hundreds of millions annually, worldwide. Uropathogenic E. coli (UPEC) is the most frequent cause of infections, with most UTIs being uncomplicated. Risk factors like immunodeficiency or catheterization, however, often lead to complicated UTIs, which are significantly harder to treat. Polymicrobial infections are strongly associated with complicated UTIs and increased disease severity, and despite their clinical significance, the impact of the infections' polymicrobial nature on disease outcomes remains poorly understood. This becomes especially relevant in catheterized patients, where the presence of the catheter enhances microbial colonization and biofilm formation. Both pathogen-pathogen and host-pathogen interactions in polymicrobial infections are implicated in modulating treatment outcomes, with phenomena ranging from dampened host immune responses to increased antibiotic tolerance.
Current clinical therapies tend to treat monomicrobial and polymicrobial infections similarly, and an in-depth study is thus necessitated for targeted and effective treatment. To understand the patient cohort and the microbial combinations which are most relevant for public health, we performed a thorough analysis of previous years' urine data (anonymized) from the Institute of Medical Microbiology, Zurich including information about age, gender, and ward distribution. From this analysis, microbial frequency across monomicrobial and polymicrobial infections, from midstream and catheterized patient urine was assessed, and co-occurring microbes enriched per condition were identified. Clinical isolates of these identified microbes are now being collected, characterized, and sequenced to build a bioinformatic landscape for microbial colonization, and for genome-wide association studies to identify genetic propensities. Our future studies aim to assess the collected strains in physiologically equivalent environments, including in-vitro catheter biofilm studies, followed by studies in bladder microtissue models. Our goal is to provide new insights into polymicrobial infections and eventually suggest new clinical treatment strategies for UTI patients.

*Student presentation

MT-02*/P-014* Investigating the mechanisms implicated in the biogenesis of aberrant bodies in Chlamydiae

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Background
The *Chlamydiales* order contains obligate intracellular bacteria (such as *Waddlia chondrophila* and *Chlamydia trachomatis*) sharing a common biphasic developmental cycle characterized by infectious, non-replicative elementary bodies and non-infectious, replicative reticulate bodies. When exposed to stress stimuli, reticulate bodies enter a persistence state called aberrant bodies (ABs). ABs are believed to play a role in chronicity and recurrence of chlamydial infections.

Methods
RNA-sequencing performed on *Waddlia* aberrant bodies allowed us to identify *ispA (iron starvation protein A)*, an upregulated gene in the *Chlamydiae* phylum, and exclusively present in that phylum. *W. chondrophila* and *C. trachomatis* ABs were then obtained with other types of stresses to assess the behavior of this gene. Bioinformatic and wet-lab analysis were also performed to characterize the protein.

Results
We confirmed that while *ispA* is upregulated upon iron-starvation in *W. chondrophila*, it wasn't the case for *C. trachomatis*. In fact, of all the stresses applied, heat-shock is the only tested condition where the upregulation was observed in both species. The secretion of IspA by the T3SS predicted bioinformatically was confirmed using *Yersinia enterocolitica* as a heterologous system, but its localization within the host cell observed by immunofluorescence is unclear. Interestingly, IspA has a conserved C-terminal domain resembling that of a cystine-knot. Cystine-knots are described as a domain of protein interaction but was only described in eukaryotes. Regulation-wise, a conserved CIRCE sequence which allows repression by HrcA, a stress regulator, was identified upstream *ispA*.

Conclusions
Our preliminary results reveal IspA, a highly conserved, *Chlamydiae*-specific T3SS effector, with a conserved cystine-knot-like domain, and a conserved CIRCE regulatory sequence. Despite these conservations, *ispA* answers differently to various stresses across the phylum, but similarly to heat-shock for *C. trachomatis* and *W. chondrophila.* Further studies should aim at identifying IspA targets and other regulatory pathways to explain these discrepancies.

*Student presentation

MT-03*/P-015* Into the Wild: Understanding Antibiotic Resistance using Clinical Strains

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In 2019 *Escherichia coli* was the bacterial pathogen causing most deaths attributed and associated with antibiotic resistance worldwide. Many *E. coli* strains carry several genes conferring resistance to multiple antibiotics. This includes extended-spectrum beta-lactamases which grant resistance to 3rd generation cephalosporins. About one third of these strains remain susceptible to the widely used 4th generation cephalosporin cefepime, but mechanisms modulating susceptibility remain unclear. In-vitro evolution of ESBL- *E. coli* results in emergence of cefepime resistance by porin OmpF loss which is rarely observed in clinical isolates.

We compared 106 *E. coli* isolates from the dominant lineage ST131 carrying the ESBL CTX-M-15 but differ in cefepime susceptibility, using mass spectrometry. We observed low CTX-M-15 protein levels in cefepime-susceptible isolates and high CTX-M-15 levels in most resistant strains, but no other significant differences including unaltered levels of OmpF and other porins. Most bacterial lysates from cefepime- susceptible strains hydrolyzed the 3rd generation cephalosporin cefotaxime, but not cefepime, whereas most lysates from cefepime-resistant strains hydrolyzed both cefotaxime and cefepime. Our data indicate increasing protein copy number of an ESBL with poor activity against 4th generation cephalosporins as a major clinical resistance mechanism.

We currently determine the frequency of occurrence and underlying mechanisms of such amplification events to estimate the risk of resistance emergence under therapy. These results will help to evaluate the controversial use of 4th generation cephalosporins for treatment of widespread ESBL-*E. coli* infections to preserve last- resort carbapenems.

*Student presentation

MT-04*/P-016* Synergistic targeting pathogenic enterococci with phage-encoded peptidoglycan hydrolases and wall teichoic acid inhibitors

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Antibiotic-resistant enterococci are on the rise and pose a great threat to humankind. Future bacterial infections will inevitably become increasingly difficult to treat due to limited drug options, intensifying the need to develop effective alternatives Endolysins are bacteriophage-derived peptidoglycan hydrolases and exhibit a great potential to kill Gram-positive bacteria in a specific and effective way. They act by cleaving the conserved bacterial peptidoglycan (PG) structure thereby inducing osmatic bacterial lysis. However, their bacteriolytic efficiency is often hampered by the presence of cell wall-associated polysaccharides, namely wall teichoic acids (WTA), which restrict the access of the endolysin to PG. To overcome this limitation, we propose to use tunicamycin, a known WTA-specific inhibitor to enhance the bacteriolytic activity of enterococcal endolysins. The enterococcal endolysin PlyV12 consists of one enzymatic active domain (EAD) and one cell wall binding domain (CBD). The EAD inherits a functional amidase cleaving the bond between the peptidoglycan sugar backbone and the peptide moiety. The combination of tunicamycin and PlyV12 in a time kill assay was significantly more effective compared to using each agent alone. Interestingly, no synergy was observed when applying tunicamycin with other non-amidase enterococcal endolysins, suggesting this synergistic effect could be amidase-dependent. To further explore the underlying mechanism and mode of action, fluorescent-tagged PlyV12 CBD and tunicamycin-derivatives were constructed and explored under the fluorescence microscope. Our data support further development of endolysins and tunicamycins as effective therapeutics against enterococcal infections.

*Student presentation

MT-05*/P-017* **Monitoring Antimicrobial Resistance in Swiss Wastewaters**

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Antimicrobial resistance (AMR) is a top 10 global health threat and the failure to address the problem lead to an estimated 10 million deaths by 2050. Wastewater surveillance is a cost-effective, non-invasive, and anonymous approach that offers a basis for providing estimates of AMR rates at the population level. Here, we investigated the prevalence of extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* (ESBL-Ec and ESBL-Kp), carbapenemase producing *E. coli* and *K. pneumonia* (CP-Ec and CP-Kp), methicillin resistant *Staphylococcus aureus* (MRSA), and vancomycin resistant *Enterococcus spp.* (VRE) in wastewater in the influent of 6 wastewater treatment plants (WWTPs) across Switzerland. In total, we collected more than 500 wastewater samples on a weekly basis from November 2021 to April 2023. ESBL-Ec and ESBL-Kp were detectable in all analyzed samples, with mean loads of $2.68x10^8$ and $1.6x10^8$ CFUs/(person-day), respectively, and mean percentages of 1.73% (±0.79) ESBL-Ec out of total *E. coli* and 0.36% (±0.38) ESBL-Kp out of total *K. pneumoniae*. CP-Ec and CP-Kp were detected in 83.3% and 91.7% of analyzed samples, respectively, with mean percentages of 0.14% (±0.49) CP-Ec over total *E. coli* and 0.18% (±0.34) CP-Kp over total *K. pneumoniae*. MRSA and VRE were detected in 92.2% and 97.7% of analyzed samples, respectively, with mean percentages of 20.3% (±23.3) MRSA over total *S. aureus* and 0.14% (±0.19) VRE over total *E. faecium/fecalis*. Loads and percentage varied significantly by WWTPs and through time on a monthly scale for all targeted resistant bacteria. Finally, screening 234 ESBL-Ec isolates for the presence of ESBL-genes using the digital Multiplex Ligation Assay identified $bla_{CTX\text{-}M1}$ as the most frequent ESBL-genes in Swiss wastewater, followed by $bla_{CTX\text{-}M9}$ and bla_{TEM}. The monitoring protocol offers insights into ESBL-genes circulating in Swiss wastewater and longer-term surveillance may provide insights into temporal trends of resistant pathogens of interest.

*Student presentation

ELEVATOR PITCH PRESENTATIONS & POSTERS P-018 – P-046

EP-01/P-018 Asymmetric expression of meiotic genes imposes distinct selective pressures on mating partners

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Anisogamous species, which produce gametes with significant morphological differences, are thought to have evolved from isogamous ancestors[1]. However, lack of evolutionary records and experimental evidence has made it difficult to understand how selection could act on isogametes to select for increased asymmetries. I will show our recent data indicating that the morphologically indistinguishable fission yeast P- and M-gametes, which differentially express only a handful of genes, experience distinct selective pressures. We initially discovered that transcription of meiosis-specific genes, including the highly conserved meiotic cohesin *rec8*, occurs ahead of fertilization. Surprisingly, only P-gametes produce Rec8 protein during mating, whereas Rec8 encoded by the M-gamete genome becomes detectable only at the time of partner fusion. This asymmetry in Rec8 production is driven by distinct pheromone signalling between partners, and P-gamete-produced Rec8 is critical for meiotic chromosome segregation. Strikingly, early expression of Rec8 also places a fitness cost on P-gametes; P-gametes that engage partners but fail to fuse exhibit increased genomic instability when allowed to reproduce asexually, which is prevented by the removal of the *rec8* gene. Finally, we show that P-gametes are at a competitive disadvantage to M-gametes in evolving populations that undergo cycles of mating and asexual reproduction, and that the observed difference in fitness depends on the *rec8* gene. Taken together, we demonstrate that distinct investments of P- and M-gametes in zygotic development impose different fitness costs. Our work provides the first example of how subtle molecular asymmetries can drive specific selective pressures on isogametes during evolution.

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EP-02/P-019 Gut microbiomes of agropastoral children from the Somali Regional State of Ethiopia show a unique bacterial composition reflecting their dietary habits

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The intestinal microbiota is a major determinant of human health and are influenced by factors such as diet, antibiotic treatment, and lifestyle. Previously, consistent differences between pre-industrial and industrial societies were shown. In this study, 59 fecal samples were obtained from children aged two to five years living a traditional agropastoral lifestyle in the Adadle district in the Somali Regional State of Ethiopia where milk and starch-rich food are predominant components of the local diet. Stool samples were subjected to both 16S rRNA gene amplicon and shotgun metagenomic sequencing. Microbiota composition, function and the resistome were described in the feces and compared to 1'384 publicly available fecal sequencing datasets from children living in other traditional, transitional, and industrial communities with different subsistence strategies. We find that samples from Adadle are distinctly low in *Bacteroidaceae, Prevotellaceae* and *Succinivibrionaceae* but high in *Akkermansiaceae, Erysipelatoclostridiaceae Bifidobacteriaceae, Lactobacillaceae* and *Streptococcaceae.* Genes participating in pathways related to the degradation of lactose, D-galactose and simple carbohydrates are enriched in samples from Adadle compared to samples from other communities. Resistances against tetracycline, fluoroquinolone, penam and macrolide antibiotic are observed in the feces of children from Adadle. Unlike other non-industrialized communities, the samples from Adadle are low in *Prevotellaceae, Spirochaetaceae* and *Succinivibrionaceae*, some of the main bacterial representatives in the feces of children living a traditional lifestyle. The high abundance of specific taxa and the enrichment of carbohydrate degradation pathways suggest that dietary choices strongly influence the microbiota and may overshadow the industrialization gradient. Our study revealed that the fecal microbiota of agropastoral children from Adadle significantly differs from the microbiota of children living a similar traditional lifestyle, highlighting the need to further characterize the fecal bacterial composition of diverse communities to better understand microbiota dynamics and better define what is a healthy microbiome on a global scale.

EP-03/P-020 A bipartite and a tripartite glycosyltransferase system controls site-specific flagellin O-glycosylation in Shewanella oneidensis MR-1

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O-glycosylation is a post-translational modification found on flagellin, the subunit of the flagellar filament that allows bacteria to swim. While modification is generally required for proper assembly of the flagellar filament, it has also been implicated in bacterial virulence and mimicry of host innate immune responses. The glycosylation is executed in the cytosol by soluble and highly specific flagellin glycosyltransferases (fGTs). Sialic acid-like sugars (i.e. pseudaminic acid and legionaminic acid) are used by fGTs as donor sugars to decorate flagellins. Two types of fGTs are known: FlmG and motility associated factors (Mafs). The latter is widespread in flagellated bacteria, however, the determinants conferring acceptor and donor specificity are not known. To investigate this, we characterized the two Maf orthologs So3273 (Maf1) and So3259 (Maf2) encoded in the flagellar gene cluster of *Shewanella oneidensis* MR-1, a nonpathogenic Gram-negative marine bacterium. By mutagenesis and complementation analyses, we showed that both Mafs glycosylate the flagellin subunits FlaA and FlaB in MR-1 cells. In addition, glycopeptide analysis by LC-MS/MS revealed that Maf1 and Maf2 act on different serine residues. We also found that Maf1 is indispensable for flagellation, while Maf2 paradoxically interferes with motility. Moreover, interaction assays in *E. coli* revealed that Maf1 interacts with flagellins via its C-terminal (tetratricopeptide repeat, TPR) domain. We also confirmed that the TPR is sufficient for binding FlaA/B. Unlike Maf1, Maf2 does not directly interact with flagellins but instead requires a specific chaperone-like protein for successful interaction. While we confirmed the glycosyltransferase activity of Maf1 on FlaA in a heterologous host producing pseudaminic acid, the activity of Maf2 and its chaperone are currently being probed in a heterologous host.

EP-04/P-021 The GENeva PHage (GENPH) collection to understand pre-adaptation to K. pneumoniae high-risk clones

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Phage therapy is the most promising solution to the antibiotic crisis that is currently threatening modern medicine. The main obstacle for developing effective phage treatment against *Klebsiella pneumoniae* is phage resistance. The advantage of phages, in contrast to antibiotics, is that they also evolve to counteract bacterial resistance. The current standard for selecting the right phage candidates for phage therapy is the inhibition or delay of bacterial regrowth during 18 to 24h. Determine the feasibility of pre-adapting phages targeting *K. pneumoniae* from different genetic backgrounds in clinically relevant international clones. Identify the genetic basis enabling phages to delay bacterial regrowth. Selected *K. pneumoniae* strains from high-risk clones, namely A58300 (ST23;KL1), P41 (ST11;KL15), P04 (ST101;KL17), P20 (ST16;KL51), and P43 (ST258;KL107), were challenged with active phages. Coevolution of six species pairs was performed by sequential passages in liquid media for 30 days. Turbidity reduction assays (OD_{600}) were performed with ancestral phages and their evolved variants at day 10, 20, and 30. The ancestral and evolved phages (day 20) were sequenced, and genetic differences were assessed. Out of the six phages, two of them (GPH11-P04 and GPH13-P20) displayed improved activity, evidenced as a 3h delay in bacterial regrowth, after 10 passages with no further improvement. There was no evidence of possible pre-adaptation on the other four phages used. We have identified genetic changes leading to improved activity in two phages against *K. pneumoniae*. Although phage pre-adaptation is possible for *K. pneumoniae* phages, it is time-consuming and costly and does not always yield enhanced activity.

EP-05/P-022 Implementation and validation of a Monkeypox virus real-time PCR on an in-house automated clinical microbiology molecular diagnostic platform for rapid, sensitive and specific syndromic testing in response to the Monkeypox 2022 Outbreak

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Background
In response to the global surge in Monkeypox virus (MPXV) infections observed in May 2022, we promptly introduced an MPXV PCR assay in our molecular diagnostic laboratory. This proactive measure aims to meet the anticipated demand for testing and provide syndromic testing capabilities.

Methods
We focused on a previously described MPXV specific Taqman RT-PCR targeting the F3L gene, whose technical characteristics were compatible with our platform, that we optimised with positive controls corresponding to MPXV DNA from infected cell cultures and a synthetic plasmid containing the target amplicons of the RT-PCR.

Results
Using the synthetic plasmid, we determined the limit of detection of the MPXV PCR assay: 100% amplification for 10 copies per reaction and 20% amplification for 1 copy per reaction. Intra- and inter-run reproducibility met diagnostic requirements, with five independent runs showing consistent results using dilutions of plasmids containing 100 and 10 DNA copies per reaction. We established a correlation between viral load (expressed as copies per ml) and infectivity towards Vero E6 cells (measured in plaque forming units per ml). The clinical performance of the PCR was evaluated using specimens from patients suspected of MPXV infection during the 2022 international outbreak and demonstrated a sensitivity of 100% (8/8) and specificity of 100% (14/14).

Conclusions
The implementation and validation of the multiplex real-time PCR assay on our in-house automated molecular diagnostic platform offer an effective syndromic testing approach during the Monkeypox outbreak of 2022. This assay enables rapid and accurate detection of Monkeypox virus, allowing for timely identification and management of infected individuals. Moreover, its ability to simultaneously screen for other relevant pathogens associated with similar clinical presentations enhances diagnostic efficiency and aids in appropriate treatment decisions. The validated assay demonstrated its potential as a valuable tool for future outbreak responses and infectious disease surveillance.

EP-06/P-023 Comparison of disk diffusion, E-test, and broth microdilution methods for testing in-vitro activity of cefiderocol against carbapenem-resistant Acinetobacter baumannii

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Background
Cefiderocol antibiotic susceptibility testing is challenging as it requires iron-depleted conditions to induce siderophore-mediated entry. The reference method is broth microdilution (BMD) with iron-depleted-Mueller-Hinton (ID-MH) medium, while breakpoints recommended for disk diffusion (DD) are based on standard MH-agar plates. Inconsistencies of DD results were reported with the reference BMD method, especially when MIC values were distant from breakpoints. We aimed to compare the agreement of the commercial BMD tests ComASP (Liofilchem) and UMIC (Bruker), and DD and E-test using MH- and ID-MH-agar plates for *Acinetobacter baumannii*.

Methods
One hundred and one whole-genome-sequenced carbapenem-resistant *A.baumannii* clinical isolates were investigated. BMD was performed based on the EUCAST guidelines. DD and E-test were performed using two commercial MH-agar plates (bioMérieux and Liofilchem) and an in-house produced ID-MH-agar plate. ComASP and UMIC were performed according to manufacturer's guidelines. AST results were interpreted according to the CLSI clinical breakpoints.

Results
Genomic analysis revealed the presence of plasmid-borne carbapenemases in 95/101 isolates (n=81 bla_{OXA}, n=4 bla_{NDM}, n=1 $bla_{GES-14,}$ n=3 harboring a combination of two bla_{OXA} genes and n=6 of bla_{OXA}/bla_{NDM} gene) and ESBLs in 2/6 carbapenemase-negative-isolates (bla_{GES-11}). Categorical agreement (CA) of DD with the reference BMD method was higher with the ID-MH-agar plate (95.1%) than with the commercial MH-agar-plates (BioMérieux 91.2%; Liofilchem 89.4%). Fewer categorization errors were observed with the ID-MH-agar compared to the MH-agar plates. E-test on ID-MH-agar plates exhibited a significantly higher essential agreement (EA, 75.2%) with standard BMD than with the two MH-agar plates (bioMérieux 56.4%, Liofilchem 43.6%), and showed a higher performance in detecting high-level resistance than ComASP and UMIC.

Conclusions
DD and E-test on ID-MH-agar plates exhibit a higher congruence with the reference BMD method than on MH-agar plates and the commercial BMD methods. Therefore, we recommend using ID-MH-agar plates for cefiderocol susceptibility testing of *A. baumannii*.

EP-07/P-024 How plant immunity shapes the coexistence of pathogenic and commensal strains during early plant infection

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Crop pathogens significantly reduce agricultural production. Yet, pathogen ecology during the infection remains elusive. In particular, the determinants underlying the pathogen-microbiota-immunity interactions during early infection steps, which are critical to determine if disease will occur, are not well understood. To successfully establish infection, bacterial pathogens use the type III secretion system to repress plant immune defenses, which in turn can facilitate the growth of co-localizing non-pathogenic strains in plant tissues. However, which properties of the commensal strains or the plant tissue influence the co-existence between pathogenic and non-pathogenic strains is unclear. Here, we use single-cell time-lapse microscopy in microfluidic chambers using the *Xanthomonas campestris* pv. *campestris* phytopathogen and combine it with an individual-based model to explore the conditions leading to pathogen establishment or exclusion. We consider a simple case where a bacterial mutant lacking the type III secretion system co-infects the plant along with a pathogenic wild-type strain and ask how different fractions of pathogen-to-mutant cells, growth rates and plant tissue constraints, affect pathogen establishment. We also explore how phenotypic heterogeneity in the expression of the type III secretion system can allow pathogens to exclude commensals and facilitate infection establishment. While the work is still in progress, our project will help to better understand strategies of plant pathogens in their natural ecological context.

EP-08/P-025 MICROBIOTA profiling for pathogen detection in routine clinical microbiology diagnostic

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Background
The etiological diagnosis of infectious diseases requires a sensitive and reliable tool for pathogen detection. Although conventional diagnostic techniques such as broad-range eubacterial PCR followed by Sanger sequencing (bPCR) or quantitative PCR (qPCR) meet the above criteria, their ability to detect polymicrobial infections is limited. This study aims to evaluate the performance of 16S rRNA amplicon next-generation sequencing for identifying bacterial pathogens in samples from physiologically sterile sites and determine its clinical value for samples with monomicrobial or potential polymicrobial infections.

Methods
For this retrospective study, 53 clinical samples from sterile tissues were selected from a collection between 2018 and 2020. Sample inclusion criterion was a request for bPCR. Of the 45 samples that tested positive, 26 exhibited multiple peaks in Sanger sequencing, suggesting polymicrobial infections. Additionally, 8 bPCR-negative samples were positive using qPCR. To assess possible contaminations, 18 negative clinical samples and 26 no-template and positive controls were included. DNA libraries were prepared by amplifying the 16S rRNA V3-V4 region before 300bp paired-end sequencing on Illumina MiSeq. Reads were processed using zAMP, a DADA2-based bioinformatics pipeline. Biomedical evaluation of the detected species was done by an advisory panel of infectious disease specialists and clinical microbiologists based on the patient hospital record and sample type, respectively.

Results
Targeted metagenomics confirmed presence of the pathogen in diagnostic samples with a sensitivity of 94% in bPCR-positives and 63% in bPCR-negatives but qPCR-positive samples. The specificity of the assay was 95% in negative samples. Moreover, 16S assay identified several species in 92% of putative polymicrobial samples. The evaluation of experts revealed that the majority (96%) of identified taxa are plausible.

Conclusions
This study demonstrated that 16S amplicon-based metagenomics profiling is a robust tool for detecting bacterial pathogens, in particular for patients with suspected polymicrobial infections and under antibiotic therapy precluding bacterial culture.

EP-09/P-026 Comparative analysis of ribosomal and functional genes among Legionella species

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Legionella are opportunistic pathogenic bacteria responsible for legionellosis, a disease of which the incidence drastically increased in recent years. While most cases have been attributed to *L. pneumophila*, the genus contains over 60 species showing large genetic variability. As a result, a notable discrepancy exists both between clinical and environmental isolates, as well as the prevalence of species across different environments. However, currently only little evidence is available to explain the discrepancies in prevalence observed experimentally. Furthermore, even though 16S rRNA sequencing is usually believed to not provide sufficient resolution to resolve such diversity, some studies suggested its possibility within this genus in light of its high genetic diversity. For this reason, we retrieved over 4900 genomes belonging to the family Legionellaceae collected from both culture- and metagenomic-based studies targeting both environmental and clinical settings. The goals were to assess the possibility to distinguish different species based on various regions of the 16S rRNA gene, and characterize the difference of their functional genes within an ecological context. The similarity of the retrieved genomes was first assessed based on their nucleotide identity in order to identify strain-level representative genomes, reduce redundancy and computational burden. After identification of 16S rRNA genes, we evaluated the degree of its similarity both within multiple copies within the same genome and across different strains and species to check the resolution provided by different hypervariable regions. Finally, a pangenomic analysis was carried out to highlight a substantial diversity of functional genes characterizing different strains and species, helping to understand their ecological differences.

EP-10*/P-027* Steering emergence of virulence-compromised Escherichia coli with bacteriophages

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The gut can serve as a reservoir for opportunistic pathogens, such as multidrug resistant *Escherichia coli* (*E. coli*). While harmless in the gut, these strains can cause severe extraintestinal infections, including recurring urinary tract infections and life-threatening sepsis in patients at risk. Prevalent sepsis strains are protected by the polysaccharide capsule K1. This capsule type promotes resistance to the host's humoral immune response by biological mimicry and thus prevents vaccination strategies. It is therefore crucial to develop new approaches to specifically target the K1 capsule and exclude these pathogens from the gut microbiota. While the capsule is an important virulence factor, it can also be a target for bacteriophages (phages) that attach to it in order to infect and kill the bacteria. In this study, we use a cocktail of capsule-specific phages to exclude a multidrug-resistant K1 *E. coli* strain from the gut. We show that our phage cocktail exerts a strong selective pressure against encapsulated bacteria in the murine gut, hence rapidly driving the emergence and fixation of capsule-less mutants.

*Student presentation

EP-11/P-028 Tagging the natural transformation machinery of Legionella pneumophila

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The human pathogen *Legionella pneumophila* is a gram-negative bacterium, that is commonly forming biofilms in a water environment lacking a constant flow. Aspiration of contaminated water droplets may cause a severe respiratory infection called Legionnaires´ disease.
Moreover, this bacterium can undergo natural transformation (NT) - it can take up exogenous DNA and integrate it into its genome by homologous recombination. This process is one of the major driving forces for bacterial evolution, environmental adaptation, and vaccine escape, but also responsible for the spread of antibiotic resistance and pathogenicity traits.
NT is mediated by a highly complex multi-protein machinery. At first, the type IV pilus binds extracellular DNA and transports it though the outer membrane. Then, a protein channel in the inner membrane transports a singe strand of this exogenous DNA into the cytoplasm. There, a third set of proteins prevents ssDNA degradation and enable its integration into the genome via homologous recombination.
Here we show that ectopic or native expression of tagged proteins still leads to the assembly of a functional NT machinery. The presence of N-or C-terminal tags opens us many doors to further characterize the stoichiometry and protein-protein interactions of this complex multi-protein machinery. Furthermore, with our work, we hope to shed more light onto the assembly process of the NT machinery in gram-negative bacteria.

EP-12/P-029 Chlamydia vaughanii: a novel chlamydia isolated from a tropical fish

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Chlamydiaceae is a family of strict intracellular bacteria that includes important human and animal pathogens of the genus *Chlamydia* such as *Chlamydia trachomatis* or *Chlamydia psittaci.* Members of this genus were isolated from birds, reptiles and mammals but their host range seems to be restricted to vertebrates and they are unable to grow in amoebae. Following the death of *Ancistrus dolichopterus* fishes in a tropical aquarium, animals were investigated for a causative infectious agent. By inoculating McCoy cells with fish samples, we were able to cultivate an intracellular bacterium and to sequence its full genome. It was classified as a new species belonging to the *Chlamydia* genus based on nine taxonomic markers and was named *Chlamydia vaughanii* in memory of late Prof. Lloyd Vaughan.
C. vaughanii has the largest genome of the *Chlamydia* genus (1.3 kb) which appears to be the consequence of multiple duplications in genes encoding putative adhesins. Like pathogenic *Chlamydia*, it infects and multiplies in different mammalian cell lines, including humans, but not in insect cells or in amoebae.
We developed a *C. vaughanii*-specific qPCR amplifying the *mutS* gene and analysed several samples from the aquarium. *C. vaughanii* was retrieved from all dead *Ancistrus dolichopterus* fishes examined but not from any other sample. Histology suggests that these bacteria induce cysts formation in fish gills.
C. vaughanii is the first *Chlamydia* isolated from fish and cultivated in mammalian cells. This capacity of spanning the species barrier, worrisome regarding human health, might be due to the expanded adhesin genes reservoir. Further investigations are needed to clarify the pathogenic potential of this novel species, its ability to create outbreaks in fishfarms and its possible zoonotic capacity.

EP-13/P-030 Mechanisms of predatory bacterium Bdellovibrio bacteriovorus to escape the surrounding bacterial prey cell remnants

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Bdellovibrio bacteriovorus is a predatory bacterium that kills and invades Gram-negative (prey) bacteria like *Escherichia coli*. Once in the prey periplasm, the predator consumes the prey, divides and needs to exit the prey cell remnants. It has been shown that a predator lysozyme specific to lyse the deacetylated peptidoglycan of the bacterial prey cell wall enables exit of the predator from prey cell remnants (Harding et al., 2020). Further, second messenger cyclic guanosine monophosphate adenosine monophosphate (cGAMP) has been shown to be critical in controlling gliding motility at exit, as in its absence *B. bacteriovorus* remains stranded inside of the empty prey cell (Lowry et al., 2022). To understand this exit process in more detail we generated a preliminary shotgun proteomics dataset to reveal predatory proteins more abundant specifically during the prey cell exit. Among the most abundant proteins we found proteins involved in gliding as well as multiple proteases. These highly-abundant gliding proteins originate from one out of four specific gliding operons. These new insights increase our understanding of predatory mechanisms specific to the bipartite interaction of predatory and prey bacterium.

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EP-14*/P-031* Peptides released by Klebsiella pneumoniae are taken up by Streptococcus pneumoniae, causing changes in pneumococcal gene expression and phenotypes

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Background

Klebsiella pneumoniae and *Streptococcus pneumoniae* are bacteria which can colonize the human respiratory tract and can cause pneumonia. We have observed *in vitro* that in co-culture the presence of *K. pneumoniae* inhibits the growth of *S. pneumoniae*. Peptides isolated from the secretome of *K. pneumoniae*, known as AmiA and AliA peptides, bind substrate-binding proteins of the pneumococcal ABC transporter Ami-AliA/AliB oligopeptide permease and lead to suppression of pneumococcal growth. Here, we determined whether the peptides are taken up and their effects on the transcriptome.

Methods

Location of FITC-labelled peptide following incubation with *S. pneumoniae* was assessed by epifluorescence microscopy. The effect of the peptides on the transcriptome was determined by RNA sequencing.

Results

AmiA peptide localized intracellularly. The AmiA and AliA peptides caused similar changes to the pneumococcal transcriptome: downregulation of expression of genes involved in branched chain amino acid synthesis and transport, the Ami-AliA/AliB permease and protein folding. CodY, a nutritional regulator, is linked to regulation of genes of these processes and was downregulated by both the AmiA and AliA peptide. Furthermore, both peptides upregulated genes of the fatty acid biosynthetic process. Additionally, AmiA peptide downregulated genes of the tryptophan biosynthetic process and upregulated genes of purine and pyrimidine metabolism.

Conclusion

Klebsiella pneumoniae peptides are taken up and locate intracellularly in *S. pneumoniae*. Transcriptional changes affecting protein and amino acid metabolism, and the oligopeptide permease itself, can explain the phenotypic changes caused by peptide treatment including reduction of growth. We conclude that pneumococci can detect *K. pneumoniae* in the environment via binding and uptake of peptides, leading to an alteration of transcriptome and phenotypes.

*Student presentation

EP-15/P-032 SYNTHETIC Nanobody-based capture assay for rapid detection of Staphylococcus aureus infections

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Staphylococcus aureus infections and their growing resistance to antibiotics are becoming a serious problem. Current strategies to detect *Staphylococcus aureus* infections and their antibiotic susceptibility require a culturing step prior to diagnosis and characterization. This could take up to a week depending on the severity of the infection. An alternative method to efficiently enrich and rapidly detect these microbes from patient samples is urgently needed. We present a synthetic nanobody-based bacterial capture method to enrich and rapidly diagnose *S. aureus* infections. We screened for synthetic nanobodies that specifically bind to Surface Protein A (SpA). We then linked selected nanobodies to magnetic beads via biotin-streptavidin conjugation coupled with a PEG11 linker. Using these beads, we could demonstrate the capture of *S. aureus* cells from clinical samples. Our capture strategy can enrich *S. aureus* cells even at the early stages of infection where the bacterial count is low. The presented method reduces the detection time from days to hours. Importantly, the isolated bacterial cells are preserved in their most natural state facilitating characterization of the infection and antibiotic susceptibility testing. The presented strategy is a proof of concept and should passage the advent of rapid and reliable diagnosis of *S. aureus* infections.

EP-16/P-033 Evaluation of next generation sequencing for the detection of first-line drug resistance of Mycobacterium tuberculosis

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Background
Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is one of the world's deadliest infectious diseases. Knowledge of the drug susceptibility pattern of an isolate is crucial for drug selection and successful TB therapy, especially for multi-drug resistant TB. Phenotypic drug susceptibility testing (DST) is still the reference standard but takes weeks to receive results. Next generation sequencing (NGS) can provide a rapid and comprehensive overview of the genotype of *M. tuberculosis*.

Methods
For 153 clinical *M. tuberculosis* isolates, collected between 2020 and 2021 at our laboratory, phenotypic and genotypic resistance testing for first-line TB drugs was compared. Phenotypic DST was performed using the BD BACTEC MGIT 960 system (BD, Allschwil, Switzerland) for the first-line drugs isoziazid (INH), rifampicin (RMP), ethambutol (EMB) and pyrazinamide (PZA). Genotypic DST was done for all isolates by NGS using the MiSeq® Illumina System (San Diego, USA) and the TB Profiler pipeline. For a subset of the isolates conventional molecular resistance testing was done with the AID TB resistance line probe assay (Autoimmun Diagnostika GmbH, Strassberg, Germany) and/or Sanger sequencing.

Results
The sensitivity of genotypic DST by NGS compared to phenotypic DST for INH, RMP and EMB was 100%. For PZA the sensitivity was 93.33%; one isolate was predicted to be PZA susceptible by NGS whereas phenotypic DST showed a PZA resistant phenotype. Subsequent Sanger sequencing confirmed a *pncA* resistance mutation. The overall specificity of genotypic DST was high for all first-line drugs resulting between 95% to 98%.

Conclusion
Resistance detection for first-line TB drugs by NGS methods was highly reliable for Swiss TB isolates. Genotypic DST by NGS can be integrated into the routine workflow and run complementary to the phenotypic DST to provide fast results.

EP-17/P034 Genome-resolved metatranscriptomics investigates the influence of carbon source complexity on microbial activity of aerobic granular sludge

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Aerobic granular sludge (AGS), a granule-forming, microbially driven process, is an emerging alternative for wastewater treatment. These AGS microbial communities have been widely studied using laboratory sequencing batch reactors (SBR) fed with volatile fatty acids (VFAs). However, VFAs are simple and easily degradable carbon substrates, in contrast to the carbon source complexity of wastewater. Our understanding of how different carbon sources modulate microbial activity and metabolism in aerobic granular sludge is limited. In this study, a column reactor in SBR mode was started using VFAs as a carbon source. The carbon source was then complexified by adding the first complex monomeric (CM) and complex polymeric substrates (CP). The return to simple carbon sources has also been studied. Genome-resolved metatranscriptomics was used to examine the overall activity of the microbial community and to investigate how the main metabolic pathways, such as dephosphatation, denitrification, and fermentation, were affected by different carbon sources. The preliminary results from this study suggest that *Candidatus* Accumulibacter phosphatis (ACC012) and four populations belonging to Haliangiaceae (MAG 1), Kapabacteriales (MAG 81), Zoogloea (MAG 85), and Spirochaetia (MAG 24) were amongst the most active members with a mean transcript per million (TPM) of 174461, 118965, 107394, 83806, and 70127, respectively, combining all three conditions (CP, CM, and VFAs). Principal component analysis suggests that the change in carbon sources drives the activity of microorganisms in aerobic granular sludge and warrants further investigation into their metabolism.

EP-18/P-035 Laboratory diagnosis of imported pulmonary Histoplasmosis in an immunocompetent patient in a Swiss hospital

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Background
Histoplasmosis is an invasive fungal infection hyperendemic in eastern states of the United States and in Latin America. *Histoplasma capsulatum* sub. *capsulatum* (HC), agent of histoplasmosis, is a dimorphic fungus present in contaminated soil. In most cases, the primary infection of the pulmonary form is latent, asymptomatic, and self-limiting except in immunodeficient patients.
The diagnosis of histoplasmosis is challenging due to its unspecific clinical presentation, suboptimal diagnostic tests, but is improved by epidemiological medical history. We report here the microbiological challenges of pulmonary histoplasmosis diagnosis in a 20-year-old immunocompetent young man. The patient experienced retrosternal pain on the inbound flight to Switzerland after a 3-month travel along the Pacific coast of South America. On admission, a CT-scan revealed bilateral mediastino-hilar adenopathies and bilateral subpleural nodular alveolar condensation.

Method/Result
Urinary antigen detection, culture from a broncho-alveolar lavage and cytological examination of two bronchial aspirates did not reveal microorganisms, whereas histological examination of parenchymal biopsies of the lingula allowed identification of spores. Serum was sent for HC immunodiffusion, yielding a positive result. Additional specific qPCR performed on the biopsy and serum confirmed the diagnostic of histoplasmosis.
Despite the arsenal of radiological, tissue- and serum-based diagnostic tests used, only the travel history, the identification of spores and their classification using a specific qPCR led to the correct diagnosis.

Conclusion
This case report illustrates the difficulties to diagnose pulmonary histoplasmosis in an immunocompetent patient and highlights the role of a multidisciplinary approach involving clinical, pathological, and microbiological expertise. Given the increasing immigration and travels from endemic regions, physicians should be aware of histoplasmosis as it is particularly feared in immunocompromised patients but can also be encountered in patients without pre-existing health problems.

EP-19*/P-036* Divergence of the intrinsically disordered RNase E C-terminal extension and implications for RNA degradosome composition

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RNA degradation is an essential cellular process that ensures the maintenance of global RNA metabolism and regulates gene expression. In γ-proteobacteria, RNA degradation is mediated by the RNA degradosome, a protein complex that is centred around the endoribonuclease E (RNase E) and often includes a metabolic enzyme, an exoribonuclease, and an RNA helicase. In *Escherichia coli* (Ec), these are enolase, PNPase, and RhlB, respectively.
The protein-protein interactions within this complex are mediated by short linear motifs (SLiMs) present on RNase E C-terminal extension (CTE), which is intrinsically disordered. Interestingly, the low sequence conservation of RNase E CTE among closely related bacteria suggests a species-specific RNA degradosome composition. This hypothesis has been confirmed in a few other organisms, though many putative SLiMs for which a ligand has not been identified remain.
To fill these gaps, we are characterising the RNA degradosome of the versatile pathogen *Pseudomonas aeruginosa* (Pa) and studying the function of its RNase E SLiMs, which differ from Ec SLiMs. Using a combination of in vivo or in vitro protein-protein interaction assays and live fluorescence microscopy, we have conclusively identified so far PNPase and RhlB as RNA degradosome components in Pa, as is the case in Ec.
The Pa RNase E regions that we have identified as necessary to mediate these interactions are located at a different (though similar) position compared to the known Ec SLiMs, which suggests an alternative complex assembly and/or dynamic. We are currently investigating the role of other Pseudomonas-specific motifs in binding potential novel RNA degradosome components and whether there is a co-evolution of RNase E SLiMs and their protein partners.
Our findings demonstrate that RNase E interactions with PNPase and RhlB are maintained in both Ec and Pa despite SLiMs sequence divergence, suggesting a fitness benefit.

*Student presentation

EP-20/P-037 Tick-Borne Encephalitis Virus Vaccines Elicit NS1-Specific Antibody Responses in Vaccinated Individuals

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Background
Besides preventing tick bites, active immunization with inactivated, whole-virus vaccines is the most important protective measure against tick-borne encephalitis virus (TBEV) infections. Vaccine-induced protection against TBEV is mediated by antibodies to the viral envelope protein. The detection of non-structural protein 1 (NS1) specific antibodies has been suggested as a marker indicative of natural infections. However, recent work has shown that TBEV vaccines contain traces of NS1, and immunization of mice induced low amounts of NS1-specific antibodies.

Methods
healthy army members (n=898) were asked to fill in a questionnaire relating to vaccination against or infection with different flaviviruses, and blood samples were collected. All samples were screened for the presence of TBEV NS1-specific IgG antibodies using a self-developed ELISA. Antibodies were quantified as percent positivity in reference to a positive control. For qualitative evaluation, we calculated the mean OD of the lower 95% of the vaccinated individuals and set the cut-off for a borderline result at +2 standard deviations (SD) and the cut-off for a positive result at +3 SD. Statistical significance was determined using a Kruskal Wallis test (quantitative results) and a Chi square test (qualitative results).

Results
both quantitative and qualitative test results significantly differed for the different groups. Antibody titers were significantly higher in individuals having received 2, 3, or 4 or more vaccine doses than in non-vaccinated individuals. Similarly, the percentage of individuals with a positive test result was higher in individuals vaccinated against tick-borne encephalitis than in unvaccinated study participants.

Conclusion
NS1-specific antibody titers increase with the number of vaccine doses, but still remain at a relatively low level. This demonstrates the importance of establishing a clear cut-off point in detection systems that allows a clear distinction between NS1-specific antibody titers after vaccination and infection.

EP-21*/P-038* Interactions of the passenger domain of the autotransporter CfaA of Baronella with antibodies

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Bartonella is a genus of facultative intracellular pathogens, which can cause acute and chronic infections in a broad spectrum of mammals. CfaA, a predicted surface autotransporter, was previously identified as a target of two neutralizing monoclonal antibodies, LS4G2 and LS5G11, generated from mice immunized with the strain *B. taylorii* IBS296. CfaA is composed of an extracellular passenger domain at the N-terminus and a C-terminal β-barrel domain embedded in the outer membrane. Structure predictions of CfaA generated with Alphafold2 indicate that the passenger domain is composed of two pertactin-like fold domains (PLF1 and PLF2). Each PLF is built by a rigid β-helix core, with several flexible loops of varying length protruding to the outside. While the sequence of the PLF1 core is well conserved between different *Bartonella* species, the loops vary even at the subspecies level. The binding sites of the antibodies were mapped to the PLF1 loops, suggesting a mechanism of immune escape via antigenic variation. The aim of the project is to determine the structure of CfaA and the structural basis of the antigen-antibody interfaces to better understand the mechanisms of immune evasion. We were able to purify the PLF1 of *B. taylorii* IBS296 and obtain different cryo-EM maps of the domain bound to Fab fragments derived from both antibodies. Our results suggest that both antibodies recognize distinct epitopes at the N-terminus of PLF1. Additional binding studies confirm the high specificity of both antibodies, which bind only the PLF1 of the immunization strain and fail to recognize its homologs from other *Bartonella* strains. Future research will determine the precise binding of both monoclonals and immune evasion determinants of other strains. This work can improve our understanding of the immune response against bacteria and be potentially helpful for the future design of vaccines.

*Student presentation

EP-22*/P-039* Production of tailor-made bacteriophages for the treatment of MDR bacterial infections using a synthetic genomics pipeline

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Phage therapy is seen as a promising alternative to antibiotics for the treatment of multi-drug resistant (MDR) bacterial infections. So far, phage therapy strictly relies on the isolation and purification of natural lytic phages. In contrast, temperate phages have the ability to integrate their genome in bacterial genomes. Recent sequencing efforts clearly highlighted the systematic presence at least one prophage sequences integrated in the genome of many MDR bacteria. However, they cannot be used as therapeutics due to their non-lytic profiles once integrated and to the presence of undesirable cargo on their genomes (i.e. resistance or toxin-encoding genes). We aim to develop a synthetic genomics pipeline employing the yeast *Saccharomyces cerevisiae* as an engineering platform to convert functional lysogenic phages into tailor-made and safe lytic phages for phage therapy.
We first focused on two members of the ESKAPE group, namely methicillin-resistant *Staphylococcus aureus* (MRSA) and ESBL-producing *Klebsiella pneumoniae.* We recently characterized i) more than 100 *Staphylococcaceae* strains isolated from dromedary camels and dogs in Africa and ii) a subset of clinical *K. pneumoniae* strains isolated in Switzerland. A great diversity of phage sequences was detected and a detailed comparative analysis of their genomes was performed. A first set of functional prophages were induced using mitomycin C and characterized in respect to their host-range and lytic properties. Additionally, we adapted the Transformation-Associated Recombination (TAR) cloning technique to the cloning and engineering of the 42-kb genome of the prophage vB_StaphS-IVB354, isolated from a CC398 MRSA strain. Different versions of the phage genome were constructed in which the lysogeny region, responsible for prophage integration, and several virulence-encoding factors were deleted. The development of rescue protocols is on-going.
Lytic conversion of temperate phages has the potential to increase the pool of phages with therapeutic potential, which will ultimately expand the impact of phage therapy.

*Student presentation

EP-23/P-040 Clinical performances of two pan-Tropheryma PCRs for the detection of new Tropheryma species and description of a clinical case

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Background
Tropheryma whipplei is responsible for the classical Whipple's disease and other localized infections. Recently, a new *Tropheryma* species was described in an immunocompromised patient with pleuritis. We developed two broad-range pan-*Tropheryma* genus PCRs that detect both *T. whipplei* and new *Tropheryma* species. Here, we present their clinical performances and a case of probable *Tropheryma sp.* infection.

Methods
Prospectively, demands for *T. whipplei* PCR were tested with both *T. whipplei* specific PCRs and *Tropheryma* broad-range PCRs (targeting *23S rRNA* and *rnpb* genes) from January 2019 to November 2022.

Results
In total, 2605 samples were tested with the broad-range *23S rRNA* and 834 with both *23S rRNA* and the *rnpB* PCRs. Sensitivity was 78.8% and 79.7% for the *23S rRNA* and the *rnpB* PCRs, respectively. Specificity was 99.9% and 99.7% for the *23S rRNA* and the *rnpB* PCRs, respectively. We identified a patient where bronchoalveolar lavage was positive with the two broad-range PCRs with $>10^5$ copies/ml. Specific *T. whipplei* PCRs were negative. Known for panuveitis, this 49-year-old male presented with a disease recurrence and underwent a CT-scan during work-up, showing multiple mediastino-hilar necrotic adenopathies. Biopsies were aspecific. Doxycyclin (1-year), hydroxychloroquin (1-year) and co-trimoxazol (1-month) treatments led to good clinical and radiological outcome.

Conclusions
Real-world sensitivity of the specific *T. whipplei* PCR was better than the broad-range PCRs close to the limit of detection (100 to 1000 copies/ml), by targeting repeated elements. However, broad-range *Tropheryma* PCRs demonstrated excellent specificity and remains the only way to identify new cases of *Tropheryma sp.* infections.

EP-24*/P-041* Infectious pestivirus clones to study endoribonucleases as IFN antagonist

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Pestiviruses, family *Flaviviridae*, include livestock pathogens of major economic importance such as bovine viral diarrhea virus (BVDV) and classical swine fever virus (CSFV). A unique feature of pestiviruses is the expression of the N^{pro} and E^{rns} proteins acting as interferon (IFN) antagonists. While N^{pro} mediates proteasomal degradation of Interferon Regulatory Factor 3 (IRF3), E^{rns} is an envelope glycoprotein additionally playing an RNase function. E^{rns} also exists in a soluble form and cleaves immunostimulatory RNA preventing an innate immune response and, hence, production of IFN. Plasmacytoid dendritic cells (pDCs) were shown to secrete large amounts of IFN upon contact with virus-infected cells, and this IFN induction is similarly prevented by E^{rns} of pestiviruses. Various positively charged amino acids in E^{rns} are essential for IFN inhibition. However, most of the studies on E^{rns} were performed using the purified viral protein and extracellular synthetic dsRNA as IFN inducer. Given the complexity of the pestiviral replication cycle and the variety of E^{rns} functions, we will study this viral glycoprotein using infectious clones of pestiviruses carrying mutations targeting key E^{rns} regions. Clones will be produced by transformation-associated recombination (TAR) cloning in yeast. Furthermore, we will investigate the function of E^{rns} in inhibiting the cell-cell contact dependent IFN induction of pDCs stimulated by wild-type pestiviruses and mutants carrying various changes in the E^{rns} protein. These studies might well extend beyond a viral infection and shed light into the possible role of RNases in self-nonself differentiation and autoimmunity.

*Student presentation

EP-25*/P-042* A flexible high-throughput cultivation protocol to assess the response of individuals' gut microbiota to diet-, drug-, and host-related factors

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High-throughput cultivation represents a promising approach for evaluating compositional and functional responses of individuals' gut microbiota to environmental conditions. However, strict anaerobic cultivation traditionally involves time-consuming, tube-based methods (*e.g.,* Hungate technique), restricting the number of microbial communities and factors that can be tested simultaneously. Based on the NicheMap™ protocol, initially designed for Hungate tubes, we developed a simple high-throughput protocol to cultivate fecal microbiota using 96-deep well plates in an anaerobic chamber. We minimized hands-on time, consumables and working volumes with sufficient volume for analysis maintained. Further, we optimized the cultivation medium comprising separately customizable parts (*i.e.,* basal medium and supplements) and carefully characterized the cultivation system (*e.g.,* pH, anaerobicity), resulting in a protocol that is fast, flexible and reproducible.
Short-chain fatty acid quantification and 16S rRNA metabarcoding of fecal cultures yielded similar metabolic activities and community compositions in plates and Hungate tubes, confirming that the protocol is a valid alternative to work-intensive, tube-based techniques. Finally, our high-throughput protocol tested with eight fecal microbiota, two dietary fibers (resistant dextrin, soluble starch), an antibiotic (ciprofloxacin) and a non-antibiotic (5-fluorouracil) drug showed consistent responses as reported *in vivo*.
Altogether, the developed protocol enables rapid testing of different factors, including dietary components (*e.g.,* fibers, proteins, vitamins), host factors (*e.g.,* pH, oxidative stress) and drugs (*e.g.,* antibiotic and non-antibiotic medication). We propose that high-throughput cultivation is a powerful tool to evaluate relevant gut microbiota-diet-drug-host interactions, thereby facilitating research and the development of tailored interventions for individuals.

*Student presentation

EP-26/P-043 Evaluation of a new point-of-care molecular test for the detection of respiratory viruses – Results from a prospective study

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Introduction
Near-patient rapid molecular testing for respiratory viruses can lead to reduced length of stay in emergency departments, earlier de-escalation of antibiotics, earlier onset of treatment and reduced time in isolation (Baron et al., Microbiol. Spec. 2022: 10: 1-8; Kuypers J, 2019 JCM 57:e01890-18.). However, besides their ease of use and rapidness, the analytical performance of rapid molecular test is a relevant determinant for their successful implementation at the point of care.

Aim
Savanna Respiratory-Viral-Panel 4 (RVP-4; QuidelOrtho) is a true specimen-to-result PCR-test for detection of SARS-CoV-2, Influenza and RSV within 20 minutes and without need for upfront specimen preparation, thus facilitating decentralised testing. Aim of this study was to evaluate the performance of this new assay.

Materials and Methods
130 prospectively collected fresh respiratory samples in transport medium were tested in parallel with the Savanna RVP-4 and the Xpert® Xpress CoV-2/Flu/RSV plus assay (Cepheid). 54 specimens tested positive in the Xpert either for SARS-CoV-2 (N=14), Flu A (N=20), Flu B (N=4) or RSV (N=16), 176 specimens were negative and the number of comparable results was 520.

Results
Positive and negative %-Agreement of the RVP-4 was 93% [N = 50 /54 concordant positive; 95%-CI: 81.3% - 97.6%] and 100% [N = 466 / 466 concordant negative; 95%-CI: 98.9% - 100%]. 4 specimens positive in the Xpert for SARS-CoV-2 [N=2]; and Flu A [N=2]; with high Ct values of 44.1, 40.1, 38.2 and 37.7 remained negative in the RVP-4. Average Ct of the RVP-4 and the Xpert in the 50 concordant positive specimens was 27.2 [95% CI:25.3-29.1] and 23.0 [95% CI: 21.5-24.6], respectively.

Conclusion
Savanna RVP-4 is a promising new alternative for rapid molecular near patient testing for the most common respiratory viruses. Further assays should be implemented on the platform with a comparable test accuracy for a further use.

EP-27*/P-044 Single cell heterogeneity in oxidative stress response influences activation of the Integrative and Conjugative Element ICEclc in Pseudomonas

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Horizontal gene transfer (HGT) is a well-known mechanism that contributes to spreading adaptative genes within bacterial communities, leading to, for example, resistance to antimicrobial compounds or pollutant metabolism. Whereas HGT mechanisms have been widely studied, the regulatory control and the influence of environmental factors on transfer rates of mobile genetic elements are much less well understood.
Here, we focus on an Integrative and Conjugative Element named ICE*clc* found in *Pseudomonas knackmussii* B13, and the potential signals that influence ICE activation. Interestingly, ICE*clc* encodes the pathway to grow with 3-chlorobenzoate (3CBA) but is also maximally activated for transfer after growth with 3CBA, and not with the related compound benzoate. ICE*clc* activation only takes place in a subset of 'transfer competent' cells (2-5%) in stationary phase. Cells grown with 3CBA accumulate high ROS levels, although their variation is considerable.
The hypothesis we aim to test is that high oxidative stress levels activate excision and transfer of the ICE, either directly (through ICE regulatory proteins) or indirectly (through e.g., the RpoS stationary phase sigma factor). Our assumption is that cells with high ROS would activate ICE*clc* whereas those with low would not. To test this hypothesis we constructed strains with double fluorescent gene reporters, one fused to an ICE*clc* promoter for transfer competence, and the other to one of six selected oxidative stress response promoters. We quantified and correlated fluorescent gene expression at single cell level as a function of growth phase in time-lapse microscopy, and by flow cytometry, as a function of growth substrate. Our results show a tendency for ICE*clc* to activate in stationary phase cells with a higher oxidative stress response compared to the whole population, which is correlated to higher oxidative stress response experienced by their ancestors.

*Student presentation

EP-28/P-045 Niche Availability Determines Inoculant Persistence in Synthetic Soil Microbiomes

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Soil microbiomes are complex and heterogenous systems involved in many ecosystem services, including soil productivity and biogeochemical cycling. Anthropogenic disruption to some soils through poor land management and systemic pollution have altered the functional capacity of the resident microbiota with implications to environmental and human health. Various approaches have been suggested to improve soil health with bioaugmentation, the inoculation of functionally beneficial bacteria, a particularly attractive option. While extensive metagenomic studies have characterised the taxonomic structure of soils such approaches are limited in identifying active functional processes contributing to the establishment of inoculants. Both biotic and abiotic factors have been shown to influence successful bacterial invasion, but the use of poorly representative, reduced model communities makes comparing results with complex natural systems challenging. Recently, a bottom-up assembled, medium complexity soil microcosm community, SynCom, was demonstrated to share assembly characteristics with a rich, semi-natural community it was designed to represent. The use of related model communities may circumvent the limitations of previously used reduced systems by validating findings across increasing levels of system complexity. In this study we investigate the response of SynCom to a pollutant, toluene, and to invasion by a toluene degrading bioaugmentation agent, *P. veronii* 1YdBTEX2. We find that SynCom is a reproducible community that can be manipulated via the engineering of niche availability, can be invaded by the inoculant, and that toluene and inoculant exposure produce alternate, reproducible community compositions. Our results demonstrate that niche availability is a driving factor in inoculant persistence and that SynCom provides a useful model soil microbiome platform with which to investigate soil health questions.

EP-29/P-046

To each his own – targeted proteomics as a tool to unveil distinct physiological states of Staphylococcus aureus and Pseudomonas aeruginosa in human tissues

Dr. Minia Antelo [1], **Dr. Richard Kühl** [2], **Dr. Benedict Morin** [3], **Dr. Mario Morgenstern** [2], **Dr. Martin Clauss** [2], **Prof. Nina Khanna** [3], **Prof. Dirk Bumann** [1]

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Staphylococcus aureus and *Pseudomonas aeruginosa* cause a wide variety of infections that are associated with substantial morbidity and mortality. Antibiotic therapy frequently fails to treat infections caused by these pathogens, even if the causative strains show full susceptibility in standard *in-vitro* antimicrobial susceptibility tests. These discrepancies indicate that the laboratory assay conditions fail to recapitulate crucial aspects of pathogens physiology in tissue microenvironments. To elucidate some aspects of pathogen in-vivo physiology, we analyzed biopsies and lung secretions of human patients using mass-spectrometry (MS). Standard MS methods are only capable of detecting the most abundant proteins. To gain detailed insights in virulence factor expression in human samples, we established a targeted approach with attomole sensitivity covering 83 and 172 different *S. aureus* and *P. aeruginosa* proteins, respectively. For *S. aureus*, we have identified three abundant *S. aureus* proteins with constant abundance across various conditions, and diverging usage of various virulence mechanisms across patients. For *P. aeruginosa* we verify a consistent high expression of two structural envelope proteins. Our results reveal high variability of *S. aureus* proteomes across patients and *S. aureus* strains in deep-seated abscesses and lung secretions. Toxins, other virulence factors and metabolism differ between infection sites. Some proteins show large discrepancies between patient samples and Mueller-Hinton broth. For *Pseudomonas*, the emerging patterns reveal commonalities and differences in metabolism, membrane transport, virulence, and motility. These results pave the way for systematic optimization of *in-vitro* conditions capable of mimicking the physiology of these two pathogens *in-vivo* and more predictive susceptibility testing.

POSTER PRESENTATIONS
P-047 – P-149

P-047 Mechanisms of resistance to azithromycin in Neisseria gonorrhoeae

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Introduction
Bacterial sexually-transmitted infections (STIs) pose a major public health problem. The emergence of antibiotic-resistant strains of *Neisseria gonorrhoeae* represents a serious threat to successful treatment and control of infection spread. The first extensively drug-resistant (XDR) strains (ceftriaxone-resistant and high-level azithromycin-resistant [HLR AZY]) have been reported. While some mechanisms of azithromycin resistance are well understood, others remain speculative.

Aims
To identify mutations implicated in azithromycin resistance in consecutive strains isolated from patients over a two-year period.

Material and Methods
From January 2021 to December 2022, 34 isolates (one per patient) were recovered from samples analyzed at the CHUV in Lausanne. Eight genes involved in azithromycin resistance were sequenced: *mtrR* repressor (mtrCDE operon repressor) and his promotor *mtrR*-pr, *rplD* gene (L4 ribosomal protein), *rplV* gene (L22 ribosomal protein) and the four alleles of the *rrl* gene (23S rRNA).

Results
With a cutoff value of 1 mg/L, 15 isolates were considered as resistant, whereas the remaining 19 were susceptible to azithromycin. The C2599T mutation in 3 or 4 of the rrl allele confer a HLR to azithromycin (MIC = 16 mg/L, N=2)). The following mutations were significantly associated with CMI values >1 mg/L: the three mutations V125A, A147G, R157Q in the rplD gene (N=10) and a substitution A->C in the mtrR promotor (N=9). Specific mutations in the mtrR repressor and its promotor were observed in both susceptible and resistant isolates.

Conclusions
Resistance to azithromycin of our isolates was explained by presence of mutations in many different copies of 23S RNA ribosomal genes, in the *rplD* gene, in the *mtrR* repressor and by substitution in the promotor of the *mtrR* repressor. Other mutations, previously reported to be associated with azithromycin resistance, were documented in both susceptible and resistant isolates, suggesting they play little role, if any in azithromycin resistance.

P-048 How much should we sequence? An analysis of the Swiss SARS-CoV-2 genomic surveillance effort

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Background
During the SARS-CoV-2 pandemic, many countries directed substantial resources towards genomic surveillance systems to track and detect both established and emerging viral variants. There is debate over how much sequencing effort is useful in national surveillance programs for SARS-CoV-2 and future pandemic threats. We aimed to investigate how important surveillance outcomes would have been affected by a reduced sequencing effort.

Methods
We analysed all genomic data available on the Swiss Pathogen Surveillance Platform (SPSP) from February 2020 until August 2022. We employed a downsampling strategy to investigate the effects of fewer available sequences on various surveillance outcomes, such as (i) first detection of variants of concern (VOCs), (ii) speed of introduction of VOCs, and (iii) first cluster detection of VOCs.

Results
A total of 143,260 SARS-CoV-2 sequences were available for a population of 8,7 million inhabitants, averaging 9.7% of all PCR-positive cases throughout the pandemic.
We find that the impact of downsampling on the detection of VOCs is dependent on individual lineage dynamics, but that most dynamics including introduction and cluster detection could be recapitulated even with a reduced sequencing effort. For example, with 50,000 sequences (1/3 of the data) there was no substantial increased delay in the detection of the VOCs. However, the effect on the observed speed of introduction is particularly sensitive to reduced sequencing effort. A stronger effort in sequencing was most accurate in capturing the respective growth of the emerging VOCs.

Conclusions
A genomic surveillance program needs to strike a balance between societal benefits and costs. While the overall national dynamics of the pandemic could be recapitulated by a reduced sequencing effort, the effect is strongly lineage dependent – something that is unknown at the time of sequencing – and comes at the cost of accuracy, in particular for tracking the emergence of potential VOCs.

P-049* The landscape and molecular underpinnings of mycobacterial drug interactions

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Combination therapies have great potential to enhance antibacterial efficacy, but their clinical benefits and performance in multi-drug trials have often been disappointing. *Mycobacterium abscessus*, a highly resistant pathogen responsible for increasing respiratory infections worldwide, exemplifies this challenge, as extensive and prolonged multi-drug regimens mostly fail. In this study, we sought to systematically assess M. abscessus drug combinations to uncover general principles and underlying mechanisms of drug interactions.

We assessed 50 drugs with antibacterial activity in *M. abscessus* individually, in every possible pairwise combination (checkerboards), and in combination with 2582 inactive compounds encompassing antibiotics, food additives, and human-targeting drugs. All interactions were experimentally tested in quadruplicate and analysed using optical densities. Synergistic and antagonistic interactions were common (antagonistic > synergistic) and frequently shared within antibiotic classes. Via clustering compounds in interaction space, we identified distinct drug characteristics driving synergistic and antagonistic effects. To reveal the genetic basis of drug interactions, we tested 28 clinically relevant pairwise drug combinations (diagonal checkerboard approach) on 271 clinical isolates from different patients with M. abscessus lung disease. While specific interactions were conserved across all clinical isolates, suggesting that drugs mainly define these interactions, other combinations had heterogeneous effects. By mapping these diverse interaction phenotypes to phylogenetic clades, as assessed with whole genome sequencing, we identified genomic clusters of synergistic and antagonistic effects, indicating that drug interactions are also driven by the bacterial genetic background.

Through our study, we characterised over 300'000 dose-specific drug combinations, providing an unprecedented glimpse into the complex landscape of drug interactions across an extensive array of drugs and genetic backgrounds. By shedding light on the chemical and genetic factors influencing antibacterial drug interactions, we confront not only current treatment limitations but also uncover novel targets and concepts to advance antibacterial approaches.

*Student presentation

P-050* In vitro activity of newly developed ß-lactam/ß-lactamase inhibitors against multidrug-resistant Gram-negative clinical isolates showing reduced susceptibility to cefiderocol

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Objectives

Although cefiderocol (FDC) is considered as a last-line treatment, particularly in metallo-ß-lactamase (MBL) producers, a wide range of FDC resistance mechanism have been described in Gram negative bacteria, and may therefore lead to therapeutic issues. However, the development of newly ß-lactam/ß-lactamase inhibitors (BL/BLI) combinations including aztreonam-avibactam (AZA), imipenem-relebactam (I/R), cefepime-zidebactam (FEP-ZID), cefepime-taniborbactam (FEP-TAN) and meropenem-nacubactam (MEM-NAC) have showed effective activity against MBL-producing isolates. This study aimed to evaluate the *in-vitro* activity of these combos against multi-drug resistant Gram-negative isolates showing reduced susceptibility to FDC, recovered from the Swiss National Reference Center for Emerging Antibiotic Resistance (NARA).

Methods

MIC values of aztreonam (ATM), AZA, cefepime (FEP), FEP-TAN, FEP-ZID, imipenem (IMP), I/R, meropenem (MEM), MEM-NAC were determined by broth microdilution method for 68, 8 and 11 clinical Enterobacterales, *P. aeruginosa* or *A. baumannii* isolates, respectively, showing reduced susceptibility to FDC (MIC value ≥1 mg/L) due to diverse resistance mechanisms (mainly MBL producers, still under investigation). MIC breakpoints of ATM, FEP, IMP and MEM in the respective species, according to EUCAST, were used to define susceptibility to the corresponding BL/BLI combinations. The concentration of all ß-lactamases inhibitors was fixed at 4 mg/l.

Results

The susceptibility rates to ATM, FEP, IMP and MEM were 7%, 3%, 24%, and 29%, respectively, among Enterobacterales. These rates were much higher for FEP-ZID (90%), MEM-NAC (81%) and AZA (79%) in comparison with FEP-TAN (34%) and I/R (37%). Among *P. aeruginosa* isolates, the best susceptibility rate was observed with I/R (67%). All *A. baumannii* isolates were resistant to these BL/BLI combos.

Conclusion

Those results showed that FEP-ZID, MEM-NAC and AZA have a significant *in-vitro* activity against multidrug-resistant Enterobacterales clinical isolates showing reduced susceptibility to FDC. By contrast, I/R was the best option among *P. aeruginosa* isolates, and worryingly, no novel BL/BLI combinations were effective against *A. baumannii* isolates.

*Student presentation

P-051* MOLECULAR characterization of shower hoses biofilms reveals genetic variability of Legionella spp. and specific associations with microbiome members

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Legionella are natural inhabitants of building plumbing biofilms, where interactions with other microorganisms influence their survival, proliferation, and death. Here, we investigated the associations of *Legionella* with prokaryotic and eukaryotic microbiomes in biofilm samples extracted from 85 shower hoses of a residential building. *Legionella* spp. relative abundance in the biofilms ranged between 0 - 7.8%, of which only 0 - 0.46% was *L. pneumophila*. Our data suggests that some microbiome members were associated with high (e.g., Chthonomonas, Vrihiamoeba) or low (e.g., Aquabacterium, Vannella) *Legionella* relative abundance. The data analysis also revealed high genetic variability in the 16S rRNA sequences assigned to the genus *Legionella* (30 unique Amplicon Sequence Variances detected). The correlations of the different *Legionella* variants with microbiome members showed distinct patterns, suggesting separate ecological niches occupied by different *Legionella* species. This study provides insights into the ecology of *Legionella* with respect to: 1) the colonization of a high number of real shower hoses biofilm samples; 2) the ecological meaning of associations between *Legionella* and co-occurring prokaryotic/eukaryotic organisms; 3) the presence of multiple species of *Legionella* in building plumbing systems, and the potential of 16S rRNA based detection of *Legionella* diversity in the environment.

*Student presentation

P-052* Reprogramming bacteriophage host range and enhancing antibacterial efficacy through structure-guided receptor binding protein modulations

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In light of the escalating antimicrobial resistance crisis and the scarcity of newly discovered antibiotics, there has been a rekindled interest in developing bacterial viruses (bacteriophages) as an alternative or adjunctive therapy to treat bacterial infections. While phage therapy has a longstanding history in Eastern Europe, and has demonstrated success in numerous compassionate care cases, it is still largely restrained from routine clinical practice. This is in part due to the limited spectrum of natural phages and the rapid development of phage resistance within clinically relevant timeframes. Here, employing comparative sequence analysis and *in silico* structural modelling, we identified specificity determining regions within the receptor binding protein (RBP) of a therapeutic *E. coli* phage (E2) and developed diverse engineering platforms to rationally reprogram its host range. By transiently grafting compatible RBPs sourced from phages targeting different Enterobacterales species, we successfully generated E2 derivatives capable of cross-genus transduction. Furthermore, through genetic modifications of the endogenous RBP using structure-guided chimeric design or site-directed mutagenesis, we engineered E2 variants with altered and/or expanded host ranges. Notably, using uropathogenic *E. coli* strains as infection targets, we demonstrated that a cocktail of E2 variants with shared tropism but differing adsorption mechanisms effectively suppressed resistance development, enabling more efficient and sustained control of bacterial growth. Leveraging standardized viral chassis with tunable host specificities, these innovative platforms offer promising opportunities for the development of next-generation phage-based therapeutics, serving as payload delivery vehicles as well as versatile antibacterial agents.

*Student presentation

P-053* Deimmunization of peptidoglycan hydrolases for therapeutic treatment of systemic S. aureus infections

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Staphylococcus aureus is an opportunistic pathogen colonizing roughly 30% of the human population, causing a wide range of diseases. Due to the emergence of resistant strains, novel antimicrobials are of high interest. Phage-derived peptidoglycan hydrolases (PGH) could be used as a treatment against drug-resistant bacterial strains. Fast lysis, high specificity and activity against drug-resistant bacteria are just a few advantages for the use of PGHs as protein therapeutics. A major drawback of protein therapeutics is the immunogenicity of foreign proteins. T cells play a key upstream role in the activation of the adaptive immune system and therefore, the immune reaction against protein therapeutics. Antigen presenting cells sample the environment and proteolytically process proteins, which are then presented as T cell epitopes on MHCII on the cell surface. This leads to the activation of CD4 T cells inducing the activation and differentiation of other T and B cells. Therefore, deimmunization approaches usually focus on T cell epitope prediction and deletion. This project applies computational tools to predict T cell epitopes and calculate epitope deleting mutations with low impact on protein activity and structure. Deimmunized variants undergo *in vitro* activity testing, *ex vivo* immunogenicity assays and *in vivo* immunogenicity and efficacy studies in humanized mice.

*Student presentation

P-054* Antibiotic susceptibility of critical priority pathogens infecting an upper airway lung model

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The antibiotic resistance crisis is becoming an increasingly serious threat to the global health. For decades, the market entry of new classes of antibiotics stagnates and more bacterial strains develop resistances, even against last resort antibiotics. We aim to support the development of new antibiotics, by developing *in vivo*-like models that mimic human infections, enabling antibiotic efficacy assessment in more physiological conditions. We have developed an air-interface lung model from healthy human bronchial epithelial cells cultured on Transwells. Using these *in vitro* systems, we have established standard infection conditions and have gained insight into the distinct infection kinetics of *P. aeruginosa*, *K. pneumonia* and *A. baumannii* using CFU assessment and confocal microscopy in live and fixed samples. We have assessed the pharmacokinetics for selected marketed compounds and determined their minimal inhibitory and bactericidal concentrations for the three species in broth and on tissue. We observed a tendency of reduced antibiotic susceptibility in the *in vivo*-like environment of the tissue models as compared to experiments in broth. Next, will assess the exact killing kinetics in broth and on tissue and we will compare these parameters with existing kinetic data from mice and humans. With these data, we aim to establish the Transwell lung model as a novel tool to assess pharmacokinetics and pharmacodynamics of antibiotics in an *in vivo*-like setting.

*Student presentation

P-055* ImmunoPhage: Targeting Recurrent Urinary Tract Infections with Genetically Engineered Immunomodulatory Bacteriophages

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Urinary tract infections (UTIs) are one of the leading causes for antibiotic prescription in primary healthcare. Recurrent UTIs affect up to 50% of female UTI patients, and current understanding suggests inadequate immune response to bladder pathogens is a contributing factor. Antibiotics are often ineffective at controlling recurrent infections as pathogenic communities can maintain themselves within the gut or intracellularly within the bladder epithelium ready to cause a relapse once treatment is terminated.
ImmunoPhage is a novel therapy that exploits the inherent bacteriolytic properties of bacteriophages (phages) to produce a unique antimicrobial and immunomodulatory combination therapy. To equip phages with immunomodulatory properties, we introduce heterologous genes, such as ones encoding for cytokines, into bacteriophage genomes. Upon ImmunoPhage infection of a bacterial host in a patient, cytokines are expressed and released alongside bacterial lysis. So far, we have engineered phages from divergent families with an array of cytokines and have shown that clinical isolates of uropathogenic *E. coli* infected by ImmunoPhage release biologically active payloads within the nanomolar range. To validate the ImmunoPhages, we are using bladder-on-a-chip and *in vivo* mouse UTI models.
The unique approach of ImmunoPhage aims at providing a lasting solution for patients suffering from recurrent UTIs and thus carries the potential to significantly reduce the burden of antibiotic use.

*Student presentation

P-056* Amino acid uptake rate determines community structure between auxotrophic E. coli strains

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The microbial world is highly structured. Spatial patterns are produced as members of microbial communities grow and interact. Using a synthetic community of engineered *E. coli* strains, we aim to understand the effect of individual interactions on pattern formation. We use *E. coli* strains passively exchanging amino acids. We follow the spatial organization and abundance of each member of the community on solid media with timelapse confocal microscopy and flow cytometry. With amino acids present, our members are competing and separate in space. However, without amino acids, the members rely on each other for growth and arrange in a mixed pattern. We observed that one member can form thicker regions isolated from its partner, i.e., the interaction range from its partner is larger. We demonstrate experimentally that the interaction range can be changed by increasing the uptake rate of the amino acids exchanged. Indeed, by overexpressing a specific proline importer, we successfully changed the spatial pattern of the community, balancing the region thickness and increasing the mixing of the community. Our methods and results lay foundations for understanding how metabolic exchanges influence microbial community structures.

*Student presentation

P-057* Deciphering Mycoplasma mycoides subsp. capri-interactions with ruminant immune cells

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Mycoplasmas are minute cell wall less bacteria that cause a variety of diseases in humans and livestock. Mycoplasma vaccines, if available at all, have low efficacy and induce often only short-term protection. The interplay between livestock mycoplasmas and host cells is not fully understood but the development of rationale vaccines would benefit from a better understanding. *Mycoplasma mycoides* subsp. *capri* GM12 (*Mmc*-GM12) is a highly virulent strain, causing severe septicemia in goats. Using synthetic genomics techniques, we engineered a spectrum of *Mmc*-GM12 mutant strains lacking one or several genes encoding candidate virulence traits and tested the virulence of these mutants *in vivo*. Considering the 3R principles, in this study we developed an *ex vivo* platform employing ruminant primary blood cells, multi-parameter flow cytometry and a multiplex immunoassay to characterize host-pathogen interactions. Fresh bovine or caprine blood cells were stimulated with capsule-deficient *Mmc*-GM12 (Δ*glf*) and a fully attenuated mutant strain lacking 68 genes (Δ*68*). Incubation with the Δ*glf* mutant exposing surface lipoproteins affected the viability of blood cells, while stimulation with the *Δ68* strain did not show significant differences when compared to *Mmc*-GM12. Moreover, the Δ*glf* mutant strongly suppressed the expression of MHC-II on antigen-presenting cells, indicating a potential immunosuppressive effect. However, at the same time, blood cells stimulated with this strain secreted a variety of pro-inflammatory cytokines and chemokines that could promote a robust T-cell mediated inflammatory response. In conclusion, the capacity of engineered *Mmc* strains to differentially modulate host immune system is a useful tool to systematically characterize candidate virulence traits and the immune responses they affect, which is likely to promote the development of more effective vaccines.

P-058* Identifying anti-Phytopthora infestans metabolites produced by a collection of Actinomycetes isolated from Sudan soil

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Actinomycetes are well-known for their ability to produce diverse specialized metabolites with various bioactivities. Despite decades of investigation, recent studies continue to unveil new bioactive compounds and shed light on novel modes of action of previously described ones. In a previous work, we examined a collection of 175 Actinomycetes strains isolated from different habitats in Sudan for their inhibitory activity against *P. infestans*, the causal agent of late blight disease in potato and tomato. Most strains exhibited significant inhibition of *P. infestans* mycelial growth in *in vitro* dual assays, with growth inhibition percentages ranging from 6% to 98%. Moreover, co-inoculation with certain strains resulted in noticeable morphological alterations in *P. infestans*. We further emphasized the protective potential of these strains and of their culture extracts in leaf disc experiments, along with evaluating the performance of select strains in whole plant infection assays. Considering the diverse inhibitory effects and observed morphological alterations induced by the strains, we aimed to identify the active metabolites responsible for these effects through a comparative metabolomic study of our Actinomycetes collection. For this purpose, we selected 63 closely or distantly related strains that displayed varying degrees of antagonistic activity against *P. infestans* and extracted their metabolites using ethyl acetate. Liquid chromatography coupled with mass spectrometry analysis and spectral network analysis were performed on the extracts. Subsequently, the metabolomes of the 63 extracts were analysed to identify metabolites associated with *P. infestans* inhibitory activity or altered morphologies. The aim of this study is to elucidate the chemical nature of compounds responsible for the observed anti-*Phytophthora infestans* activity both in terms of *in vitro* mycelial growth and of *in planta* disease spread inhibition. The identification of these compounds could potentially lead to the discovery of new bioderived molecules with significance for sustainable crop protection.

*Student presentation

P-059* Relative inhibitory activities of taniborbactam, a novel class B ß-lactamase inhibitor, against metallo-ß-lactamases

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Objectives
Metallo-ß-lactamases (MBLs) are the most problematic carbapenemases, conferring resistance to all ß-lactams except monobactams, and not inhibited by all clinically-available ß-lactamase inhibitors (BLI). By contrast to other newly-developed BLI, taniborbactam (TAN) possesses the ability to inhibit MBL hydrolytic activity. However, we recently demonstrated that the activity of TAN can be significantly affected by a single amino acid substitution either in the NDM-1 (NDM-9) or the VIM-2 natural sequence at position 163. Here the relative inhibitory activity of TAN against a wide range of acquired MBLs.

Methods
Genes encoding a series of subclass B1 MBLs (VIM-1, VIM-2, VIM-5, VIM-6, VIM-19, VIM-34, VIM-42, VIM-53, NDM-1, NDM-5,NDM-7, NDM-9, NDM-19, NDM-30, NDM-47, IMP-1, IMP-2, IMP-13, DIM-1, GIM-1, SPM-1) or B3 (AIM-1) were amplified by PCR and corresponding amplicons cloned into plasmid pUCp24, and then transformed in *Escherichia coli*. Susceptibility testing was performed for cefepime (FEP), ceftazidime (CAZ) and their combinations with TAN (FEP-TAN and CAZ-TAN) at a fixed concentration of 4 mg/L. Fifty percent inhibitory concentrations (IC_{50}) of TAN were also determined for all MBLs.

Results
When considering TAN-based combinations, MICs values were reduced by more than 4-fold dilution for all MBLs except for IMP-like, NDM-9, NDM-30, VIM-83, SIM-1 and AIM-1 compared to the MIC of the corresponding ß-lactam alone. In line with these results, IC_{50} of TAN were found to be higher than 15 mM for these latter enzymes, in comparison with values at 1 mM or less for the others MBLs.

Conclusion
This study further highlights the in-vitro inhibitory activity of TAN against a wide range of MBLs. Nevertheless, apart from its inefficacy against IMP-like enzymes previously reported, we showed here that some specific variants of MBLs such as NDM-9, NDM-30, NDM-19, and VIM-83, or some specific MBLs such as SIM-1 or AIM-1 are not inactivated by this novel inhibitor.

*Student presentation

P-060* Wide dissemination of Gram-negative bacteria producing the taniborbactam-resistant NDM-9 variant; a One-Health concern

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Objectives
Metallo-ß-lactamases (MBL) of the NDM group are the most disseminated acquired carbapenemases worldwide. These enzymes hydrolyze all β-lactams (BL) except monobactams, and are not inactivated by the recently-developed ß-lactamases inhibitors (BLI), except taniborbactam (TAN), supposed to be marked soon with cefepime (FEP). However, a previous study showed that NDM-9, differing from NDM-1 by a single amino acid substitution (E152K), was resistant to TAN in a recombinant *Escherichia coli*. Here we report the *in-vitro* activity of FEP-TAN and other newly-developed BL/BLI combinations against NDM-9 producers.

Methods
A collection of 8 isolates carrying bla_{NDM-9} in *Escherichia coli, Klebsiella pneumoniae, Klebsiella variicola,* and *Acinetobacter baumannii*, recovered from different origins and countries, were included. Susceptibility testing for FEP, aztreonam (ATM), Aztreonam-avibactam (AZA), ceftazidime (CAZ), ceftazidime-avibactam (CZA), imipenem (IPM), imipenem-relebactam (I/R), meropenem (MEM), meropenem-vaborbactam (MVB), meropenem-nacubactam (MEM-NAC), cefepime-zidebactam (FEP-ZID), FEP-TAN, and cefiderocol (FDC), were performed. All BLI were used at fixed concentration of 4 mg/L, except vaborbactam (8 mg/L). Results were interpretated using EUCAST guidelines, or according to the breakpoints of ATM, FEP, or MEM for the corresponding BL/BLI.

Results
All isolates showed high resistance to ATM, CAZ, CZA, FEP, IMP, and I/R, and a reduced susceptibility to MEM and MVB. All NDM-9 producers displayed high MICs values for FEP-TAN (≥ 128 mg/L). Interestingly, MICs of FEP-ZID and MEM-NAC showed were very low for *E. coli* strains (≤0.125 mg/L), variable (from 0.5 to 8 mg/L) in *Klebsiella* spp. and high in *A. baumannii* (>32 mg/L). AZA and FDC remained highly effective against *Enterobacterales*strains.

Conclusion
This study highlights the in-vitro ineffectiveness of FEP-TAN against NDM-9-producing isolates, regardless the species of concern. Worryingly, the potential of the NDM-9 encoding gene to successfully spread among many different species and many different environments is also well reported here, as a good example of a One-Health critical issue.

*Student presentation

P-061* Hunting for probiotics against Legionella in building plumbing systems: potential candidates and natural compounds

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Legionella is a genus of bacteria comprising more than 60 species, half of which are known as human opportunistic pathogens, causative agents of Legionnaires' disease. *Legionella* are ubiquitous in freshwater and are often found in building plumbing systems, where they inhabit biofilms and increase the risk of infection for humans. Conventional control of *Legionella* include water treatments with high temperature boiler settings and disinfectants (e.g. chlorine), but they are sometimes not effective. Here, we explore the inhibitory potential of 10 selected waterborne bacteria towards 10 pathogenic *Legionella* species. As a first result, spot-on-lawn agar assays showed differential inhibition patterns of the *Legionella* species (e.g., only two antagonists were able to inhibit all the *Legionella* species), suggesting different attack/defense mechanisms involved in the specific interactions. Genome mining of the antagonists revealed a range of interesting biosynthetic gene clusters encoding for natural products that could be involved in the inhibition, including pathways for lipodepsipeptides and siderophores. We further co-cultured the *Legionella* species and the antagonists and confirmed the biological activity of the extracellular compounds towards *Legionella*. Preliminary LC-MS data detected the presence of the lipodepsipeptide viscosin suggesting its potential role in the inhibition of *Legionella*. Further experiments are currently in progress in order to isolate and test the biological activity and potential of viscosin and other natural products. Similarly, the activity of the live antagonistic strains is being tested in microcosm assays where *Legionella* can grow in biofilms on plastic material, thus resembling more natural conditions. These strategies combined offer the opportunity to explore the potential of alternative strategies to control *Legionella* in plumbing systems.

*Student presentation

P-062* antifungal effects of water-filtered infrared a (wira) in skin fungi

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Fungal skin infections affect up to 25% of the general population worldwide. The skin commensal yeast *Malassezia* are associated with common skin disorders such as dandruff, pityriasis versicolor, seborrheic dermatitis and atopic dermatitis, which all lead to a loss in the patient's quality of life. Antifungal drugs have a beneficial effect in many cases, but disease recurrence post-treatment and resistance to the limited number of effective antifungals has been described, which calls for novel effective therapeutic options.

Water-filtered infrared A (wIRA) irradiation is a promising new therapy against skin fungal infections. The high penetration properties of the heat field qualify wIRA irradiation as a localized, non-invasive and contact-free therapy against infections in superficial tissues. Importantly, the antimicrobial and wound healing properties of wIRA have already proven effective and safe in a clinical setting.

In this project, the effect of wIRA irradiation on *Malassezia furfur* was assessed in host-free conditions and in presence of keratinocytes and dendritic cells. Different conditions of irradiation and different readouts for fungal viability and function were tested.

The metabolic activity of *M. furfur* was systemically decreased in response to wIRA under host-free conditions. When combining wIRA-irradiated *M. furfur* with non-irradiated dendritic cells, we observed a decrease in fungal load, suggesting that uptake and/or degradation of *M. furfur* by immune cells might be enhanced by wIRA irradiation. Likewise, the simultaneous irradiation of keratinocytes and *M. furfur* resulted in reduced fungal growth, while *M. furfur*-induced cytokine production was not affected.

These results indicate that wIRA irradiation may be an applicable therapy in skin disorders associated with *M. furfur*. However, further optimization of the conditions in preclinical settings and a description of the mechanism of action is required for its application in a clinical setting.

*Student presentation

P-063*

Uncovering the phosphorus redox biogeochemical cycle: A high-throughput phosphite sensor for investigating phosphorus reduction by Nitrosopumilus maritimus and Streptomyces viridochromogenes

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While current evidence supports that biological phosphorus (P) reduction plays a significant role in the marine phosphorus cycle, direct observation of phosphate (+5 oxidation state) reduction to phosphite (+3 oxidation state) has never been made in pure culture. Phosphite also serves as an important P source for certain bacterial species by way of the *ptxD* gene coding for phosphite dehydrogenase (PTDH), notably by *Prochlorococcus* in low-P oligotrophic ocean waters. To further interrogate the role of phosphite in the phosphorus redox biogeochemical cycle, we have developed an enzymatic, fluorescence-based phosphite assay method that has been optimized for sensitivity, processing time, and robust performance on a variety of complex biological and environmental samples. This sensor has been applied to the investigation of marine archaeon *Nitrosopumilus maritimus* and *Streptomyces viridochromogenes*. Here, we investigate the effect of P-deplete and P-replete conditions on phosphite production in concentrated *N. maritimus* cultures and culture supernatant, as well as phosphite production from phosphonate precursors in *S. viridochromogenes*. This work will serve not only to facilitate high-throughput phosphite monitoring in ocean sampling, but to launch our investigation into the thermodynamically-challenging biochemistry involved in phosphite production.

*Student presentation

P-064*

Bioinformatics in phage therapy: from phage collections characterization to final products quality assessment

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The PHAGO-CHUV project aims to propose phage therapy to patients in a therapeutic dead-end due to persistent infection by multi-drug resistant bacteria. Here, we explored how bioinformatics can help establishing a well-characterized phage collection and assessing the quality and safety of the final products that will be administered to patients. An academic collection of 200 lytic phages is currently used to select bactericidal phages specific to patient strains for clinical use. To assess their composition, phages were sequenced and assembled *de novo*. Clusters of similar phages were defined based on alignment coverage and nucleotide identity before refinement of SNP distances and determination of population heterogeneity. The presence of virulence factors (VFs) and prophages was inferred among phage replicons and within the bacterial production strain ATCC 15442. A quality assessment pipeline reporting phage purity, genetic distance to the expected phage and the presence of prophages and bacterial DNA contaminants was built and applied to three final products. Among the first 58 samples, 68 *Pseudomonas aeruginosa* lytic phages were identified. Fourty-eight and 10 samples contained respectively, one and two replicons. 78% replicons presented a unique nucleotide sequence, while 22% were redundant. The presence of phage subpopulations was suggested by fragmented assemblies in 11 replicons. No replicon carried a VF gene. Ultimately, all final products reported an identical match to the expected phage and on average only 0.18% of reads were of bacterial origin. Despite four intact prophages and four VF genes were detected in the chromosome of the production strain, none was identified in the contaminant bacterial DNA. The genomic analyses supported the cleaning of the academic collection towards a pure and non-redundant phage collection. The bioinformatics pipeline allowed us to validate the quality of the product and suggested potential improvements by selecting/engineering production strains devoid of VFs and prophages.

*Student presentation

P-065* Dynamic behaviour of the type IV secretion system subunits encoded by the integrative conjugative element ICEclc in Pseudomonas

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Conjugation is one of the three major mechanisms driving horizontal gene transfer in bacteria. Although plasmid conjugation has been widely studied, transfer of Integrative and Conjugative Elements (ICE) is much less understood. We study here the conjugative system of ICE*clc,* a mobile element discovered in *Pseudomonas knackmussii* B13. ICE*clc* conjugation is atypical, because only appears in a small proportion of so-called transfer competent (tc) cells in stationary phase (3-5%). In tc cells the ICE excises from its chromosomal location, temporarily replicates to several copies, which can be transferred to one or multiple recipient cells. DNA transfer requires a multisubunit protein machinery known as the type IV secretion system (T4SS), which spans both inner and outer membranes of the donor cell. The ICE*clc* T4SS is phylogenetically distant from plasmid conjugative systems and is representative for a wide family of ICEs found in β- and γ-proteobacteria. The goal of this study was to understand whether donor cells can actively 'scan' for new recipients or 'stochastically' build T4SS complexes around the cell and be able to transfer in any direction. For this we constructed translational fusions between T4SS subunits and fluorescent proteins, and monitored their localization in live cells using super-resolution time-lapse microscopy. In addition, we investigated T4SS dynamics in live donors alone or in presence of recipients. Our results show that components of the presumed T4SS inner and outer membrane channel only partly colocalize in tc cells and that outer membrane components are less dynamic compared to those in the inner membrane (on a minutes–to–hour time scale). This suggests that T4SS complexes occur as incomplete units in the outer and inner membrane, and perhaps form the full complex only upon specific triggers. Ongoing preliminary experiments indicate that contact to recipient cells can lead to stable fluorescent foci, suggestive for active transferring complexes.

*Student presentation

P-066 Disseminated gonococcal infection: A case report

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Neisseria gonorrhoeae is one of the most common agent of sexually transmitted infection (STI). Most clinical presentations are uncomplicated, however, serious complications such as disseminated gonococcal infection can occur.
A 59-year-old man presented to the emergency department with a highly febrile state with diffuse arthromyalgias predominant on the left ankle. The patient recently travelled to Spain and Italy where he reported several unprotected sexual relationships with multiple partners.
Dermatological examination highlighted several petechial lesions on arms, legs and the back. Neither meningism nor confusional state were observed. The laboratory revealed an important inflammatory syndrome (CRP 160 mg/dl). Due to the high suspicion of disseminated *Neisseria* infection, empirical treatment with ceftriaxone was started. Blood cultures grew gram-negative diplococci, identified as *Neisseria gonorrhoeae*. Gonococcal sepsis with articular and cutaneous involvement was diagnosed and the patient was treated with ceftriaxone for 7 days. The clinical evolution 10 days after leaving the hospital was favourable. However, one month after, the patient came back with a recurrent fever and arthralgias on the right ankle, elbow and knee. Petechial lesions also recurred on lower extremities. Transthoracic echocardiographic examination showed a suspicion of aortic valve vegetation, later non-confirmed using transoesophageal echography. Right knee punction came positive for *N. gonorroheae* polymerase chain reaction (PCR) and the diagnosis of recurrent gonococcemia with possible gonococcal endocarditis was retained. The patient was given ceftriaxone for 6 weeks and was free of recurrence 2 monthes later.
Severe disease after genital gonorrhoeae is rare. However, in front of a typical petechial rash and arthritis in a context of unprotected sexual relationships, disseminated gonococcemia should also be considered and empirically treated. Although gonococcal endocarditis is exceptional, it should also be investigated when gonococcemia is diagnosed. Rapid diagnostic and antibiotic susceptibility testing are essential for appropriate therapy.

P-067 Effect of zinc oxide and copper on antibiotic resistance plasmid transfer in Escherichia coli

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The spread of antimicrobial resistance is highly accredited to the excessive usage of antibiotics. However, molecules such as heavy metals zinc (Zn) and copper (Cu) have been also suggested to promote antibiotic resistance dissemination. Our aim was to investigate whether sub-dosage of Zn and Cu, metals widely used in veterinary and agricultural settings, could enhance plasmid transfer, and subsequently resistance genes dissemination. Plasmid conjugation frequencies (PCF) were determined with *Escherichia coli* strains carrying the IncL (bla_{OXA-48} gene), IncA/C (bla_{CMY-2}), IncI1 ($bla_{CTX-M-1}$), IncF ($bla_{CTX-M-1}$), and IncX3 (bla_{NDM-5}), representing various plasmid scaffolds and clinically relevant broad-spectrum β-lactamase genes. Mating-out assays were performed in presence or absence of sub-dosages of zinc oxide (ZnO) and Cu. Quantification of the SOS-response associated genes expression levels (qRT-PCR), and of the production of reactive oxygen species (ROS) (fluorescence detection) were determined to evaluate the metals as bacterial stress inducers, contributing to resistance dissemination. Increased PCF was observed for IncL (13.8-), IncA/C (9.9-), and IncX3 (18.4-fold) when treated with ZnO. Increase in PCF was observed only for IncL when treated with Cu (17-fold). The ROS production presented an overall positive correlation with PCF increase after treatment with ZnO for IncL (22.8-), IncA/C (59.3-), and IncX3 (47.7-fold). For Cu treatment, same correlation was observed only for IncL (1.3-fold). No increase was observed for expression of SOS-response associated genes for all strains under Cu treatment, and under ZnO treatment, an increase in both genes was observed only for IncX3 (recA, 38.1-; sfiA, 45.9-fold). Our data showed that sub-dosages of ZnO and Cu, could significantly enhance PCF in *E. coli*, with a more marked effect observed with plasmids possessing IncL, IncA/C, and IncX3 scaffolds. Our study suggested that use of certain heavy metals is not the panacea for avoiding use of antibiotics in order to prevent the dissemination of antibiotic resistance.

P-068 Attach and conquer: studying the molecular biology of phages to overcome antibiotic-resilient bacteria

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The discovery and study of bacteriophages, the viruses of bacteria, has greatly contributed to our understanding of life and molecular biology. Due to the rise of antimicrobial resistance, phages are being increasingly used as an alternative treatment option. However, their unreliable performance *in vivo* despite their high *in vitro* potency has hindered their adoption as a reliable treatment option.
Similarly, survival of drug-sensitive bacteria to antibiotic treatment has been linked to persistence of non-growing, dormant cells inside patients and evolution of antibiotic resistance. In this study we report the isolation of phage Ercole, a small podovirus capable to effectively lyse dense cultures of *Pseudomonas aeruginosa* PAK harbouring high numbers of antibiotic-tolerant bacteria. Interestingly, this phenotype relies on the same stress responses associated with antibiotic tolerance, as previously observed for another phage studied in our lab.
Moreover, we show that phage Ercole is the first known *P. aeruginosa* phage that utilizes the tip of the type 4 pili (T4P), surface appendages with important roles in virulence and ecology of this bacterium, as receptor to initiate infection in an allele specific manner. Furthermore, phage isolation experiments performed with allelic variants of pili components demonstrated that more, rare phages with a specificity towards distinct pili alleles exist.
Our findings provide valuable insights into the molecular arms race of phages with their hosts and illustrate how phage predation is a driver of T4P diversification and therefore also evolution. Our results might inform future efforts in developing phage cocktails for therapy, such as by choosing phage variants with broad specificity that overcome the allelic variation of T4P. In conclusion, we anticipate that the continued study of Ercole and similar phages holds promise for inspiring the development of new antibacterial treatments.

P-069 Synergistic activity of KL51-depolymerase and GENPH collection phages against ST16 Klebsiella pneumoniae

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Introduction
Phage therapy has been gaining interest as a promising alternative therapeutic option for emerging *Klebsiella pneumoniae* clones that are both multidrug-resistant and hypervirulent, such as ST16. A major hurdle in against *K. pneumoniae* is its large capsule diversity that requires careful investigation in each major lineage. The phage PWKp9B active against ST16 presented a characteristic depolymerization halo surrounding its lysis plaques, signing for the presence of a depolymerase able to degrade the pathogen's thick polysaccharide capsule.

Objective
Identify and purify PWKp9B anti-KL51 depolymerase and determine if it could synergize with phages from the GENeva PHage (GENPH) collection.

Methodology
The depolymerase was identified by similarity searches using a combination of BLAST analysis, an in-house depolymerase database, and HHpred tools. The candidate gene was cloned in pET24a with a C-terminus-His-tag, expressed in *Escherichia coli* and purified on nickel-affinity column. The depolymerase anti-capsule activity was confirmed by spot assay on double layered agar, Percoll density gradient and fluorescence exclusion microscopy. Synergy with phages was assessed by liquid-infection growth curves.

Results
We successfully identified and purified the depolymerase encoded in the genome of PWKp9B. The recombinant depolymerase showed highly effective capsule removal activity on ST16 and ST231 isolates, both harboring a KL51 capsule. A strong synergy was observed between the depolymerase and phages able to infect only the non-encapsulated bacterial subpopulation.

Conclusion
The capsule in *K. pneumoniae* plays key roles in virulence as well as in phage recognition and interaction. Depolymerases targeting clinically relevant capsules are an attractive tool to improve anti-*Klebsiella* phage activity.

P-070 Cardiovascular consequences of COVID-19: SARS-CoV-2 interaction with the human endothelium

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Although the main target of SARS-CoV-2 infection is the pulmonary organ, damage to the cardiovascular system, especially in severe cases, is frequently reported. Moreover, SARS-CoV-2 infection also manifested in persistent symptoms beyond the acute illness, a condition referred to as long-COVID or post-acute sequelae of COVID-19. Therefore, elucidating the extent and mechanisms underlying SARS-CoV-2 pathogenesis in the vasculature is needed to understand the connection between COVID-19 and cardiovascular disease. Here we characterized the interaction of SARS-CoV-2 with the human endothelium using a biologically relevant *in vitro* model that resembles the constant exposure of human endothelial cells (hECs) to physiological blood flow and shear stress using microfluidic technology. We observed that hECs were not susceptible to SARS-CoV-2 infection, either under static- or physiological flow conditions. However, exposure of hECs to the spike protein of SARS-CoV-2 could trigger cell activation, as shown by the increase in the expression of cell-surface molecules and shedding of the endothelial glycocalyx, indicating the vascular inflammatory effect of SARS-CoV-2. We also used the microfluidic EC culture to investigate whether transient exposure to SARS-CoV-2 leads to long-term dysregulation of the inflammatory state of the endothelium. We observed that transient treatment of hECs with SARS-CoV-2 spike protein led to the expression of pro-inflammatory responses, with the expression of ICAM1 persisting for at least 72h after treatment. We are currently performing transcriptomic change analysis as well as functional analysis to characterize the changes in complement activation, coagulation, and inflammatory states to further investigate the impact of acute and post-acute exposure of SARS-CoV-2 to hECs. Taken together, our data contribute to understanding the pathogenesis and the range of acute and post-acute impact of SARS-CoV-2 on the vasculature, as well as the possible mechanisms underlying the cardiovascular manifestation of COVID-19.

P-071 Enzymatic approach to improve sonication-culture-guided diagnosis of implant-associated infections – An in vitro study

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Background
The diagnosis of implant-associated infections is challenging due to microbial biofilm formation on the implant. Sonication of explanted devices improves the microbiological diagnosis, due to physical biofilm detachment. However, sensitivity is low and time-to-positivity is delayed. Therefore, we asked whether combination of sonication with biofilm-matrix degrading enzymes could improve recovery of bacteria from sonication culture.

Methods
We investigated the effect of an enzymatic mixture (EM) in combination with sonication for recovery of bacterial colony forming units (CFUs) from infected prosthetic materials (polyethylene, titanium alloys, cobalt-chrome-molybdenum and bone cement) *in vitro*. Biofilms from clinical PJI isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* (all 6 days), or *Cutibacterium acnes* (8 days) were grown on 1 cm diameter discs. Next, we treated materials with a control saline solution, EM (1 mg/ml, 15 min) or sonication (1 min) alone, or EM followed by sonication. The amount of recovered CFUs from the biofilms, as well as the proportion of so-called non-stable small colonies (nsSCs) for *S. aureus* were determined.

Results
All tested treatment groups were superior to the control solution for CFU recovery from all implant materials for *S. aureus* (2.5-15-fold) and for all further tested bacteria on polyethylene (1.2-120-fold increased recovery). EM in combination with sonication led to increased CFUs recovery as compared to EM (1.6-4-fold) or sonication (1.3-3-fold) alone for all tested pathogens and materials, the strongest effect for polyethylene and bone cement. EM alone and in combination with sonication led to significantly lower proportions of nsSC of *S. aureus* as compared to sonication alone (2-3-fold less).

Conclusion
EM might enhance the diagnostic value of sonication culture, potentially leading to increased sensitivity and decreased time-to-positivity for IAI diagnosis. Further clinical studies will be required to evaluate an advantage of EM alone or in combination with sonication in microbiological laboratories.

P-072 Enterococcus faecium PBP5 mutations: a tradeoff between ampicillin resistance and thermal stability

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Background
Antimicrobial resistance is a serious threat to modern medicine. In Gram-positive bacteria, beta-lactam resistance is mainly driven by the production of low affinity penicillin binding protein (PBPs) belonging to the structural class B1. *Enterococcus faecium* expressed PBP5, a class B1 PBP, that has acquired additional mutations leading to ampicillin resistance.

Methods
PBP5 from ampicillin sensitive (Com15) and resistant (TX0334) strains were cloned in pET24a, recombinant proteins were expressed in *E. coli* and purified. Competition experiments using bocillin-FL, followed by SDS-PAGE and image analysis (ImageJ, NIH) were performed to calculate the ampicillin half maximal inhibitory concentration (IC_{50}). Chimeric recombinant proteins were created by overlap PCR to investigate the role of specific amino-acid mutations. Protein stability was investigated by differential scanning fluorimetry using Sypro Orange (Sigma).

Results
The PBP5-TX0334 ampicillin resistant variant presented 17 non-synonymous mutations and one serine insertion in position 466 compared to the WT Com15-PBP5. Ampicillin IC_{50} were confirmed to be 20.4-fold higher in PBP5-TX0334 compared to PBP5-Com15. Differential scanning fluorimetry revealed that the protein stability of the resistant variant was dramatically decreased compared with WT (Δtm -13.5°C). Analysis of chimeric recombinant proteins showed that the insSer466, in the loop gating the active site, played a key role in ampicillin resistance but strongly impaired protein stability. Insertion of Ser 466 in Com15-PBP5 sequence increased ampicillin IC_{50} by 7.9-fold and reduced stability (Δtm=-4.5°C); deletion of Ser466 in TX0334-PBP5 reduced ampicillin IC_{50} and increased protein stability. Other mutations were also shown to be important for ampicillin resistance and protein instability.

Conclusion
Acquisition of mutations reducing ampicillin affinity in PBP5 is associated with a high cost in terms of protein stability. The unique biochemical properties displayed by the ampicillin resistant PBP5 variant could be exploited to restore beta-lactam activity in *Enterococcus faecium*.

P-073 Investigating Human and Staphylococcus aureus interactions using dual RNA-Seq and CRISPRi-Seq approaches

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Deep-seated *Staphylococcus aureus* (SA) infections cause major morbidity and mortality. Despite appropriate antibiotic therapy guided by susceptibility testing, treatment failure rates remain high, ranging from 20% to 50%. Novel and more effective therapies are thus urgently needed. While SA is usually not considered an intracellular pathogen, recent studies have demonstrated its ability to survive within macrophages, potentially contributing to persistent infections and treatment failures.
In this study, we performed dual RNA-Seq analysis on fresh biopsies obtained directly from patients undergoing surgery for SA deep-seated infections. By comparing healthy and infected tissues from the same patient, we identified up-regulation of genes involved in response to bacteria, humoral immune response and phagocytosis specifically at the site of infection. Moreover, analysis of bacterial transcripts within the infected site indicated downregulation of metabolic and virulence genes supporting the hypothesis of dormant intracellular SA. Additionally, we performed dual RNA-Seq in an *in vitro* infection model mimicking human infection using the THP-1 monocyte cell line and a clinical SA strain isolated from a deep-seated infection. Data suggested that monocytes upregulate genes involved in monocyte activation and inflammatory response while SA upregulates genes encoding factors inhibiting host's innate immune response.
To investigate the intracellular lifestyle of SA, we performed a CRISPRi-Seq screen
to assess the set of genes essential for its survival within human monocytes/macrophages. Image stream data obtained on our *in vitro* infection model demonstrated that up to 20% of the cells contained intracellular SA. Analysis of the CRISPRi-Seq data suggested potential targets for intracellular survival including genes involved in fibronectin binding, pH adaptation and virulence. Overall, our findings provide new insights into the intracellular behavior of SA during human infection and offer important information about both human and SA factors at the site of infection in biopsy samples.

P-074 How reliable is serology after the omicron SARS-CoV-2 variant wave?

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Purpose
Assess the validity of selected tests for SARS-CoV2 serology on patients infected by Omicron variant.

Introduction
Surveillance of serologic tests on the market is less stringent than genomic tests Companies rapidly developed serology at the beginning of Covid-19 pandemia. We decided to survey assays with sera from patients infected with the highly mutated Omicron in Switzerland.

Material and Method
Sera were collected from non-vaccinated healthcare workers weekly checked for SARS-CoV-2 infection and having a positive PCR in saliva[CA1] . We have no serological status before infection. Blood sampling was required at day 14 and 28. Sera were analysed on Cobas (Roche) and Alinity (Abbott) for S-antigen and N-antigen, on VirCLIA (Vircell) for quantitative S and combined S-N, finally on Seraspot (Seramun) for a dot-blot on 4 antigens.

Results and Discussion
In January and February 2022, 23 sera were collected within 12 to 40 days post positive PCR from 13 workers fulfilling the inclusion criteria of the study. First sera (S1) were collected at an average of 16.5 days (n=11 sera), and second sera (S2) were collected at an average of 32.9 days (n=10 sera). Rate of positivity of S1 sera for S-protein was 17% for Roche, Abbott, Vircell and higher for N-protein from 58% to 70% for Seraspot Vircell and Roche. Rate of positivity of S2 sera was higher for S-protein with Roche (64%) but still low for Abbot (20%) and Vircell (10%) whereas N-protein gave enhanced rate of positivity for Vircell (91%) than Roche (82%) or Seraspot (70%). Serology response showed a different kinetics than previous SARS-CoV-2 variants as most patients were not positive 2 to even 4 weeks after positive PCRs,

Conclusion
Serology assays are not adapted to the highly mutated SARS-CoV2 Omicron variant. We show that post-marketing surveillance is a useful mandatory CE-IVDR requirement.

P-075 Fecal microbiota transplantation (FMT), a lyophilized formulation

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Faecal microbiota transplant (FMT) oral capsules are the standard of care for individuals recurrently infected with *Clostridioides difficile*. While oral capsules are much easier to administer than the previous methods of endoscopic or naso-duodenal tubes, some individuals, particularly the elderly, may have difficulty swallowing 30 capsules. A novel formulation encapsulating FMT into 2mm size alginate particles may be an alternative solution. We collected 12 faecal donations from 4 healthy FMT donors and performed 16S amplicon sequencing to compare the composition of the alginate particles to the standard of care capsules and evaluated the stability of the alginate formulation. No difference in Chao1 index, Shannon index, or Bray-Curtis distance was observed, however, there was significant difference in Jaccard distance. Looking at the hierarchical relationship, samples clustered into their respective donor groups and next to the capsule produced from the same visit, suggesting the formulation did not significantly alter the profile. Two *Ruminococcaceae* species were identified to be differentially reduced in the capsules compared to the particles. Over an average of 5 months, no change in alpha or beta diversity was observed. However, several differentially abundant taxas were identified. The composition of the alginate particles was comparable to the capsules and the stability of the formulation was maintained. These particles can be mixed into food and beverages for easier ingestion. We will like to continue to evaluate the stability of this formulation until 24 months after production and their stability at room temperature.

P-076 IgA immunoassay as a potential tool for the diagnosis of syphilis

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Objective
Serological tests for syphilis detect mainly total Ig, IgM or IgG antibodies. We aimed to standardize and evaluate a specific IgA immunoassay (EIA) for the diagnosis of syphilis in patients with different clinical stages.

Material and Method
A new IgA EIA was developed using commercially available microplates coated with recombinant treponemal antigens and an anti-IgA-conjugate from DIA.PRO (Italy). To define a cut-off, we used 91 syphilis positive and 136 negative sera previously defined by the rapid plasma reagin (RPR) and the *Treponema pallidum* particle agglutination (TPPA) results. Then we determined the intra- and inter-assay precisions, diagnostic sensitivity according to clinical stage with 66, 55 and 42 sera from primary, secondary and latent syphilis patients, respectively. We defined specificity with 211 sera from donors, and patients with other infection or false positive syphilis reactivity. We further tested 71 sera from patients previously treated for syphilis.

Results and Discussion
The newly developed IgA-EIA showed a good discrimination between negative and positive samples with intra- and inter-assay variation coefficients <20%. The sensitivity was 80.30%, 100.00% and 95.24% in primary, secondary and latent syphilis, respectively, and the specificity was 98.10%. Further, IgA values were negative in 61.3% (38/62) of patients with previously treated syphilis.

Conclusion
Our findings suggest the *Treponema pallidum* IgA-EIA is a sensitive and specific tool for the diagnosis of syphilis. Further evaluations in prospective longitudinal field studies are necessary to assess its role in diagnostic.

P-077 Fever-like temperature impairs the bovine ex vivo response to Mycoplasmopsis bovis

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Although cattle are the mammalian species with most planetary biomass associated with a huge impact on our planet, their immune system remains poorly understood. Notably, the bovine immune system has peculiarities such as an overrepresentation of γδ T cells that requires particular attention, specifically in an infectious context. In line of 3R principles, we developed an *ex vivo* platform to dissect host-pathogen interactions. The experimental design was based on two independent readouts: firstly, a novel 12-14 color multiparameter flow cytometry assay measuring maturation (modulation of cell surface marker expression) and activation (intracellular cytokine detection) of monocytes, conventional and plasmacytoid dendritic cells, natural killer cells, γδ T cells, B and T cells; secondly, a multiplex immunoassay monitoring bovine chemokine and cytokine secretion levels. The experiments were conducted on primary bovine blood cells exposed to *Mycoplasmopsis bovis* (*M. bovis*), a major bovine respiratory pathogen. Besides reaffirming the tight cooperation of the different primary blood cells, we also identified new key players such as strong IFN-γ secreting NK cells, whose role was so far largely neglected. Additionally, we investigated the influence of the fever-like temperature. Strikingly, high fever temperature attenuated the capacity of most immune cell subsets to respond to *M. bovis*. Our experimental approach, phenotypically delineating the bovine immune system provided us with a deep insight of the bovine immune response towards *M. bovis* as well as new insights into the influence of high fever towards that immune response. A better understanding of host-pathogen interactions will foster the development of rationale vaccines.

P-078 Evaluation of the RESIST ACINETO multiplex immunochromatographic assay for detection of OXA-23-like, OXA-40/58-like and NDM carbapenemases in Acinetobacter baumannii

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Background
Acinetobacter baumannii is a major cause of hospital-acquired infections. Due to its ability to rapidly accumulate antimicrobial resistance traits, multidrug-resistant isolates have been reported worldwide. Carbapenemase-resistant *A. baumannii* (CRAB) are a concern, as only few options, including colistin, tigecycline/eravacycline, and cefiderocol, are currently available. Rapid diagnostics is crucial to guide best antibiotic treatment and to prevent the dissemination of CRAB. In this study, we evaluated the performance of "RESIST ACINETO" lateral flow immunoassay (BioConcept, Belgium) for the detection of the most prevalent acquired carbapenemases in *A. baumannii.*

Methods
Hundred-thirty-two *A. baumannii* clinical isolates were characterized using whole-genome-sequencing and acquired carbapenemases were identified, respectively. "RESIST ACINETO" was performed on overnight cultures according to the manufacturer's instructions.

Results
The strain collection comprised 25 carbapenemase-negative and 107 carbapenemase-producing isolates. Ninety-two isolates produced an OXA-type carbapenemase (n=72 OXA-23, n=18 OXA-72, and n=3 OXA-58), while three showed a combination of two oxacillinases (n=2 OXA-23/OXA-58 and n=1 OXA-23/OXA-72). Four isolates produced NDM-1, one NDM-2, while the remaining carbapenemase-producers harbored a combination of NDM-1 and an oxacillinase (n=2 NDM-1/OXA-23, n=3 NDM-1/OXA-72, and n=1 NDM-1/OXA-58) The isolates represented 34 different STs, with ST-2 being the most prevalent (47/132, 36%). "RESIST ACINETO" LFIA correctly identified all the six different carbapenemase variants in all the strains, including those producing two carbapenemases (sensitivity 100%). OXA-72 was correctly detected as OXA-40 variant. No false positive result, also possibly due to cross-reactivity with one of the 24 detected different intrinsic oxacillinases, was observed (specificity 100%).

Conclusions
"RESIST ACINETO" shows a high and reliable performance for the detection of most prevalent carbapenemases in *A. baumannii*.

P-079 Antibiotic susceptibility testing of bacilli Gram negative by Micronaut®, Vitek®, and gradient strip method

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Introduction
Infections of multi-drug resistant bacilli gram negative bacteria pose a serious public health concern with increasing incidence. Precise and reliable antibiotic sensibility testing is crucial for a successful treatment. To respond to this situation in our lab, we addressed the *in vitro* antibiotic susceptibility determined by MICRONAUT® system and compared with gradient strip method (GSM) or VITEK®-2. A total of 36 strains were tested, including *Enterobacterales*, *Pseudomonas aeruginosa*, and *Acinetobacter baumanii*. We tested colistin (CT), Fosfomycin (FOS), Ceftolozane-tazobactam (CTZ), Ceftazidime-avibactam (CZA), Imipenem (IPM), Meropenem (MEM), and Aztreonam (AZT).

Objectives
The goal of this study is to determine the antibiotic sensibility by MICRONAUT® system and compare it with GSM and Vitek®-2 for testing multi-drug resistant *Enterobacterales*, *P. aeruginosa* and *A. baumanii*.

Methods
Our selected strains (n=36) included 65.7% (23/36) of *Enterobacterales*, 25.7% (9/36) of *P. aeruginosa*, and 8.6% (3/36) of *A. baumanii*. The selected strains were 57.1% AZT/R, 34% CT/R, 48.6% CTZ/R, 34.4% CZA/R, 13.5% FOS/R, 43.2 IPM/R, and 35.1% MEM/R as tested by GSM or Vitek-2 AST, and of which 25% were ESBL and 72.2% harbored a carbapenemase. We tested all the strains with MICRONAUT® system and compared the results with those obtained by GSM or Vitek®-2. We considered as categorical agreement (CA) those results that differed no more than 1 $\log_2$ between results and those with identical interpretation by EUCAST criteria. In case of major disagreements between results (Major errors or very major errors), we compared our results with those provided by the National Reference Centre of Antibiotic Resistance (NARA).

Results
For those analysis with specific EUCAST criteria available (n=231), our results showed 89.6% of CA, 0.4% (1/231) of VME, and 1.7% (4/231) of ME. For those results lacking specific EUCAST criteria (n=21), 4 analyses differed more than 1 $\log_2$ between results.

P-080 Infection due to Mycobacterium riyadhense mimicking tuberculosis: first report in Switzerland

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INTRODUCTION
Mycobacterium riyadhense is a new slow-growing non-tuberculous mycobacterium (NTM) pathogen. It was originally isolated in 2009 in Riyadh and most of the few reported cases in literature were from Saudi-Arabia. *M. riyadhense* is capable of causing a range of respiratory and non-respiratory infections, in immunocompromised and immunocompetent patients, clinically indistinguishable from tuberculosis.

CASE PRESENTATION
A 60-year-old man presented with history of productive cough for one year and a chest X-ray very suggestive for tuberculosis with caverns. The patient lived in Riyadh. According to the clinical presentation, he was admitted and isolated as a case of tuberculosis (TB) and a standard anti-TB treatment was started. A sputum was performed, Ziehl-Neelsen direct examination was strongly positive for acid-fast bacilli but surprisingly the MTB GeneXpert was negative. A 16S rRNA gene pan-mycobacterial PCR was performed directly from the specimen and sequencing provided the identification at the species level, i.e. *M. riyadhense,* 48-hours later. After 6 days, the MGIT culture turned positive, the identification by MALDI-TOF MS and by 16S rDNA sequencing confirmed *M. riyadhense*. The standard TB regimen was continued.

CONCLUSION
To our knowledge this was the first case of *M. riyadhense* in Switzerland. In this case, direct examination was the trigger signal to rapidly test for NTM as the MTB GeneXpert was negative. Due to this accurate diagnosis at the species level, treatment was not changed as *M. riyadhense* is described to well respond to the standard TB regimen so far, whereas relapse of treatment were reported after NTM treatment regimen. Rapid proper distinction of TB and NTM to the species level is of the utmost importance, because this has profound consequences for patient management and treatment.

P-081

Population-based phenotypic inference of molecular resistance mechanisms to provide a coherent genotype/phenotype categorization system for quinolone resistance in Escherichia coli

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Background

Quinolone resistance in *Escherichia coli* occurs mainly due to mutations in the quinolone resistance determining regions of *gyrA* and *parC*, which encode the drugs' primary targets. In addition, mutational alterations affecting drug permeability or efflux and plasmid-based resistance mechanisms can contribute to resistance. A single mutation in *gyrA* results in low-level resistance towards fluoroquinolones, while clinical high-level resistance is associated with two mutations in *gyrA* plus one mutation in *parC*. Both low- and high-level resistance can be detected phenotypically using nalidixic acid and fluoroquinolones, such as ciprofloxacin. The aim of this study was to develop a decision tree based on disc diffusion data and to define epidemiological cut-offs to infer resistance mechanisms and to predict clinical resistance in *E. coli*.

Results

Phenotypic susceptibility of 553 clinical *E. coli* isolates towards nalidixic acid, ciprofloxacin, norfloxacin and levofloxacin was determined by disc diffusion and the genomes were sequenced. Based on epidemiological cut-offs we developed a QUInolone Resistance Inference Algorithm (QUIRMIA) to deduce underlying resistance mechanisms responsible for observed phenotypes, resulting in the categorization as "susceptible" (wildtype), "low-level resistance" (non-wildtype) and "high-level resistance" (non-wildtype). Congruence of phenotypes and whole genome sequencing (WGS)-derived genotypes was then assigned using QUIRMIA- and EUCAST-based AST interpretation. Inference of resistance mechanisms by QUIRMIA was highly congruent with WGS data (542/553, 98%). In contrast, EUCAST-based classification with its binary classification into "susceptible" and "resistant" isolates failed to recognize and properly categorize low-level resistant isolates.

Conclusions

QUIRMIA provides a coherent genotype/phenotype categorization and may be integrated in the EUCAST expert rule set, thereby enabling reliable detection of low-level resistant isolates, which may help to better predict outcome and to prevent the emergence of clinical resistance.

P-082

Tailored liposomal nanotraps for the treatment of bacterial infections

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In view of the alarming increase in severe infections caused by antibiotic-resistant pathogens, the development of novel therapeutic approaches is crucial. Bactericidal treatment increases the likelihood of positive selection for drug-resistant mutants; hence, the benefit of agents that do not directly target the pathogen. Virulence factors, such as exotoxins, play an important role in the fitness of pathogens; yet, they are not essential for bacterial survival. Secreted exotoxins kill or modify the behaviour of host cells allowing bacteria to erode epithelial barriers and to evade host immune responses by damaging the plasma membrane of host cells.
We engineered mixtures of empty liposomes composed exclusively of naturally occurring lipids, which were tailored to serve as nanotraps for bacterial membrane-damaging toxins. We showed that liposomal nanotraps sequester the released toxins of various Gram-positive pathogens (*Streptococcus pneumoniae, Staphylococcus aureus*) and efficiently protect mice from fatal septicaemia and pneumonia. This compound (CAL02, Combioxin, Geneva) proved safe and successful in a human phase I/II clinical trial that showed a faster resolution of severe pneumonia caused by *S. pneumonia* when used as an add-on therapy to antibiotics.
Here we show that in addition to neutralizing the exotoxins of *Streptococcus pneumoniae,* liposomal nanotraps can be further tailored to achieve complete neutralization of the whole palette of membrane-damaging toxins that are secreted by *Streptococcus* species such as *Streptococcus pyogenes* (group A streptococcus) and *Streptococcus dysgalactiae* (group G streptococcus).
Since sequestration of bacterial exotoxins provides the host with an increased chance of survival and a reduced rate of clinical complications, and moreover, non-bactericidal liposomal therapy drastically decreases the likelihood of eliciting drug resistance, anti-infective liposomal nanotraps with a wide target range are potential game-changers in the fight against deadly antibiotic-resistant infections.

P-083 Comparison of the SmartGene Centroid database and NCBI Genbank for fungal species determination in clinical settings

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Sequencing specific parts of the genome is one of the key approaches in medical microbiology for accurate identification of fungal species. Matching a DNA sequence to a fungal taxon relies on two essential aspects: 1) reference databases made up of quality-controlled entries and up-to-date nomenclature; 2) algorithms that calculate a statistical comparison between a queried DNA sequence and the database. SmartGene's IDNS® solution incorporates these features, using a patented centroid approach for species identification. At present, the CHUV molecular diagnostics laboratory is targeting 2 regions of the fungal genome: a panfungal PCR, generating a small 240 bp sequence in the 18S rDNA gene which is used to detect fungi directly from clinical samples, and a 600 bp sequence in the D1-D2 region of 28S rDNA gene, used essentially for identification of isolated strains. Sequences are submitted to NCBI GenBank database for identification.
In this project, we compared the ability of the SmartGene Centroid database to identify accurately fungal species versus the current routine use of GenBank.
To do this, we analysed fungal sequences obtained between January 2021 and November 2022 at the CHUV, i.e. 253 and 89 for 18S and 28S rDNA genes respectively.
We showed a better accuracy when using the SmartGene solution. Based on 18S, identification at the species level was achieved in 6.1% versus 9.7% using respectively NCBI Blast and the centroid approach, and in 42.7% versus 58% for 28S rDNA PCR. The same trend was observed for identification at the genus level.
We then focused on the discrimination of cryptic species of *Aspergillus* using the 28S rDNA PCR, this discrimination being often of clinical importance as some cryptic species have intrinsic resistance to antifungals.
Our results suggest the use of 28S rDNA sequences and the centroid database to improve fungal species identification in clinical settings.

P-084 High occurrence of Enterococcus faecalis, Enterococcus faecium, and Vagococcus lutrae harbouring oxazolidinone resistance genes in raw meat-based diets for companion animals

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Introduction
Enterococci harbouring genes encoding resistance to florfenicol and the oxazolidinone antimicrobial linezolid have emerged among food-producing animals and meat thereof, but few studies have analysed their occurrence in raw meat-based diets (RMBDs) for pets.

Aim
Our aim was to examine to what extent RMBDs may represent a source of bacteria harbouring oxazolidinone resistance genes.

Methods
Fifty-nine samples of different types of RMBDs from ten different suppliers were screened for florfenicol resistant Gram-positive bacteria using a selective culture medium. Isolates were phenotypically and genotypically characterised.

Results
Twenty-seven *Enterococcus faecalis*, *Enterococcus faecium*, and *Vagococcus lutrae* were found in 24 of the 59 samples. The *optrA*, *poxtA*, and *cfr* genes were identified in 24/27, 6/27 and 5/27 isolates, respectively.
The minimum inhibitory concentrations of chloramphenicol and linezolid ranged from 24 mg/L–256 mg/L, and 1.5 mg/L–8 mg/L, respectively. Twenty-six of 27 isolates were resistant to chloramphenicol according to the CLSI breakpoint of ≥32 mg/L, and two of 27 were resistant to linezolid using the CLSI breakpoint of ≥8 mg/L. MLST analysis of the 17 *E. faecalis* identified ten different STs, with ST593 (n=4) and ST207 (n=2) occurring more than once, and two isolates assigned to novel STs. *E. faecium* isolates were assigned to four different STs (168, 264, 822, and 1846).

Conclusion
The high occurrence of Gram-positive bacteria harbouring genes encoding resistance to the critically important linzeolid is a matter of concern since such bacteria could easily spread from companion animals to humans with close contact with their animals.

P-085* Induction, resuscitation, and characterization of viable but non-culturable Legionella pneumophila

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Many bacteria, including the human pathogen *Legionella pneumophila*, respond to a variety of stresses by entering a dormant "viable but non-culturable" (VBNC) state. In the VBNC form, the cells remain viable, but can no longer be cultivated on conventional laboratory media, and therefore, cannot be detected by standard, cultivation-based diagnostic tools.
L. pneumophila is a ubiquitous, waterborne bacterium that can be found not only in natural freshwater systems but also in artificial water supplies, where it infects and kills free-living amoeba and other protozoa. Relying on the same mechanisms used in amoeba, *L. pneumophila* is also able to infect and destroy human macrophages. After inhalation of contaminated aerosols, *L. pneumophila* replicates intracellularly within alveolar macrophages and can cause a severe pneumonia termed Legionnaires' disease.
In this project, we explore the induction of VBNC *L. pneumophila* through heat stress as well as their ability to resuscitate upon contact with different amoeba species. In initial experiments, the capability of VBNC *L. pneumophila* to infect *A. castellanii* and *D. discoideum* amoeba was analyzed in great depth, by analyzing the uptake rate, the ability to form *Legionella*-containing vacuoles (LCVs) and the intracellular replication rate. In the future, we want to characterize bacterial as well as host factors implicated in the VBNC state. Initially, we focus on the pivotal role of ribosomes during the process of dormancy/hibernation.
The project bears the potential to detect and identify not only culturable *L. pneumophila* but also persistent VBNC forms, and thus, could lead to a better prevention and control of Legionnaires' disease outbreaks.

*Student presentation

P-086* Interaction of the Legionella effector protein RidL with host large fission GTPases

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Legionella species are Gram-negative, environmental bacteria, which cause a severe pneumonia termed Legionnaires' disease in humans. *Legionella pneumophila* infects protozoan and mammalian host cells through an evolutionarily conserved mechanism and replicates within a unique compartment termed the *Legionella*-containing vacuole (LCV). LCVs avoid fusion with bactericidal lysosomes, but extensively communicate with the endosomal, secretory, and retrograde vesicle trafficking pathways and finally associate with the endoplasmic reticulum (ER). Formation of the LCV is a complex process and requires the bacterial Icm/Dot type IV secretion system (T4SS), which translocates more than 300 different "effector" proteins into eukaryotic host cells. Upon translocation of these proteins, critical cellular processes are subverted, including signal transduction and vesicle trafficking. Accordingly, some effectors target host components directly involved in these pathways, such as phosphoinositide (PI) lipids or small GTPases.
The L. pneumophila effector RidL (***R***etromer ***i***nteractor ***d***ecorating ***L***CVs) interacts, through its N-terminus, with the Vps29 subunit of the retromer coat complex and inhibits the retrograde vesicle trafficking pathway in an unknown manner. Preliminary results indicate that the C-terminus of RidL interacts with dynamin-like large GTPases, such as Vps1, involved in retrograde trafficking in S. cerevisiae, and Drp1, which is required for mitochondrial fragmentation. The interaction of RidL with Vps1 and Drp1 has several consequences, including the inhibition of tubule formation and GTPase activity. The dual interaction of RidL with the retromer complex and large GTPases is unique, and a mechanistic understanding of this process will provide insights of broad impact into a novel bacterial virulence strategy as well as the elucidation of a pivotal cell biological pathway. To uncover the molecular mechanism of RidL function we use biochemical, structural, and cell biological approaches.

*Student presentation

P-087 Genomic study on Bacillus anthracis from wildlife animals in South Africa revealed a unique genetic cluster of B-clade strains

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The bacterium *Bacillus anthracis*, which causes anthrax, has three genetic subgroups: A, B, and C. Clade-A is distributed worldwide, whereas B-clade has been found in South Africa's Kruger National Park (KNP). One of South Africa's endemic anthrax regions with significant genetic diversity is the KNP. Historical culture collection isolates (n = 67) of *B. anthracis* strains from the 1970s to 2015 were subjected to whole genome sequencing. On 175 global *B. anthracis* genomes, whole genome single nucleotide polymorphisms (wgSNPs) and pan-genomics analyses were employed. In this study, KNP possessed several *B. anthracis* strains that clustered in the A-clade lineage, with the ABr.005/006 (Ancient A) SNP lineage being particularly dominant. Through non-stabilized genetic evolution, this SNP lineage exhibits a genetic cluster that has prevailed. This was augmented by non-parsimony informative SNPs found in a few Ancient A minor subclades. Pan-genomics of *B. anthracis* revealed 11,374 predicted clusters of protein-coding genes, clearly separating the A and B-clade genomes. Biosynthetic cell wall and sporulation genes (mepH, BclaA, trypABCD) and a second copy of Fosfomycin's multidrug resistance gene, fosB, were distinctive accessory genes found in B-clade genomes. The B.Br.009 genetic SNP lineage is what distinguishes the KNP B-clade strains. The sequenced *B. anthracis* strains from this study will help researchers better understand how the disease spreads and the evolution of this bacterium throughout the world, particularly in South Africa.

P-088 Rapid detection of imipenem/relebactam susceptibility/resistance in Enterobacterales

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Background
Carbapenem-resistant Enterobacterales (CRE) are a great concern for global public health. The treatment options for infections caused by CRE are extremely scarce and subsequently, the development of new antibiotics is an urgent necessity to combat the global spread of carbapenem-resistance genes. Imipenem/relebactam (IPR) has been recently introduced for the treatment of severe urinary tract infections, pyelonephritis, and complicated intra-abdominal infections in adults. Despite its rarity, IPR resistance has already been reported in Enterobacterales, and its rapid detection may be crucial for clinical decision-making.

Objectives
To develop a rapid and accurate test, namely the Rapid IPR NP, for the identification of IPR resistance among MDR Enterobacterales.

Methods
The Rapid IPR NP test is based on the detection of glucose metabolization due to bacterial growth in the presence of imipenem 12 mg/L and relebactam 4 mg/L. Bacterial growth is visually detectable by a color change of red phenol, a pH indicator, which goes from red to yellow, subsequent to the acidification of the medium upon bacterial growth. A total of 76 Enterobacterales isolates were randomly selected for evaluating the performance of the Rapid IPR NP test.

Results
The sensitivity and specificity of the test compared with the reference method were found to be 95% (95% CI 83.5%-98.6%) and 97.2% (97.2% CI 85.8%-99.5%) respectively. All the results were obtained within 3 h incubation time at 35°C ± 2°C which is a gain of time of at least 15 h (a day in practice) compared with currently used antimicrobial susceptibility testing including broth microdilution. Moreover, the test displayed only one major error and two very major errors.

Conclusions
The Rapid IPR NP test is simple to perform and interpret, and exhibited excellent sensitivity and specificity. Thus, it is suitable for implementation in clinical microbiology routine laboratories.

P-089 Diphtheria outbreak among young asylum seekers in a national asylum centre in Basel, Switzerland

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Since June 2022, European disease surveillance authorities report an increase in diphtheria cases among migrants. We report on *Corynebacterium diphtheriae* isolated from 19 patients housed in a single asylum centre in Basel, Switzerland.
The first case was detected through a toe wound swab from August 28, 2022. Subsequently, through November 15, 2022, we analysed a total of 214 swabs from 115 migrants and staff from the same asylum centre. Twenty-two samples (10.3%) from 18 individuals (15.7%) tested positive for *C. diphtheriae*. Including the index case, we identified altogether nine patients with positive throat swabs, nine with positive wound swabs, and one patient with both. The patients were mainly unaccompanied minor asylum seekers from Afghanistan aged 14 to 25. One patient was 34 years old. Twenty-one isolates from 19 patients were subjected to further genotypic and phenotypic analyses.
Phylogenetically, we identified three distinct cgMLST clusters of toxigenic isolates corresponding to ST377, ST384, and ST574, well separated from a single toxin-negative isolate of sequence type ST858, a pattern confirmed by SNP tree analysis.
All isolates were tested sensitive or intermediate to penicillin, ceftriaxone, meropenem, ciprofloxacin, vancomycin, and erythromycin, except for one erythromycin-resistant ST377 strain that harbours the macrolide resistance gene *ermX* as well as the bla_{OXA-2} β-lactamase gene. The MIC distribution for ciprofloxacin was bimodal with ST384, ST574 and ST858 isolate MICs at least three twofold dilutions lower than for ST377 isolates that harbour *gyrA* mutations at serine 89, a position associated with quinolone resistance in *C. diphtheriae*.
We confirmed known sequence types of *C. diphtheriae* found on Swiss and EU asylum seekers, suggesting importation from different regions with poor vaccine coverage. Local transmission within Swiss asylum centres may have occurred, supporting the importance of preventive measures against diphtheria in European migrant shelters.

P-090 Impact of the Seegene NovaplexTM Dermatophyte Multiplex PCR Assay for Laboratory and Clinical Routine

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Aim
The Seegene Novaplex™ Dermatophyte Multiplex PCR assay is able to detect 28 dermatophyte species. We compare this assay to mycological cultures for diagnosing skin, nail and hair mycoses and aim to assess accordance of PCR and culture results, effect on turn-around-time (TAT) and its clinical impact.

Methods
We structured our study in two parts: 1.) Verification of the whole assay, and 2.) evaluation of its clinical impact (still ongoing). Since the manufacturer currently does not give instructions how to handle dry specimen, we established a first extraction step with overnight incubation of sample in proteinase K and ATL lysis puffer™ (Qiagen) (56°C, 500 rpm). The following extraction and PCR steps were performed according to the manufacturer's protocol for the Nimbus™ extractor (Seegene) and CFX96™ thermocycler (Bio-Rad) automat respectively.

Results
Of 47 specimen (68% skin dandruff, 36% nail, 2% hair) analyzed by culture and PCR, the methods provided identical results in 31 cases (66%): 14 were positive with seamless corresponding fungus identification, 17 negative. PCR and culture were solitarily positive in each eight cases (17%). Three of eight PCR-negative, but culture-positive cases were due to fungi not included in the multiplex assay; in five of these cases PCR was most likely affected by insufficient amount of sample material. Positive PCR reduced TAT from maximally 28 days to one day. 3.) So far, early availability of a positive PCR result led to a change in patient's management in 3/6 cases (50%).

Conclusion
Sufficient specimen provided, the Seegene Novaplex™ Dermatophyte Multiplex PCR assay seems to enhance sensitivity and reduce TAT essentially compared to mycological culture. Nevertheless, cultures cannot be omitted due to infection by fungal species currently not included in the PCR assay. Shortened TAT of positive PCR results seems clinically impactive, further results are pending.

P-091* Single-cell dynamics of Staphylococcus aureus agr quorum-sensing upon synthetic autoinducing peptide stimulation and during community interactions

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The *accessory gene regulator* (*agr*) quorum sensing system of *Staphylococcus aureus* is critical for virulence regulation, yet understanding of its dynamic operation at the single-cell level and across different *agr* types remains incomplete. Our study utilizes advanced techniques including microfluidics, microscopy, and deep-learning image analysis to explore this dynamic system in detail.
We identified significant differences in AIP sensitivity across the four known *agr* types. Our investigation revealed that the response time to AIP concentration changes is dependent on both the AIP concentration itself and the *agr* type, thereby highlighting a nuanced picture of quorum sensing modulation. Notably, we found the activation of the *agr* system exhibited a strong intergenerational stability, suggesting cellular responses have lasting impacts on lineage behaviors. This was evidenced by early non-responding bacteria producing successive generations of *agr*-inactive offspring even when exposed to high AIP concentrations.
In addition, we validated the known cross-inhibitory effect of heterologous AIPs at the single-cell level, further elucidating the antagonistic interactions within the *agr* system. Extending beyond previous research, we engineered neighboring communities of different *agr* types to interact via AIP diffusion, unveiling distinct classes of interaction outcomes. While one *agr* type often dominated the other, our data also unveiled instances of dual-activation and dynamic switching of *agr* activation states. Importantly, these interactions exhibited variability based on the *agr* type combination and were influenced by early *agr* states and community sizes.
Our findings significantly advance our understanding of the intricate dynamics of the *agr* system and reveal distinct behaviors across *agr* types. We believe this work paves the way for future studies focusing on manipulating these systems for novel therapeutic interventions, either via synthetic drugs or by harnessing the natural antagonism produced by other bacterial species.

*Student presentation

P-092* CELLular determinants of permissiveness to HIV: a 6-day correlation analysis of cell stimulation and infection

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Cell permissiveness to HIV varies among individuals and among cells of the same individual. This heterogeneity can be linked to CD4+ T cell subpopulation diversity and activation state. *In vivo*, the immune system changes and evolves according to sex and age. We thus wondered if those factors could also influence cell heterogeneity and permissiveness to HIV *ex vivo*, and thereby identify novel players involved in HIV replication.
We selected 20 HIV-negative blood donors that diverge in age and gender. Primary CD4+ T cells were activated through T cell receptor-mediated stimulation over six days, and analyzed every 24h for (i) HIV infection efficiency and (ii) cell surface protein expression involved in HIV entry, activation or exhaustion. Cells were infected with three pseudotyped HIV-based vectors displaying either the amphotropic VSV-G envelope, or the CCR5- or CXCR4-tropic HIV envelope, and harboring both an HIV LTR-controlled GFP reporter and a constitutive EF1α-driven mKO2 reporter.
Preliminary results suggest that infection success peaked between 24 and 72h post-stimulation depending on viral envelope. Furthermore, infection success correlated significantly with the expression of CTLA-4 for the three vectors, while that of CD69 and PD-1 was vector-specific. Finally, men tended to exhibit higher levels of infection compared to women, which might be explained by cellular activation. These differences will be further investigated by transcriptomic analyses.
Further understanding cell heterogeneity linked to permissiveness to HIV replication should provide additional insights about the heterogeneity of the latent reservoir cells, and help designing strategies targeting cellular determinants common to all latently-infected cells.

*Student presentation

P-093* Assessing the physiological function of intracellular lactonases of the mushroom Coprinopsis cinerea

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Lactone rings are common structural features of natural products. In bacteria and fungi, lactone-based natural products, or lactones, can serve as signaling mediators of intra-species communication, e.g. quorum sensing in Gram-negative bacteria, but also as antimicrobial agents and virulence-promoting toxins. Lactones can be inactivated by enzymes that hydrolyze the ester bond of the lactone ring. These enzymes are commonly referred to as lactonases.
The saprophytic mushroom *Coprinopsis cinerea* dwells in herbivore dung and competes with several bacterial and fungal species for this ecological niche. Hence, *C. cinerea* is a model system to study fungal antagonism and defense. *Coprinopsis cinerea* possesses two intracellular lactonases, which are known to be active against the lactones of Gram-negative bacteria. In this study, we aim to establish whether the same enzymes are relevant to the interactions of *C. cinerea* with other microbes, including gram-positive bacteria and fungal competitors. To address this question, we will test the activity of *C. cinerea* lactonases against lactones with antifungal activity of different origins. These will include the antifungal agent rapamycin, produced by the Gram-positive bacterium *Streptomyces hygroscopicus,* and the mycotoxins patulin and zearalenone produced by plant-pathogenic fungi.
The model yeast *Saccharomyces cerevisiae* was used for assaying the activity of the *C. cinerea* lactonases against the selected lactones. Firstly, the working concentrations of the antifungals against *S. cerevisiae* were determined. Secondly, *S. cerevisiae* was transformed to express *C. cinerea* lactonases and tested for growth at inhibitory lactone concentrations. Lactonase activity against the compounds was assessed by the ability of transformants to grow.
Transformants did not grow on either rapamycin- or patulin- supplemented medium, suggesting that C. cinerea lactonases may be specific to lactones of Gram-negative bacteria.'

*Student presentation

P-094* Environmental cues controlling integrative and conjugative element transfer

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Horizontal gene transfer (HGT) is the process by which DNA is transferred from a donor cell to a recipient that is not part of its progeny. Mechanisms of HGT in prokaryotes include transformation, transduction, and conjugation; the latter of which transfers plasmids or chromosomally excised DNA like integrative and conjugative elements (ICEs) from a donor to a recipient in a cell-to-cell dependent contact. ICEs are widespread autonomous mobile DNA, containing the genes necessary for their excision, conjugative transfer, and insertion into the genome of a new host cell. ICE*clc* of *Pseudomonas knackmussii* B13 has been widely studied in our lab and is characterized by the presence of the *clc* genes for chlorocatechol degradation. ICE*clc* transfer is initiated from a small subpopulation of cells, that become transfer competent (tc). The proportion of tc cells is the highest when cultures have been grown on 3-chlorobenzoate as a sole carbon source and when they enter stationary phase, suggesting a certain degree of environmental control over transfer competence development. However, the link between growth on 3-chlorobenzoate and ICE*clc* transfer competence activation is unknown. To address this, we used single cell transcriptomics to precisely identify differences in transcriptional states of cells growing on different carbon sources, and between tc and non-tc cells. We were able to differentiate the genome-wide transcription fingerprints of tc- and non-tc cells under 3-chlorobenzoate growth conditions, as well as global carbon source and growth phase signatures. Candidate genes and pathways will be followed up in genetic and reporter studies.

*Student presentation

P-095 Effective Biofilm Eradication on Orthopedic Implants with Methylene Blue Based Antimicrobial Photodynamic Therapy In Vitro

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Periprosthetic joint infections (PJI) are difficult to treat due to biofilm formation on implant surfaces, often requiring removal or exchange of prostheses along with long-lasting antibiotic treatment. This in vitro study investigated the effect of methylene blue photodynamic therapy (MB-PDT) on PJI-causing biofilms on different implant materials. MB-PDT (664 nm LED, 15 J/cm^2) was tested on different *Staphylococcus aureus, Staphylococcus epidermidis*, *Escherichia coli* and *Cutibacterium acnes* strains in both planktonic form and grown in early and mature biofilms on prosthetic materials (polyethylene, titanium alloys, cobalt-chrome-based alloys, and bone cement). The minimum bactericidal concentration with 100% killing ($MBC_{100\%}$) was determined. Chemical and topographical alterations were investigated on the prosthesis surfaces after MB-PDT. Results showed a $MBC_{100\%}$ of 0.5-5 µg/mL for planktonic bacteria and 50-100 µg/mL for bacteria in biofilms-independent of the tested strain, the orthopedic material, or the maturity of the biofilm. Material testing showed no relevant surface modification. MB-PDT effectively eradicated common PJI pathogens on arthroplasty materials without damage to the materials, suggesting that MB-PDT could be used as a novel treatment method, replacing current, more invasive approaches and potentially shortening the antibiotic treatment in PJI. This would improve quality of life and reduce morbidity, mortality, and high health-care costs.

P-096 DEVELOPING A RAPID PHENOTYPIC ASSESSMENT OF PHAGE SUSCEPTIBILITY USING A NANOMOTION TECHNOLOGY PLATFORM

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Question
Alternatives to antibiotics, such as bacteriophages, become essential. However, clinical implementation of phage therapy requires fast and reliable phage susceptibility testing (PST) of the infecting strain.
Methods: We propose nanomotion technology[1-3] measuring the vibrations of bacterial cells based on their metabolic activity and viability changes for PST. To measure vibrations, we have developed the Phenotech device currently in clinical studies for antibiotic susceptibility testing (AST) in bloodstream infections (NANO-RAST and PHENOTECH-1).

Results
We present the first data obtained with our device measuring *Pseudomonas aeruginosa* isolates from lung infections. Our recordings show the dose-dependent lysis in real-time. For this, we analyzed the changes in the variance of the cantilever deflections over time. While decrease indicated phage-susceptibility, increase indicated phage-resistance. Results were compared to empirical drop test assays (DTA). Depending on the phage-bacterium pair, PST with the Phenotech was as fast as 4h, compared to 16-24h for the DTA.

Conclusions
We plan to extend our experiments to a larger set of *P. aeruginosa* isolates and phages to train classification models with supervised machine learning using common features from the nanomotion signal. These models will contribute to a rapid and reliable PST for optimal selection of phages in clinical protocols of phage therapy.

P-097* Understanding how Pseudomonas aeruginosa effectively colonizes surfaces by balancing pili-mediated adherence and dissemination

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To infect their human or animal hosts, bacterial pathogens need to be able to effectively colonize and breach epithelial barriers. During this process they attach to tissue surfaces, adapt their motility behavior and induce virulence factors. The opportunistic human pathogen *Pseudomonas aeruginosa* uses type IV pili (T4P) as primary adhesins and motility organelles to adhere to and disseminate on host mucosa. How T4P are differentially regulated to coordinate distinct surface processes and behavior is not well understood. The second messenger c-di-GMP (cdG) has emerged as an important coordinator in regulating T4P activities during early surface sensing. Here, we analyze the regulation of two cdG-dependent effectors, FimW and FimX, that differentially control T4P activity. While FimW stimulates T4P-dependent surface adherence and appears to act as a motility break, FimX was shown to promote T4P-dependent twitching motility. However, how cdG individually controls distinct T4P functions has remained unclear. Here, we attempt to understand how FimW and FimX dynamics is differentially controlled by cdG and how this directs distinct T4P-dependent behavior of *P. aeruginosa* on surfaces. Our studies suggest that FimW and FimX are activated by different sources of cdG to balance attachment and motility.

*Student presentation

P-098 New dog, old tricks: conserved regulation mechanism in domesticated phage Bartonella gene transfer agent

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Gene transfer agents, or GTAs, are domesticated genetic elements mediating the exchange of bacterial and archaeal genes by bacteriophage-like particles. GTAs emerged from phages and are widely present both in bacteria and archaea. A notable example is GTA of pathogenic bacteria *Bartonella* (BaGTA). *Bartonella* shows a unique example of a sequential domestication of two prophages which resulted in the linkage of a capsid morphogenesis module BaGTA and a phage-derived origin of replication BaROR. Consequently, the joint function of BaGTA and BaROR mediates the transfer of genes involved in host-pathogen interactions and is suggested to be one of the key innovations underlying the evolutionary success of these pathogens. In this work, we investigated the genetic regulatory network enabling the coordination of these two distinct components. We found that the BaROR encodes a set of regulatory proteins that control the expression of the BaGTA locus. The control is achieved by premature termination of the BaGTA locus transcription which is counteracted by processive antitermination – a mechanism inherited from the phage ancestor.
Collectively, these results provide insight into the regulation of BaGTA-mediated gene transfer in the pathogenic bacteria *Bartonella.* GTAs are present in a wide range of prokaryotes and are prevalent in up to 60% of alphaproteobacterial genomes. Thus, GTAs claim to be a widespread mechanism of HGT whose full potential is yet to be uncovered.

P-099 Evaluation of two rapid commercial assays for detection of Streptococcus agalactiae from vaginal samples

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Background
Streptococcus agalactiae, also known as group B streptococci (GBS), is associated with invasive infections in neonates. Identification of GBS vaginal colonization of pregnant women before delivery is essential for treatment with antibiotics to prevent intrapartum vertical transmission to the newborn. We aimed to compare two PCR based assays against our culture-based reference standard in samples of pregnant women.

Methods
This study was designed to evaluate applicability of the rapid real-time PCR Xpert® GBS (Xpert; Cepheid, Sunnyvale, California, U.S.). The test was compared with GenomEra® GBS PCR (GenomEra; Abacus Diagnostica, Turku, Finland). Culture-based detection served as reference standard. Culture was performed on CNA agar (bioMérieux, Marcy l'Étoile, France), GBS agar (Brilliance GBS, Thermo Scientific, Waltham, MA, USA) and GBS enrichment bouillon containing casein and starch (Merck, Darmstadt, Germany) at 37°C for 24h. Identification was done with MALDI-TOF MS.

Results
We analyzed vaginal samples of 260 pregnant women; 42 (16.2%) samples were tested GBS-positive by culture, 30 (11.5%) by Xpert, and 37 (14.2%) by GenomEra. Xpert and GenomEra assays performed with sensitivities of 71.4% and 88.1%, as well as specificities of 98.6% and 99.1%, respectively. Twelve vaginal samples were false-negative by Xpert and 5 samples by GenomEra. By inclusion of higher Ct-values, the sensitivity of Xpert increases up to 78.6%.

Conclusions
The GenomEra assay showed a higher sensitivity (88.1%) than the Xpert PCR (71.4%). However, the Xpert assay needed less hands-on-time for a sample preparation and required substantially less repetitions compared to the GenomEra assay. The Xpert assay showed an overall acceptable sensitivity and high specificity, which make it applicable as rapid intrapartum point-of-care test. However, culture in the 35-37 week of gestation clearly remains the gold standard to detect vaginal colonization. PCR based detection should be complemented with culture.

P-100 High-fluence accelerated photoactivated chromophore for keratitis cross-linking (PACK-CXL) to treat porcine corneas infected with Staphylococcus aureus or Pseudomonas aeruginosa

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Background
Infectious keratitis results from micro-damage to the cornea structure e.g., via contact lenses and a subsequent superinfection with bacteria. Infectious keratitis can be a therapeutic challenge. We aimed to investigate whether high-fluence accelerated PACK-CXL can effectively treat porcine corneas infected with *Staphylococcus aureus* or *Pseudomonas aeruginosa*.

Methods
We established a bacterial keratitis model on fresh porcine corneas using *S. aureus* and *P. aeruginosa*, respectively. Two high-fluence accelerated PACK-CXL protocols with different chromophores (riboflavin, exposed to 365 nm UV-A light: 30 mW/cm^2, 8 min 20 secs, 15 J/cm^2; rose bengal exposed to 522 nm green light: 15 mW/cm^2, 16 min 40 secs, 15 J/cm^2) were applied to one quarter of infected corneas. All corneas were incubated for 24 hours and 8 mm corneal trephine was applied to get corneal buttons. The bacteria were released by vortexing the corneal buttons in 0.9% saline solution. The irradiated and unirradiated solutions were diluted, plated and inoculated on Columbia agar + 5% sheep blood (bioMérieux, Marcy l'Étoile, France) for quantification. Bacterial killing ratios (BKR) were calculated from at least 3 biological replicates with at least 3 technical replicates for each condition.

Results
In this *ex-vivo* porcine infectious keratitis model, we successfully applied PACK-CXL with riboflavin plus UV-A light and observed an average BKR for *S. aureus* and *P. aeruginosa* of 55.5% and 54.3%, respectively. When we applied PACK-CXL with rose bengal plus green light, the average BKR for *S. aureus* and *P. aeruginosa* was 83.0% and 73.3%, respectively. The BKR is significantly increased by applying rose bengal as compared to riboflavin.

Conclusion
Both types of high-fluence accelerated PACK-CXL can significantly decrease the bacterial load of *S. aureus* and *P. aeruginosa* in a porcine cornea model. The results are promising to further optimize the treatment and have a high translation potential for treatment in humans.

P-101 Characterization of Burkholderia cenocepacia ST-250 isolates in patients with cystic fibrosis (CF) in Switzerland

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Background
The *Burkholderia cepacia* complex (Bcc) comprises a group of genetically distinct bacteria. Colonization of the respiratory tract in CF-patients is associated with poor clinical outcome. We aimed to investigate Bcc strains from CF-patients and non-CF patients obtained in our center over a period of 20 years on a genomovar, genetic, and epidemiological level.

Materials
We analyzed a total of 100 Bcc strains collected from 2001-2021 in our routine diagnostics using whole-genome sequencing (Illumina MiSeq, paired-end 150nt). We determined the multi-locus sequence types (MLST) from PubMLST. We performed single nucleotide polymorphism (SNP)-analysis in CLC Genomics Workbench v22.0.2. We measured antibiotic susceptibility with disk diffusion using clinical breakpoints from the CLSI (M100 32th edition).

Results
The genomovars identified were *Burkholderia cenocepacia* n=44, *B. multivorans* n=32, *B. contaminans* n=15; *B. cepacia* n=3; *B. anthina* n=2; *B. stabilis* n=2; *B. gladioli* n=1; and *B. vietnamensis* n=1. MLST revealed *B. cenocepacia* ST-250 in 18 clinical samples obtained from eight CF-patients, with multiple isolates collected over up to nine years from five patients. SNP-analysis of these ST-250 strains showed clustering of isolates from patients. A within-patient genomic variation of up to 114 SNPs and a between patient variation of 68-252 SNPs were observed, respectively, suggesting that this cluster has a recent common ancestor.
B. cenocepacia ST-250 isolates exhibited high resistance towards meropenem (15/18, 83.3%), minocycline (14/18, 77.8 %), and trimethoprim-sulfamethoxazole (17/18, 94.4%). Only 12/18 (66.7%) were resistant towards ceftazidime. Isolates derived over time from the same patients displayed a progressive increase in resistance towards different antibiotics.

Conclusions
We present whole-genome data from *B. cenocepacia* ST-250. *B. cenocepacia* ST-250 isolates from five CF-patients showed within-host diversity over several years of colonization and evolution of antimicrobial resistance. Further analyses are underway to infer the timing of a common ancestor and possible transmission of *B. cenocepacia* ST-250 isolates.

P-102 Emergence of blaSHV-12 and qnrS1 encoded on IncX3 plasmids: changing epidemiology of extended-spectrum ß-lactamases among Enterobacterales isolated from broilers

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Objective
The occurrence of extended-spectrum ß-lactamase (ESBL) producing Enterobacterales in broilers represents a risk to public health because of the possibility of transmission of ESBL-producers and/or bla_{ESBL} genes via the food chain or within settings where human-animal interfaces exist.
Methods: This study assessed the occurrence of ESBL-producers among faecal samples of broilers at slaughter. Isolates were characterised by multilocus sequence typing (MLST), antimicrobial susceptibility testing (AST), and whole genome sequencing (WGS).

Results
The flock prevalence, determined by sampling crates of 100 poultry flocks, was 21%. The predominant bla_{ESBL} gene was bla_{SHV-12} identified in 92% of the isolates. A variety of *E. coli* and *K. pneumoniae* sequence types (STs) were identified, including extraintestinal pathogenic (ExPEC) *E. coli* ST38, avian pathogenic *E. coli* (APEC) ST10, ST93, ST117 and ST155, and nosocomial outbreak clone *K. pneumoniae* ST20. WGS characterized a subset of 15 isolates, including six *Escherichia coli*, four *Klebsiella pneumoniae*, one *K. grimontii*, one *K. michiganensis*, one *K. variicola*, and one *Atlantibacter subterranea*. Fourteen isolates carried identical or closely related 46338-54929 bp IncX3 plasmids encoding bla_{SHV-12} and *qnrS1*. One *E. coli* isolate carried the 46338 bp IncX3 plasmid integrated chromosomally into *ydbD*.

Conclusions
The bla_{SHV-12} gene has replaced the previously predominant $bla_{CTX-M-1}$ in ESBL-producing Enterobacterales from broilers in Switzerland. Broilers may play a role in the dissemination of bla_{SHV-12} and *qnrS1*associated with epidemic IncX3 plasmids, representing a risk to human and animal health.

P-103 Rapid Aztreonam/Avibactam NP test for detection of aztreonam/avibactam susceptibility/resistance in Enterobacterales

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Background
Aztreonam-avibactam, a newly developed β-lactam/β-lactamase inhibitor combination, is a treatment option for infections due to carbapenem-resistant Enterobacterales including metallo-ß-lactamase producers, regardless additional production of broad-spectrum serine-ß-lactamases. However, aztreonam-avibactam resistance has already been reported in Enterobacterales and its early detection could be a valuable tool for faster and accurate clinical decision-making.

Objectives
To develop a rapid culture-based test for the identification of aztreonam-avibactam resistance among multidrug-resistant Enterobacterales.

Methods
The Rapid Aztreonam-Avibactam NP test is based on resazurin reduction when bacterial growth occurs in the presence of aztreonam-avibactam at 8/4 µg/ml (protocol 1) or 12/4 µg/ml (protocol 2). Given the absence of guidelines on aztreonam-avibactam susceptibility testing, two tentative breakpoints were indeed used to categorize aztreonam-avibactam-susceptible isolates: ≤4 µg/ml in protocol 1, ≤8 µg/ml in protocol 2. Bacterial growth was visually detectable by a blue-to-purple- or blue-to-pink color change of the medium. A total of 78 (among which 35 aztreonam-avibactam-resistant) and 51 (among which 14 aztreonam-avibactam-resistant) enterobacterial isolates were used to evaluate the test performance using protocol 1 or protocol 2, respectively.

Results
The sensitivity and specificity of the test were found to be respectively 100% and 97.7% with protocol 1, and respectively 92.9% and 100% with protocol 2, in comparison with broth microdilution. All results were obtained within 4.5 h corresponding to a time saving of ca. 14 h compared with currently-available methods for aztreonam-avibactam susceptibility testing.

Conclusion
The Rapid Aztreonam-Avibactam NP test is rapid, highly sensitive, specific, easily interpretable, and easy to implement in routine.

P-104 Rapid detection of temocillin susceptibility/resistance in Enterobacterales

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Temocillin has been largely overlooked as a treatment option for infections caused by Gram-negative bacteria due to its poor activity against non-fermenters. However, temocillin exhibits remarkable stability against a plethora of beta-lactamases including ESBLs and AmpCs, leading to its indication in several countries for the treatment of urinary tract, blood stream, and lower respiratory tract infections. This study describes a rapid test to determine susceptibility/resistance to temocillin in ≤ 4 hours. One-hundred clinical Enterobacterale isolates were tested. Susceptibility testing was performed by broth microdilution and the beta-lactamase content of the tested isolates was determined by PCR. Thirty-eight tested isolates were temocillin susceptible, with MICs ranging from 2-16 mg/L, and the remaining 62 were resistant (32->256 mg/L). The Rapid Temocillin NP test solution was prepared with 21.3 mg/L of temocillin and 150 µl was added into one well of a 96-well plate, and 150 µl of test solution without temocillin was added into a second well. Inocula were prepared from overnight agar cultures, as follows; a 0.5 MacFarland suspension was prepared then diluted 1/10 in MH broth, 50 µl of this suspension was then used to inoculate the plates (approx. 1.5×10^7 bacterial cells per well), resulting in a final temocillin concentration of 16 mg/L, before incubation at 37°C for 4 hours. After 3- and 4-hours, plates were read and a colour change from red to yellow was interpreted as positive (resistant), confirming acid production via the metabolism of glucose in the test solution, and red was interpreted as negative (susceptible). Overall, the test exhibited 100% specificity and 98.4% sensitivity. As the use of temocillin becomes more common place, such a test can prove useful in determining targeted rather than simply empirical therapy in a relatively short time frame.

P-105 Streptococcus pneumoniae metabolizes human milk oligosaccharides as an exclusive carbon source

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Background
Streptococcus pneumoniae is a human pathogen, that can reside asymptomatically in the upper respiratory tract. The pneumococcus easily uses different monosaccharides for growth. It is less clear, if and how polysaccharides can be metabolized. However, during its lifespan, *S. pneumoniae* is exposed to various polysaccharides, which are e.g. attached to proteins or freely available. We aimed to investigate the growth of *S. pneumoniae* in the presence of human milk oligosaccharides (HMOs), which might be taken up by infants during breastfeeding and may subsequently reside in the mucus.

Methods
The growth of wild-type *S. pneumoniae* strains and deletion mutants (*ΔbgaA* and *ΔnanA*) was examined *in vitro.* The HMOs 2'-Fucosyllactose (2FL), 3'-Fucosyllactose (3FL), Lacto-N-tetraose (LNT), Lacto-N-neotetraose (LNnT), 6'-Sialyllactose (6SL), and 3'-Sialyllactose (3SL) were added as sole C-source to a semi-defined medium. In addition, the effect of different pre-growth conditions (using Glucose versus Galactose) was investigated.

Results
The wild-type strains were able to use 6SL and LNnT as single C-source. The HMOs 3FL, 3SL, and LNT led to growth in both, wild-type and mutants, while 2FL could not be metabolized. We found that the enzymes BgaA and NanA are needed for metabolizing LNnT and 6SL, respectively. The pre-growth with Galactose (in contrast to Glucose) allowed the metabolization of specific HMOs as the sole carbon source.

Conclusions
Using Galactose in the pre-growth leads to increased expression of relevant genes like *bgaA* and *nanA* facilitating subsequent metabolization of two of the HMOs. The presence of HMOs in the upper airways may influence the colonization rate of *S. pneumoniae.*

P-106 Novel macrolide-lincosamide-streptogramin B resistance gene erm(56) in Trueperella pyogenes

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Suppurative infections caused by *Trueperella pyogenes*, a commensal and opportunistic Gram-positive pathogen of animals, are occasionally treated using macrolides and lincosamides posing the risk of antimicrobial resistance selection. Acquired resistances to macrolide, lincosamide and streptogramin B (MLS_B) antibiotics in *T. pyogenes* have been so far associated with erythromycin ribosome methylase genes, *erm*(B) or *erm*(X), located within mobile genetic elements.
T. pyogenes strain 09KM1269, isolated from a dog abscess, exhibited constitutive resistance to erythromycin and clindamycin. Whole genome sequence analysis identified a novel gene, *erm*(56), that coded for a 23S rRNA methylase and showed the closest relatedness to Erm(X) with only 54% nucleotide and 58% amino acid identity.
Functionality of the new gene was demonstrated by cloning *erm*(56) and its promoter sequences into pJRD215. The resulting *erm*(56)-containing plasmid pJEM1269 was subsequently electrotransformed into susceptible strains of *E. coli* AG100A and *T. pyogenes* 13OD0707. When *erm*(56) was expressed from pJEM1269 in *T. pyogenes* 13OD0707, the MIC increased by more than 256-fold for erythromycin and clindamycin and by 16-fold for pristinamycin IA. Increased MICs of erythromycin (64-fold) and clindamycin (8-fold) were also measured for *E. coli* AG100A containing pJEM1269.
The *erm*(56) gene was integrated into the chromosome between two IS*6100*, situated next to a class 1 integron containing the sulfonamide resistance gene *sul1*. The *erm*(56) gene associated with IS*6100* was also detected in strains from livestock in China, namely in another *T. pyogenes* and a *Rothia nasimurium*. Although a circular conformation containing one copy of IS*6100* was detected by PCR, the *erm*(56) gene could not be transferred by either filter mating or electroporation of genomic DNA into MLS_B-susceptible and plasmid-free *T. pyogenes* strains.
The detection of *erm*(56) in unrelated bacteria from different animal sources and geographical origins suggests that it has been independently acquired and likely selected by the use of antibiotics.

P-107 Lipoprotein BamB protects against porin-dependent antibiotic permeability in Pseudomonas aeruginosa

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The role of the BamB lipoprotein, a non-essential component of β-barrel assembly machinery (BAM) that inserts proteins into the outer membrane (OM) of Gram-negative bacteria, is not well understood. Here we show that *Pseudomonas aeruginosa* BamB sustains OM barrier function towards several large antibiotics. We identified bamB mutant cells in a Tn-Seq screen for mutants hypersensitive to vancomycin, a glycopeptide antibiotic that predominantly acts on Gram-positive bacteria, and we show that loss of BamB synergizes with crippled LptD function, an essential BAM substrate that is required for the insertion of lipopolysaccharide (LPS) into the OM. Moreover, we find that removal of all detectable porin gene paralogs mitigates the antibiotic susceptibility of bamB mutant cells, providing evidence that BamB substrate competition or substrate activity control extends to a myriad of concurrently engaged OM client proteins. Thus, BamB represents a suitable novel antibiotic target for combinatorial chemotherapeutic treatment against the ESKAPE pathogen *P. aeruginosa*.

P-108 Rapid Meropenem/Vaborbactam NP test for detecting susceptibility/ resistance in Enterobacterales

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The treatment options for infections caused by carbapenem-resistant Enterobacterales (CRE) are extremely scarce nowadays and the development of new antibiotics does not follow the exponential increase in the dissemination of carbapenem resistance determinants worldwide. Meropenem/vaborbactam (MEV) was recently approved for clinical uses and it has been indicated for treating several infections. MEV has been preferred over ceftazidime-avibactam (CZA), other β-lactam-β-lactamase inhibitor, because of the increasing reports of acquired resistance to CZA observed among Enterobacterales (producing distinct KPC variants or CMY-185). However, although relatively rare, MEV resistance has already been reported in Enterobacterales and its early detection could be a valuable tool for faster clinical decision-making. The aim of this study was to develop a rapid test, namely the Rapid MEV NP, for identifying MEV resistance in Enterobacterales. The Rapid MEV NP test is based on the detection of glucose metabolization occurring upon bacterial growth in the presence of MEV at a concentration of 16/8 µg/ml. Bacterial growth is detectable by a color change of red phenol (from red to yellow) subsequent of the acidification of the medium upon bacterial growth. A total of 75 Enterobacterales isolates were randomly selected for evaluating the performance of the Rapid MEV NP test. The test showed 97.2% (95% CI 85.8%-99.5%) of sensitivity and 92.3% (95% CI 79.7%-97.4%) of specificity when compared with the reference method. The results are obtained after 3 h of incubation at 35°C ± 2°C, which is a gain of time of at least 15 h (one day in practice) compared with currently-used antimicrobial susceptibility testing including broth microdilution method. The Rapid MEV NP test is easy to perform and to interpret, showed a remarkable performance while providing fast results, and is therefore suitable for implementation in routine among clinical microbiology laboratories. It will be ideal for identifying MEV susceptibility.

P-109 Clinical evaluation of the fully automated NeuMoDx HIV-1 Quant Assay

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Introduction
Quantification of HIV-1 RNA is critical for monitoring viral load and to guide patient management. Here we describe the clinical performance of the novel NeuMoDx HIV-1 Quant Assay (QIAGEN GmbH) in EDTA plasma samples in comparison to a validated CE-IVD assay, the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (Roche Diagnostics).

Methods
310 samples were assessed: 148 were previously tested using Roche and stored at -20°C before using NeuMoDx HIV-1 Quant Assay. 162 samples were prospectively analyzed with both methods. Qualitative analysis, linear regression (Deming) and a Bland-Altman test were performed for paired quantitative results.

Results
220/310 samples had a qualitative result concordant between both assays (91 HIV-1 positive and 129 HIV-1 not detected). 11 samples were HIV-1 not detected by NeuMoDx and HIV-1 positive by Roche (range 20-68 c/mL). 14 samples were HIV-1 not detected by Roche but HIV-1 positive by NeuMoDx (20-178 c/mL). In addition, 59 samples were HIV-1 RNA detected but below the lower limit of quantification (LLOQ) by Roche: 23 and 36 of these samples were HIV-1 positive and HIV-1 not detected by NeuMoDx (12-199 c/ml), respectively. 6 samples were HIV-1 RNA detected below LLOQ by NeuMoDx: 2 were HIV-1 positive and 3 HIV-1 not detected by Roche. HIV-1 positive samples with results within the linear range of both assays were highly correlated across the quantifiable range (correlation coefficient R^2 of 0,9). A Bland-Altman plot showed a mean log difference of 0,14 log10 IU/ml between both methodologies.

Conclusion
This study demonstrated the excellent performance of the NeuMoDx HIV-1 Quant Assay, which accurately detects and quantify HIV-1 RNA. Low-level discrepant samples were equal across both methods and were all below 200 c/mL. Data from this study were used to obtain Swiss HIV Cohort approval for the routine use of the NeuMoDx HIV-1 Quant Assay in Switzerland.

P-110 Searching for conformational states of Bacillus subtilis Smc complex using synthetic antibodies

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Bacillus subtilis is a gram-positive bacterium widely used as a model organism for studying sporulation and cell differentiation due to its versatility and the availability of gene editing techniques. During the cell cycle, the chromosome is organized by tightly regulated factors that ensure its proper compaction and faithful segregation. The Structural Maintenance of Chromosome (SMC) complex, composed of Smc, ScpA, and ScpB, is required for these processes in *Bacillus subtilis*. The complex loads onto the chromosome at specific *parS* sites near the origin of replication through the adapter protein ParB and aligns the chromosome arms by actively and progressively enlarging DNA loops, a process known as loop extrusion. *ΔsmcscpAB* mutants exhibit chromosome segregation defects and fail to grow on rich medium, indicating the importance of this complex in cell viability.
SMC complexes are ATPase motors that actively translocate along DNA, but the exact mechanism remains unclear. Recent studies from our group have shown that SmcScpAB exhibits different conformational states ranging from open to gradually closed. However, these conformations and their association with DNA throughout the ATPase cycle are challenging to detect due to their transience. Therefore, capturing intermediate states is crucial to understanding how SMC performs its cellular role.
Conformation-specific synthetic nanobodies, or "sybodies," are useful tools for stabilizing such states. We selected sybodies that stabilize the complex with sufficient affinity to induce a *ΔsmcscpAB* phenotype *in vivo*. The presence of each sybody strongly altered the ATPase activity of the complex, and we were able to hypothesize open- or closed-state specific binding. Genetic mapping studies using Smc fragments demonstrated that sybodies bind to a defined region of Smc, which is essential for SmcScpAB function. Ongoing *in vivo* and *in vitro* work, as well as future cryo-EM experiment will provide additional insights into the stabilized Smc conformation.

P-111* Patterns of Microbial Arctic Tundra Ecosystems and Potential Drivers of Community Composition in Light of Arctic Greening

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The Arctic has been warming at an unprecedented rate. Consequently, Arctic native plants and soil microbial communities are experiencing prolonged growth seasons and higher plant productivity, resulting in the “greening” of the Arctic. However, these changes are not homogeneous and it is not clear what is driving this heterogeneity. To evaluate the role microorganisms play in Arctic greening, we sampled and analysed different sites on the island of Svalbard. These sample sites consist of (i) native tundra soils that are characterized by low nutrient input, (ii) soils around bird cliffs that are heavily amended with nutrients such as nitrogen and phosphorus by bird droppings, and (iii) soils with influence and disturbance from humans and agriculture. A selection of topsoil samples from the three site types were preliminarily sequenced for 16S rRNA and a quantitative PCR of 16S rRNA was performed. The alpha diversity of the sequenced communities of soils with influence from humans and agriculture was lower than the diversity in the other samples. The beta diversity showed a trend for distinct communities in each site, nevertheless there are more similar communities in the native tundra and the soils around bird cliffs, the soil with human disturbance having a more distinct community composition. The 16S rRNA copy numbers per gram of soil were highest in the site of human disturbance. These preliminary results, based on a small fraction of the samples will further be explored and completed. By analysing these datasets we will close important gaps in the understanding of spatial structuring of Arctic tundra microbial topsoil community and the drivers of heterogeneity across different Arctic tundra ecosystems. This more complete understanding of the processes that shape these microbes will further be important in elucidating their role in Arctic greening and how it will progress with climate change.

*Student presentation

P-112 Structural mechanisms of prokaryotic immune defence systems

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Bacteria use diverse defense mechanisms for protection against infections from phages and foreign genetic elements. Effective cellular defense involves the programmable and highly regulated action of protein complexes. These complexes can exist in two modules, including a reader module which recognizes foreign DNA/RNA and the effector module which either degrades the foreign genetic element or facilitates host abortive immunity. We unraveled the mechanisms of defensive action of a Type-IIIE CRISPR and argonaute defense complexes by cryo-electron microscopy (cryo-EM) revealing diverse mechanisms of abortive immunity among different prokaryotic immune complexes.

P-113* Third-generation cephalosporins resistance plasmids in E. coli isolated from poultry and retail meat in Switzerland

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Resistance to third-generation cephalosporins (3GCs) in *E. coli* represents a serious public health problem. For instance, 3GC resistance plasmids contribute to the dissemination of multiple resistance traits in Enterobacterales highly limiting the therapeutic options in the clinical setting.
Food-producing animals play a role in the dissemination of antimicrobial-resistant bacteria and poultry has been recognized as a reservoir of 3GC-resistant *E. coli*. Handling of contaminated meat or direct contact with livestock poses the risk of *E. coli* transmission to humans, where plasmids may further be transferred to the human gut flora. In this study, the MGEs containing 3GC resistance genes were identified for 350 E. coli from poultry and poultry meat using hybrid assemblies of whole genome sequences obtained by ONT and Illumina technologies and analyzed.
The predominant 3GC-R genes detected in the investigated *E. coli* were bla_{CMY-2} (n=113) and $bla_{CTX-M-1}$ (n=104). Phylogenetic analysis of the plasmids revealed that the bla_{CMY-2} and $bla_{CTX-M-1}$ genes were mostly carried by plasmids, which belonged to two major plasmid families. The first family consisted of plasmids of the incompatibility group I1 (IncI1) harboring either bla_{CMY-2} or $bla_{CTX-M-1}$. They had sizes ranging from 100 to 146 kb, shared a common backbone and also carried, in most cases, additional resistance genes such as *sul2*, *dfrA17* or *tet*(A). The second family of plasmids only carried bla_{CMY-2} and belonged to the IncB/O/K/Z group. They had a more variable size range (85-189 kb) and also carried additional resistance genes such as bla_{TEM-1}, *sul1*, *tet*(A), *aadA1* and *aac(3)-Via*.
This study provides an insight of complete 3GC resistance plasmids present in different *E. coli* from poultry origin and will serve as a basis for further comparative analysis with plasmids of strains from human origin following a One-Health approach.

*Student presentation

P-114 Engineering the outer membrane of the purple bacterium Rhodospirillum rubrum for surface display presentation of potential vaccine epitopes

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The outer membrane of the Gram-negative purple bacterium *Rhodospirillum rubrum* contains 11 major proteins, two of which are the outer membrane porins, Por39 and Por41. Both porins exhibit extensive sequence similarity to each other, but only limited sequence identity to the only two porins from photosynthetic bacteria (2POR of *Rhodobacter capsulatus* (PBD: 2POR) and *Rhodopseudomonas blastica* (PDB: 2PRN) for which X-ray structures are available. All of the latter structures show no sequence identity to well-known enterobacterial porins. *R. rubrum* has no known pathogenic potential, and the lipopolysaccharide of the outer membrane has very low endotoxicity. In addition, the organism can be grown to extremely high cell densities (ODs> 160 are achievable) semi-aerobically, at low aeration, and with a cheap well-defined medium. These factors make the organism interesting for development as a surface display platform, where epitopes inserted into the loops of either Por39 or Por41 can be employed as vaccines.
For this purpose, we created a deletion mutant of *R. rubrum* (S1ΔPor3941) lacking the major porins Por39 and Por41, and performed a physiological characterization. The S1ΔPor3941 mutant can grow well both photosynthetically,anaerobically, or semi-aerobically in the dark, with slightly reduced growth rates relative to wild-type, We expressed the individual porins separately. Finally, in our initial study we constructed Por39 variants, where key ACE2 binding sites of the spike protein from the pandemic coronavirus Cov2 were incorporated into a predicted outer membrane loop of Por39, which was chosen on the basis of an extensive modelling study[1], where the putative 3D structure of Por39 was predicted by a comparison with the weakly related homologue, 2POR. Results will be presented.

[1] Markthaler, D., and Ghosh, R. (2023) Computational prediction of extracellular loops of the Por39 outer membrane porin of *Rhodospirillum rubrum* suitable for epitope surface display. Comp. Struct. Biotechol. J., 21, 2483-2494.

P-115 Comparing qPCR and culture-based methods for enumeration of Legionella pneumophila in drinking water: a meta-analysis

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Characterizing the relationship between monitoring results obtained with qPCR and culture-based methods can help inform the development of preventive risk-based approaches for the management of Legionella in water systems. This study presents a framework to compile, review, and model the qPCR:culture relationship for *Legionella pneumophila* concentrations measured in water from full-scale potable water systems. Statistical models were developed to evaluate the distributions of the qPCR:culture ratios from published data. Three applications of the model are demonstrated: 1) site-specific analysis of a ratio distribution, 2) meta-analysis of mean ratios from multiple studies, and iii) subgroup analysis to evaluate the influence of environmental factors on ratios. Although meta-analysis results show an arithmetic mean qPCR:culture ratio lower than 10:1 for around 60% of site-specific datasets, a single adjustment factor of 1:1 would be overly conservative for some sites. Collecting water quality parameters and documenting water quality profiles within buildings would be valuable to develop system-specific adjustment factors.

P-116 Degree of enrichment determines PHA accumulation potential in activated sludge

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Efforts continue to scale up microbial community-based polyhydroxyalkanoate (PHA) production. Microbial community-based PHA production can be categorized into enrichment accumulation and direct accumulation depending on biomass source. Enrichment accumulation applies optimal feast-famine regimes to grow functional biomass. Essentially 100% of the biomass will be PHA-accumulating microorganisms, but maximal PHA contents ranged between 0.4 and 0.9 gPHA/gVSS. Direct accumulation uses waste activated sludge from wastewater treatment plants (WWTP) as the biomass. Biological wastewater treatment processes can inherently apply selection pressures which also promote the growth of PHA-accumulating microorganisms. Direct accumulation with activated sludge has also reached PHA contents in excess of 0.5 gPHA/gVSS. However, the distribution and fraction of the PHA storing biomass in activated sludge has not been well studied. Therefore, methods of selective staining of PHA, with counter-staining other biomass components, were developed with microscopy and image analyses for visualization and quantitative assessment of degree of enrichment for activated sludge.
Direct PHA accumulation was performed with full-scale municipal activated sludge from six Dutch WWTPs. 48-hours accumulation assays resulted in biomass average PHA contents from 0.18 to 0.42 gPHA/gVSS between respective WWTPs. Parallel samples were processed to evaluate PHA distribution in biomass. In distinction to average PHA content measurements, image analysis was applied to explicitly distinguish between phenotype enrichment (i.e. degree of enrichment) for PHA storing bacteria in open cultures, and the polymer accumulating potential of the enriched fraction of the biomass. Image analysis revealed that between WWTPs, 16% to 51% of the biomass was active in PHA storage. However, from the estimated degree of enrichment and average biomass PHA content, the accumulating potential of the accumulating fraction was similar and 0.58 gPHA/gVSS on average. Selective growth of the PHA storing fraction during direct accumulation could promote to reach maximum levels of PHA as typically observed with enrichment accumulation outcomes.

P-117* Characterization of novel candidate factors impacting HIV life cycle

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Throughout its life cycle, HIV interacts with host factors that can either promote (HIV dependency factors, HDF) or inhibit (HIV Inhibitory factors, HIF) viral replication. The success of HIV replication relies on the balance between HDF and HIF. Epitranscriptomics, i.e., the field of RNA modifications such as N6-methyladenosine (m^6A) methylations, provides a new layer of gene regulation and an additional opportunity to uncover novel cellular players involved in HIV replication.
We performed a differential analysis on m^6A epitranscriptomic landscape of CD4+ T (SupT1) cells, infected or not with an HIV-based vector over time (12h, 24h, 36h), and identified 59 m^6A hypermethylated transcripts [1]. We selected the top 10 consistently m^6A hypermethylated transcripts at the three time points, -for further investigation of their impact on HIV life cycle using a CRISPR-Cas9-mediated knock-out (KO) approach in Jurkat T cells.
The top 10 hypermethylated candidates revealed an enrichment for GTPases of the GIMAP protein family (known to be involved in T cell survival and T cell development), with GIMAP1, GIMAP5 and GIMAP7. We successfully generated and validated KO Jurkat cell lines for each GIMAP candidate, as well as for known HDFs as positive controls. We are currently assessing their impact on HIV life cycle upon infection with a GFP reporter HIV vector. Preliminary data so far suggests that GIMAP1 may inhibit HIV replication.
Finding novel actors modulating HIV replication through novel opportunities, such as epitranscriptomics, may improve current understanding of HIV biology and potentially provide an array of new therapeutic targets.

[1]Cristinelli, S.; Angelino, P.; Janowczyk, A.; Delorenzi, M.; Ciuffi, A. HIV Modifies the m6A and m5C Epitranscriptomic Landscape of the Host Cell. *Frontiers in Virology* **2021**, *1*, doi:10.3389/fviro.2021.714475

*Student presentation

P-118* Characterizing the initial trigger of the anti-nematode defense response of Coprinopsis cinerea using omnivorous nematodes

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Nematodes are the most abundant soil metazoans. Some species predate on plant roots and fungal mycelia. The chemical defense of the model mushroom *Coprinopsis cinerea* against these predators includes an inducible part. The vegetative mycelium of this fungus produces a series of nematotoxic intracellular proteins upon attack by the fungivorous nematode *Aphelenchus avenae*. *A. avenae* feeds on mycelia by inserting a protrusible stylet into the hyphal cell and extracting the cellular content in a syringe-like manner. Based on a variety of experiments including Fluid-FM, we hypothesize that the abrupt loss of hyphal turgor pressure caused by this type of feeding, triggers the production of nematotoxins in *C. cinerea.*
We recently isolated several unidentified nematodes from environmental samples. These nematodes possess a stoma typical for bacterivores and lack a stylet. Feeding tests revealed that these nematodes are capable of thriving on various food sources, including *Escherichia coli*, *Saccharomyces cerevisiae*, *Botrytis cinerea*, and *Coprinopsis cinerea*, suggesting an omnivorous feeding habits. Interestingly, the *C. cinerea* vegetative mycelium does not allow propagation of *A. avenae*, but it is permissive for the propagation of these unidentified omnivorous nematodes. This phenomenon may be attributed to the nematotoxins produced by *C. cinerea* being less toxic to these nematodes, and/or the different feeding mode of these nematodes which may not sufficiently trigger the inducible defense response of *C. cinerea*. The goal of this study was to find out whether non-stylet omnivorous nematodes can induce the anti-nematode defense in *C. cinerea*. To achieve this, we challenged *C. cinerea* mycelium with live fungivorous and omnivorous nematodes and comparatively assessed the expression of reporter genes for anti-nematode defense response. Additionally, we examined the feeding behaviour of these nematodes microscopically using a customized microfluidic device. These results will deepen our understanding of the mechanisms triggering the anti-nematode defense response in basidiomycetes.

*Student presentation

P-119* effects of dynamic substrate supply on methanogenesis

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Bioelectrochemical power-to-gas presents a promising technology for CO_2 reduction and long-term storage of excess renewable energy in the form of methane. The core of this technology- biocatalytic conversion of CO_2 to methane was carried out by (mainly hydrogenotrophic) methanogen. The successful application of the technology can be promoted by a better understanding of the physiology of methanogen, especially methanogenesis activities under different substrate conditions and intermittent supplies.
In batch cultures, we determined K_m for a mesophilic methanogen *Methanococcus maripaludis* and a thermophilic methanogen *Methanothermobacter marburgensis*. We then cultivated *M. maripaludis* in chemostat reactors and showed that H_2 and CO_2 limitations did not affect the yield or methanogenesis activity. To explore the effect of intermittent substrate on methanogen, we investigated *M. maripaludis*' recovery after being starved for H_2 *vs.* CO_2 using batch cultures. We found that *M. maripaludis* is more robust for H_2 starvation as compared to CO_2. Yet, the difference is only evident when cells are starved for more than 3 days: longer lag phases and lower initial methanogenesis rates were observed when *M. maripaludis* was starved for CO_2 *vs.* H_2 for 3 days and 5 days, respectively. These characterizations show methanogenesis is a robust platform for power-to-gas connected to intermittent power supplies from solar or wind.

*Student presentation

P-120 Prevalence of macrolide and fluoroquinolone resistance-associated mutations in Mycoplasma genitalium in urine and genital clinical specimens

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Mycoplasma genitalium, a sexually transmitted bacterium, is a common cause of non-gonococcal urethritis in men, and is associated with cervicitis and pelvic inflammatory disease in women. According to the European guideline, azithromycin is recommended as the first-line treatment and moxifloxacin as second-line treatment. However, a significant proportion of *M. genitalium* strains have acquired macrolide and fluoroquinolone resistance-associated mutations thus compromising the treatment effectiveness. As *M. genitalium* culture is fastidious, conventional bacterial culture-based resistance are not used in routine diagnostics. Fortunately, resistance-associated mutations can be detected by different commercial real-time PCR kits for macrolide and fluoroquinolone resistance. In this study, the prevalence of resistant bacterial strains was assessed using two kits, (1) Allplex™ MG & AziR Assay and (2) Allplex™ MG & MoxiR Assay (Seegene, Korea), on positive *M. genitalium* urine and genital clinical specimens. Among tested samples, 12/38 (31.6%) were resistant to macrolide and 2/49 (4.1%) were resistant to fluoroquinolone. One sample harboured resistance-associated mutations for both antibiotic classes (2.6%). These results are similar to those found in literature, confirming the need of implementing molecular resistance tests for each *M. genitalium* positive sample, especially for macrolide.

P-121* Establishing an infection model for studying Brucella antibiotic persistence and treatment using Brucella microti

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The gram-negative, facultative intracellular pathogens *Brucella melitensis, B. abortus and B. suis* are causative for major bacterial zoonoses. Brucellosis prevalence is high especially in low and middle income countries with insufficient surveillance and infection control in life stock. Infections are mostly caused by unpasteurized dairy products or occupational contact with infected animals. Treatment consists of a combination of doxycycline and either rifampicin or streptomycin for 6 weeks up to several months. Despite this harsh treatment, relapses occur in 5-15 % of the cases. Normally, work with *Brucella spp.* requires a BSL3 laboratory, however in the scope of this project we establish the rodent pathogen *Brucella microti* as a BSL2 surrogate for studying human Brucellosis. In this line an infection model based on human THP-1 cells differentiated to macrophages is being established. Using this model we aim at investigating the molecular basis of the high relapse rate and test for more efficacious drug combinations to treat human Brucellosis. In particular, we aim for investigating antibiotic persistence of *Brucella spp.* and repurpose existing antibiotics. Antibiotic treatment efficacy will be assessed by detecting survivor bacteria using a cumate-inducible dual reporter plasmid in fluorescence microscopy and by CFU plating. Finally, enriching the survivor population by flow cytometry will allow us to perform proteomic analysis for identifying potential targets playing key roles in antibiotic persistence.

*Student presentation

P-122 Methicillin-resistant Staphylococcus aureus among asylum seekers from a national asylum center in Basel, Switzerland

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Background
Asylum seekers are at increased risk for colonization with multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA). During an outbreak of *Corynebacterium diphtheriae* in a national asylum center in Basel, Switzerland in summer 2022, several patients with cutaneous diphtheria were diagnosed with MRSA-coinfection. To explore potential transmission pathways, we determined genetic relatedness of MRSA and *C. diphtheriae* strains isolated from the same patient population.

Methods
From 08/2022-11/2022, mainly cutaneous and respiratory samples were cultured for detection of MRSA and *C. diphtheriae* according to standard bacteriological methods. Isolates were whole genome sequenced (WGS) using Illumina NextSeq 500. Sequences were aligned and phylogenetic trees were generated using either Ridom SeqSphere+ or CLC genomics workbench. Epidemiological and clinical data are currently being collected and analyzed.

Results
Among 43 MRSA-strains from 35 patients, we identified 11 distinct sequencing types (ST), predominantly ST 672 (32%) and ST 1 (23%). Panton-valentine leucocidin (PVL) was confirmed in 27%. We were unable to identify a correlation between ST, PVL positivity, or *mecA* type. For seven patients in which both MRSA and *C. diphtheriae* were co-isolated from the same sample, we could not detect a relatedness between the clustering of *C. diphtheriae* and MRSA isolates. But the genetic difference between MRSA strains (range 36 to 182 alleles) was clearly larger than between *C. diphtheriae* strains (range 2 to 6 alleles).

Conclusions
We provide first data on the epidemiology of MRSA in asylum seekers that currently travel to Western Europe. The lacking correlation of clusters and the greater diversity of MRSA strains compared to *C. diphtheriae* strains isolated from co-infected asylum seekers, suggests MRSA-colonization precedes acquisition of *C. diphtheriae*. *C. diphtheriae* was more likely transmitted during travel or within asylum centers than MRSA. Subsequent analyses using detailed clinical and epidemiological data will expand the understanding of current transmission pathways.

P-123 Comparison of sensitivity of E-swab and Medical Wire and Equipment Sigma transwab utilising the Roche Liat in the context of an outbreak scenario of S. pyogenes in the United Kingdom

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Supply chain issues are commonplace within pathology, and it is good practice to have contingency for the various solutions to diagnostic testing we offer. An important element of any diagnostic test is the pre-pre-analytical component. The Roche Liat is validated for use as per instruction for use (IFU) utilising the Copan E-swab for the detection of S. pyogenes. Manufacturing facility for the Copan devices are in Italy and experience has taught that outbreaks can result in stock-outs. In addition, all collection devices have key raw material associated with them and again experience has shown that these raw materials can be unobtainable or delayed. As such it is prudent of all large pathology service to ensure there is contingencies built into the key components of the testing pathway. The efficacy of an alternative to the Copan E-swab was evaluated, namely the Sigma transwab utilising the Cobas Strep A Assay on the Roche Liat. An 0.5 McFarland suspension of group A Quantimetrix control and prepare serial 1:10 dilutions to 10^{-8} in phosphate buffered water was prepared. For each concentration a Copan E-swab (1ml) and a Sigma Transwab (1 ml purple top) was inserted for 30s and then returned to the tube. Each swab was vortexed for 30s and cultured onto CBA agar using swab to inoculate agar and liquid media for the Roche Cobas Liat Strep A assay (200µl). Testing was performed on the Roche Liat in triplicate and results demonstrated a similar performance for the detection of *S. pyogenes* between the swab types for the contrived samples and PCR results confirmed by culture up to 10^{-5}/10^{-6}dilution.

P-124* The effect of soil antibiotic contamination on the soil microbial community and its resistome

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Human waste-based fertilizers contribute to sustainable agriculture by reusing valuable nutrients. However, these fertilizers may contain antibiotics and antibiotic resistance genes (ARGs) which spread among soil microorganisms by horizontal gene transfer using mobile genetic elements (MGEs). The goal of this study was to investigate the effect of antibiotics, commonly found in human waste-based fertilizers, on the soil microbial community and its resistome.
A greenhouse trial was conducted where spinach and radish were exposed to a mix of five antibiotics (clarithromycin, sulfamethoxazole, trimethoprim, chlortetracycline and enrofloxacin). This mix was applied in four concentrations (0, 0.1, 1.0 and 10.0 mg/kg soil dry weight). The crops were grown for 6 weeks, after which samples were collected for metabarcoding (16S and ITS) and qPCR analysis. The metabarcoding data was analysed using a customised pipeline. For the qPCR, four ARG and two MGE targets were selected, namely *sul1*, *dfrA12*, *tetQ*, *qnrS1*, *intI-1* and *intI-2*.
Preliminary results of the metabarcoding show a shift of the soil bacterial community for both crops. Here, the 0 mg/kg treatment was significantly different than the 0.1 and 1.0 mg/kg treatment. For the resistome, *sul1* and *intI-1* were consistently found across all samples. Moreover, their abundance increased in the 0.1 and 1.0 mg/kg treatments compared to the 0 mg/kg control treatment. Detailed analysis of the soil microbial community, to be performed, will link the antibiotic distribution to the soil microbial community and its resistome.
Our study shows that low antibiotic concentrations have the potential to increase ARG abundance. While these genes are not necessarily present in pathogens nor do they usually spread to clinical settings, soil antibiotic resistance has been linked to antibiotic resistant clinical pathogens. Therefore, it is important to better understand how these genes behave and disseminate in agriculture with waste-based fertilizers potentially containing antibiotics.

*Student presentation

P-125* Investigation 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 successfully replicate in alveolar macrophages. During the intracellular state, *L. pneumophila* secrets over 300 “effector proteins” into host cells. This enables the formation of a *Legionella*-containing vacuole (LCV) by subverting phagosome maturation and other cellular processes. While this step is essential for the survival and replication of the pathogen, virtually all host cellular processes are targeted to further support the survival and successful replication of intracellular bacteria.
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. To this date, 3 effectors were found to localize to mitochondria, and at least 3 additional effectors effect mitochondrial dynamics by presumably acting outside of the mitochondria.
Preliminary results of a bioinformatic analysis show that an additional 16 effectors have a high probability of localizing to the mitochondrial network, with 7 effectors comprising a canonical mitochondrial targeting sequence at their N-terminus. To experimentally validate this finding, mitochondria from infected cells and associated effectors will be investigated by biochemical, and cell biological approaches. These studies will provide mechanistic insights into the virulence strategy of *Legionella* and will potentially make novel probes available for specific activation or inhibition of mitochondrial components.

*Student presentation

P-126* Clusters of patients with Pneumocystis jirovecii pneumonia harboring similar repertoires of genes encoding the major surface glycoproteins

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The surface antigenic variation system of the pathogenic fungus *Pneumocystis jirovecii* relies on reassortment of the repertoires of ca. 80 allelic genes encoding the major surface glycoproteins of family I (*msg*-I; Meier et al. submitted). The characterization of the repertoires present in 29 patients with *Pneumocystis* pneumonia from five different cities evidenced one cluster of three patients with identical repertoires, one cluster of four patients with similar ones, and one cluster of two patients with weakly similar ones. A novel genotyping technique consisting in sequencing the ITS1-5.8S-ITS2 region with PacBio circular consensus sequence confirmed only the former and the last clusters. The former cluster presented the peculiarity that one patient was diagnosed seven years after the two other ones. The transmission of the strain to this third patient may result from two not mutually exclusive hypotheses : (i) a chain of transmission involving unknown patients, suggesting a slow dynamics of reassortment of the *msg*-I repertoires, and (ii) acquisition of dormant *P. jirovecii* cells that were in the environment. The cluster with four patients was difficult to interpret. It might result from several transmission events of the fungus between the patients that led to the accumulation in each patient of numerous strains, coupled to an enrichment with specific *msg*-I alleles in the geographical area. The last cluster with weakly similar repertoires might reflect interhuman transmission followed by relatively fast dynamics of reassortment of the *msg*-I repertoires.

*Student presentation

P-127 Chlamydia trachomatis/Neisseria gonorrhoeae co-infection in men-who-have-sex-with-men: preliminary findings from the Swiss PrEPared cohort study

Dr. Enrique Rayo [1], **Dr. Delia Onorini** [1], **Dr. Cory Leonard** [1], **Dr. Hanna Marti** [2], **Mrs. Theresa Pesch** [1], **Dr. Benjamin Hampel** [3], **Dr. Nicola Low** [4], **Prof. Nicole Borel** [1]

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Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (NG) are more prevalent among men-who-have-sex-with-men (MSM) than among heterosexual men and women. According to epidemiological data, CT/NG co-infections are more frequent than would be predicted by chance. Interaction between these pathogens might therefore increase either susceptibility, transmission, or both. To better understand anorectal bacterial loads and viability of single and CT/NG co-infections, we studied MSM enrolled in the Swiss PrEPared program, an ongoing multicentre cohort of individuals seeking pre-exposure prophylaxis (PrEP) to prevent HIV infection. We present findings from 236 anorectal swabs from MSM suspected of having CT and/or NG. Using qPCR, we found CT in 71 (29.6%, Ct-mean cutoff <38) of the swabs and NG in 69 (29.2%, Ct-mean cutoff <36), with 16 (6.8%) of this subset containing both CT and NG. For PCR-positive samples, NG was isolated in plate culture for 65/69 samples (94.2%) and CT was isolated in cell culture in 29/42 samples (69%, processing still ongoing and evaluated in parallel by viability-PCR). In contrast to a previous study among MSM not taking PrEP, in our study bacterial load in CT/NG co-infections did not differ from single infections (CT Log10 loads mean = 3.75; SD (Standard Deviation) = 1.08 vs. CT/NG mean = 3.72, SD = 0.89, p-value 0.76; NG Log10 loads mean = 4.58, SD = 1.22 vs CT/NG mean = 4.32, SD = 1.02, p-value 0.44). In conclusion, CT/NG co-infections in anorectal infections in MSM warrant further investigation. We will continue collecting samples from MSM on PrEP while simultaneously enrolling a new cohort of non-PrEP users. The follow-up aim is to compare CT and NG anorectal bacterial loads and pathogen viability in a) single infection versus co-infection and b) PrEP versus non-PrEP MSM users.

P-128* Causes and Consequences of Early Life Malnutrition in Later Life

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Recent years have seen a rapid global surge in deaths linked to diet-related noncommunicable diseases such as type-2 diabetes, cardiovascular disease and metabolic syndrome (MetS). Currently, 38.9 million children are overweight and over 40% of all adults are overweight or obese. Recent evidence suggests that both early life under- and overnutrition contribute to an increased risk of metabolic disease in later life. Both forms of malnutrition share common pathophysiological hallmarks and their prevalence is of particular concern in low- and middle- income countries, where both forms of malnutrition can exist within individuals concomitantly or at different stages during their lifetime (“double burden of malnutrition”). Many aspects of health are driven by the gut microbiota and dysbiosis due to early life malnutrition may contribute to the physiological responses to nutrition-related disorders. Currently, the factors linking early-life malnutrition to MetS development in later-life remain unclear. We aim to elucidate the molecular mechanisms underlying the double burden of malnutrition through a neonatal mouse model of dietary *in utero* growth restriction or macrosomia. We will investigate a causal role of the gut microbiota and their metabolites in the trajectory from early life over- and undernutrition to MetS. Further, we focus on whether nutrition-induced maternal dysbiosis directly leads to inheritable changes in gene expression though DNA methylation patterns of relevant genes linked to nutrient absorption, metabolism, and inflammation and/or if these changes are induced by the early-life microbiota of malnourished subjects.We observed that offspring born to undernourished dams show an increased weight gain upon exposure to a high-fat diet at weaning compared to overnourished or control mice. It is essential to understand these underlying molecular mechanisms in a bid to design targeted preventable measures and treatments.

*Student presentation

P-129 C. difficile abundance and gut microbiota composition in the first three weeks after antimicrobial treatment for C. difficile infection

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Background
Antimicrobial treatment is a major risk factor for development of *Clostridioides difficile* infection (CDI) and antimicrobial treatment for CDI further disturbs the endogenous gut microbiota, contributing to the risk of recurrent CDI (rCDI). Asymptomatic *C. difficile* colonization can be observed in 4-15% of healthy people. Yet, little is known about asymptomatic *C. difficile* colonization directly after treatment of a CDI episode. We here studied *C. difficile* abundance and gut microbiota composition in the first three weeks after antimicrobial treatment for CDI.

Methods
14 CDI patients were enrolled for the study and fecal samples were collected on a weekly basis during the first three weeks after antimicrobial treatment for CDI. Clinical data, frequency of bowel movement and stool consistency were retrieved during telephone-follow ups on the same days as fecal sample collection. Gut microbiota composition and relative *C. difficile* abundance were studied by amplicon-based 16S rRNA sequencing (HVR 1-9, QIAseq® 16S/ITS panel, Qiagen) on an Illumina MiSeq instrument (paired end 2x300 nt). Sequencing data was processed with an in house developed pipeline and NAMCO.

Results
C. difficile was present with varying abundance (>0.01 % to 37 %) in 85 % of participants/ 65% of samples one to three weeks after antimicrobial treatment for CDI. At the last time-point 58 % of samples remained positive for *C. difficile*. *C. difficile* abundance > 0.02% agreed well with *C. difficile* NAAT(GeneXpert). Mean gut microbiota alpha-diversity increased over the three weeks study period, but with high individual variation.

Conclusion
Our preliminary results show that *C. difficile* can be detected with varying abundance in the majority of participants (85%) and samples (65%) in the first three weeks after CDI. Our findings provide the basis for further research into asymptomatic *C. difficile* colonization and the role of the endogenous gut microbiota for protection against rCDI.

P-130 Prevalence and genetic characteristics of ESBL-producing E. coli ST131 in wastewater and environmental water in Switzerland

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The *E. coli* lineage ST131 is a major cause of multi-drug resistant urinary tract and bloodstream infections worldwide. Recently emerged ST131 sublineages spread globally within few years, but their dissemination routes are incompletely understood. In this study, we investigate the potential role of wastewater and surface water in the spread of extended-spectrum β-lactamase (ESBL)-producing ST131. Streams, lakes, and two wastewater treatment plants (WWTPs) in the canton of Zug, Switzerland, were consecutively sampled over 1.5 years. ST131 was detected in 38 % of the samples taken downstream (1 to 5 km) of WWTP discharge sites, but usually absent in water bodies distant from urban areas or WWTP discharge. Strains recovered from wastewater were often re-isolated (≤ 5 pairwise cgSNP distance) from flow-connected rivers, indicating their repeated entry or residency in WWTPs in large concentrations. Genetic characterization of the ESBL-producing water isolates revealed a predominance of clades A and C1 and an emerging ciprofloxacin-resistant sublineage with mutations in quinolone-resistance determining regions within clade A. Multiple isolates belonged to internationally circulating sublineages, including C1-M27 and *papGII* sublineages with chromosomally encoded ESBLs. In summary, our study shows that ST131 is commonly released into the environment through wastewater discharge, which may lead to the contamination of wildlife and food products and thereby contribute to the dissemination of this lineage.

P-131 Effect of photodynamic therapy on skin microbiome (PHOMIC-III)

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Background
Current skin antisepsis protocols to prevent surgical site infections are ineffective to eradicate all skin-colonizing bacteria in up to 50% of patients. Photodynamic therapy (PDT) with the photosensitizer methyl-aminolevulinate (MAL) and a red light source has shown bactericidal effects *in vitro* and improvement of skin antisepsis clinically, achieving complete eradication of skin colonizing bacteria in all subjects, although with local side effects. Therefore, we explored the application of MAL-PDT with daylight (DL) in healthy participants for its potential use to sterilize skin without causing irritation.

Methods
We investigated the effect of MAL-PDT-DL (Daylight PDT-1200) versus no PDT in combination with skin antisepsis (povidone-iodine/alcohol) on the groin skin of 5 healthy study participants. Skin swabs were taken at baseline, immediately after PDT and after skin antisepsis treatment to cultivate bacteria. At day 3, bacterial cultures were repeated before and after antisepsis treatment without PDT.

Results
Interim results from 5 study participants using MAL-PDT-DL showed the presence of skin-colonizing bacteria in all participants at baseline sampling. Immediately after MAL-PDT-DL, no bacterial growth was detected in 2 (40%) participants before and in 5 (100%) participants after skin antisepsis treatment. In contrast, we found skin-colonizing bacteria in 2 (40%) participants of the control group receiving only skin antisepsis. After 3 days, skin sterility was similar to the baseline irrespective of group. MAL-PDT-DL was well tolerated without any skin irritations.

Conclusion
MAL-PDT-DL with skin antisepsis treatment improved bacterial reduction on the skin immediately after its use compared to antisepsis only. However, the effect did not persist for 3 days post-treatment; skin bacteria similar to baseline were found. Importantly, MAL-PDT_DL was well tolerated. This proof-of-concept study shall provide the fundament for a prospective study of patients with planned orthopedic surgery to investigate safety and efficacy of MAL-PDT-DL to prevent infections.

ClinicalTrials.gov Identifier: NCT05676801

P-132 Comparison of two methods of susceptibility testing on Vancomycin resistant Enterococci

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Enterococcus can cause urinary infections, sepsis and endocarditis with an ability to easily spread among hospitals. Glycopeptides such as vancomycin and teicoplanin are effective against *Enterococcus* resistant to Beta-lactam antibiotics. However, resistance can be found, in particular with Vancomycin-resistant *Enterococcus* (VRE) expressing *van* genes.
In our laboratory, antibiotic susceptibility testing of *Enterococci* is performed using the VITEK®. In case of VRE suspicion, a MIC gradient tests was performed in addition to a *vanA/B* PCR using the GeneXpert system. However, EUCAST issued a warning that MIC gradient tests fail to detect VRE in low-level resistant enterococci. An additional method for MIC determination and confirmation was therefore required.
Our objective is to evaluate and compare MICRONAUT® (a microplate-photometry reading) with VITEK®2 on VRE strains to detect glycopeptides resistance.
28 strains of *Enterococcus,* including *E.faecium*(n=17), *E.faecalis*(n=11) were selected and *van* genes were molecularly characterized. MIC were determined using MICRONAUT® and VITEK®2 method.
Essential agreement (EA), categorical agreement (CA), minor error (MI), major errors (ME) and very major errors (VME) were determined as well as reproducibility and repeatability testing.
Our data shows a CA of 89% and 96% and a EA of 79% and 82% for vancomycin and teicoplanin with no VME and MI detected. Five strains carrying *vanB*, *vanL* and *vanE* genes shows discordances. Two (*vanL* and *vanE*) were confirmed as sensitive due to a lower level of resistance, three *vanB* strains were detected as resistant only by MICRONAUT®.
In conclusion, MICRONAUT® shows promising results with increased detection of VRE on *vanB* strains, suggesting a lack of sensitivity of our actual system. However, the system has limitations i)*E.faecalis* and *E.faecium* were the only species able to grow, ii)due to its long procedure,its implementation in the laboratory routine would be difficult but could be reserved to particular situations where glycopeptides MIC are clinically relevant.

P-133 Canine Staphylococcaceae circulating in a Kenyan animal shelter

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Animal shelters bring together pets from different regions and history. This study investigated nasal Staphylococcaceae of sheltered dogs in Kenya, to determine their genetic relatedness, the presence of dominant clones and antibiotic resistance profile. Nasal swabs were collected from >100 crossbreed dogs in 2015 and screened for Staphylococcaceae using standard cultivation techniques. Complete genomes of 92 strains isolated were obtained using PacBio long-read sequencing. Strains encompassed nine validated species, with S. aureus (n=47), S. pseudintermedius (n=21) and Mammaliicoccus (M.) sciuri (n=16) being the most dominant species. Two S. aureus clones of ST15 (CC15) and ST1292 (CC1) were isolated from 9 and 37 dogs, respectively. Strains were tested for their antimicrobial susceptibility by determining the minimum inhibitory concentrations (MICs). Eighty six strains had non-wildtype minimal inhibitory concentrations to at least one of the following antimicrobials: tetracycline, benzylpenicillin, oxacillin, erythromycin, clindamycin, trimethoprim, gentamicin or streptomycin, encoded by tet(K)/tet(M)/tet(L), blaZ, mecA/mecA1, msrA/mphC/erm(A)/erm(B), salA/lnu(A)/lnu(B)/lsa(E), dfrG/dfrK, aac(6')-aph(2'') and str, respectively. Many virulence-encoding genes were detected in the S. aureus strains, other Staphylococcaceae contained less virulence-encoding genes. Plasmids and prophage sequences were linked to distinct resistance and virulence-encoding genes. The unsuspected high presence of S. aureus clones in many dogs suggests dissemination within the shelter and a human source.

P-134 Fourier-transform infrared spectroscopy for typing of vancomycin-resistant Enterococcus faecium – performance analysis and outbreak investigation

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Background

Vancomycin-resistant Enterococci, mainly *E. faecium* (VREfm), are causing several nosocomial infections and outbreaks in hospitals worldwide. Bacterial typing methods are used to assist outbreak investigations. Most of them, especially the genotypic methods like multi-locus sequence typing (MLST), whole genome sequencing (WGS) or pulsed-field gel electrophoresis, are quite expensive and time consuming. Fourier-transform infrared (FT-IR) spectroscopy assesses the biochemical compositions of bacteria, such as carboxy bond in polysaccharides. It is cheaper and has a faster turnaround time. Thus, the aim of this study was to evaluate FT-IR spectroscopy for VREfm outbreak investigations.

Methods

Basic performance requirements like reproducibility and effects of incubation time were assessed in distinct sample sets. After determining a usable cut-off range, the clustering agreement within a retrospective (n: 92 isolates) and a prospective outbreak (n: 15 isolates) were investigated and compared to MLST and WGS by using an average nucleotide identity (ANI) cut-off score of 0.999.

Results

Basic performance analysis showed reproducible results. Moreover, FT-IR spectroscopy readouts showed a high agreement with WGS-ANI analysis in clinical outbreak investigations (V-measure 0.772 for the retrospective and 1.000 for the prospective outbreak). FT-IR spectroscopy had a higher discriminatory power than MLST in the outbreak investigations.

Conclusion

After determining cut-off values to achieve the optimal resolution, FT-IR spectroscopy is a promising tool to assist in outbreak investigation as an affordable, easy-to-use tool with a turn-around-time of less than one day.

P-135* how are bacterial telomeres protected from cellular nucleases?

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In all living cells, genome integrity is constantly threatened by various types of DNA damage. Double-strand breaks (DSBs) are amongst the most harmful DNA lesions. A key player in the recognition, signaling, and nucleolytic end processing of DSBs is the Mre11-Rad50 (MR) complex. Previous studies provided a unified mechanism for MR endonuclease, exonuclease, and hairpin-opening activities. Due to their similarity to DSBs, the ends of linear chromosomes can be mistaken for broken or damaged DNA and therefore need to be protected from the MR end-processing machinery. While hairpin telomeres are an ideal substrate for MR, little is known about how these structures are protected from nuclease degradation. The *E.coli* bacteriophage N15 is not integrated into the bacterial genome but exists as a linear DNA molecule with covalently closed hairpin ends. Hairpin telomere resolution is catalyzed by the phage-encoded protelomerase TelN using a cleaving-joining activity. Our work implicates TelN in hairpin telomere protection from MR in bacterial cells. We are developing genetic tools and biochemical assays to better understand the role of TelN in hairpin telomere protection. We envisage several possible mechanisms for hairpin end protection that may be partially overlapping.

*Student presentation

P-136 stabilisation of microbial gut microbiome composition in preanalytics: a problem solved!

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Because of its strong influence on human health, the gut microbiome has become a hot topic in fundamental research, clinical studies and routine diagnostics. To achieve reliable, consistent and comparable results, the microbial composition of faecal samples requires stabilisation immediately after specimen collection. The Calex® NGS sampling device from Ortho-Analytic meets the preanalytical expectations for reliable and reproducible microbiome analysis. The device enables easy sampling, preservation of the microbiome and standardized automatable next generation sequencing – thus exemplifying a new golden standard in microbiome analysis.
The Calex® NGS was adapted from Bühlmann' well-known Calex® Cap for Calprotectin measurements. 10 mg of stool per specimen are stabilised in a buffer solution. The special buffer inactivates bacteria, viruses and yeasts while simultaneously stabilizes RNA/DNA at room temperature for at least 30 days. Upon arrival in the laboratory, samples are ready for nucleic acid extraction without further treatment. Bacterial DNA for NGS analysis can be extracted automatically on the Tecan DreamPrep® NAP using the ZymoBIOMICS™-96 MagBead DNA Kit.
To demonstrate robustness and reproducibility, 3 persons collected stool samples in 5 Calex® NGS at 5 different locations per specimen. The correlation value average for each person was > 99.7 %. A study with the HUG, taking triplicated samples of 3 patients and performing DNA extraction, library preparation and sequencing in two laboratories showed > 98 % correlation, even though library preparation and DNA sequencing was done using different protocols. Furthermore, using the Calex® NGS, the external quality control trial for gut microbiome analysis by INSTAND e.V. was passed with 60/61 points (98.5%).
In summary, the Calex® NGS allows for robust and standardized results of microbiome analysis. It combines very easy sampling and stabilisation, thereby allowing high quality analytics. Thus, the Calex® NGS might be used in clinical research as well as in diagnostic laboratories.

P-137

The GENeva PHage (GENPH) collection for phage therapy against difficult-to-treat K. pneumoniae

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Klebsiella pneumoniae is the second most common uropathogenic Gram negative bacteria, associated with both community-acquired and catheter-associated urinary tract infections (UTI). Phage therapy (PT) is regaining interest as an alternative or adjuvant therapy to antimicrobials for the treatment of recurrent UTIs. The emergence of phage-resistant *K. pneumoniae* after phage infection is a major hurdle for developing such approaches. The GENeva PHage (GENPH) Collection is a phage biobank against antibiotic resistant and hypervirulent *K. pneumoniae* prevalent lineages, available as a resource for researchers and clinicians to develop efficient phage combinations for treatment. Establish an efficient in vitro phage combination against a clinical ESBL-producing *K. pneumoniae* isolate (ST869, KL23) causing recurrent UTI in a female patient to enable personalized PT using the GENPH Collection. A phagogram, comprising 160 phages, was performed to select phages active against the provided clinical isolate. Encapsulated and non-encapsulation subpopulations were separated and proceeded to confirm which phages were active against each fraction in double-layer agar. Turbidity reduction assays (OD_{600}) of selected phage pairs targeting each subpopulation were tested in 24-well plates against the *K. pneumoniae* clinical isolate at different MOIs. Synergistic activity was observed for combinations of capsule-dependent and -independent phages. While single phages resulted in bacterial regrowth after 4h, growth inhibition continued for >16h with the combination of phages GPH149 and GPH154. The best synergistic combination was chosen for future patient treatment. This strategy allows to predict and mitigate the risk of phage resistance emergence under treatment in *K. pneumoniae*.

P-138

Cancelled

P-139 Risk assessment of alimentary transmission of tick-borne encephalitis viruses from goats to humans by means of milk and milk products in Swiss alpine regions

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Viral zoonosis tick-borne encephalitis (TBE) is usually transmitted to humans by the bite of an infected tick. Another possibility to become infected with the viral pathogen is through the consumption of raw milk and raw milk products. Based on the seroprevalence of antibodies against TBE viruses in goats in the Valais canton in a recently published study, a risk assessment for viral contamination of goat milk was performed for this area. The probability of virus-contaminated milk was calculated to range between 0.0012% and 0.024% of household milk.

P-140* CELL wall deficient L-Forms as persisters enabling survival of bacterial populations under adverse conditions

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The cell wall of Gram-positive bacteria is an important target for various antibacterial agents, ranging from antibiotics such as β-lactams to bacterial viruses (bacteriophages) and cell wall targeting enzymes. Interestingly, under osmoprotective conditions bacteria may lose the cell wall allowing them to survive the lysis induced by cell wall antagonists. These cell wall deficient bacteria, termed L-Form, are suspected to sustain chronic and recalcitrant infections such as urinary tract infections. Previous work in our laboratory showed that *Enterococcus faecalis* cultured in human urine can evade lysis from without by cell wall targeting, recombinant phage-endolysins by converting to L-Forms. The L-Form conversion may also occur within human host cells, as shown by another researcher in our laboratory using the invasive, foodborne pathogen *Listeria monocytogenes*. It could convert to L-Forms within human placenta cells upon treatment with a β-lactam, indicating that evasion of antibiotic treatment in this way may be possible. However, most virulent bacteria are crucially dependent on cell wall associated virulence factors and indeed L-Forms are often heavily attenuated in virulence or even completely avirulent. Therefore, a persisting L-Form is required to revert to a walled, virulent state. We aim to experimentally validate that L-Form conversion and reversion can provide an escape mechanism during antimicrobial treatment in a human host. Especially with phage-derived therapy on the rise, we anticipate that L-Forms may become more important in chronic or recalcitrant infections. The elucidation of genetic and physiological prerequisites for the persistence as L-Forms in Gram-positive bacteria may incite research on improved antibacterial agents or novel targets altogether.

*Student presentation

P-141* New Horizons of the BASEL Phage Collection: A Comprehensive Resource for Exploring Bacteriophage Biology and Phage-Host Interactions

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Bacteriophages, the viruses infecting bacteria, are the most numerous biological entities on Earth and possess an overwhelming genetic diversity. However, our understanding of the molecular mechanisms underlying phage-host interactions has predominantly focused on a few classical phage models, leaving a vast reservoir of viral diversity and antiviral/antibacterial defenses largely untapped. Additionally, the lack of systematic, interconnected data on phage genomes and phenotypic properties hinders a comprehensive understanding of the mechanistic complexity driving phage biology.
To overcome these problems, we have composed the BASEL collection and its new expansion pack that together form a reference set of 105 newly isolated phages infecting *Escherichia coli* K-12. Notably, the expansion pack now includes phage groups that have previously been missed, e.g., because they depend on O-antigen glycans as host receptor which are absent in laboratory *E. coli* strains. The assorted diversity of BASEL phages makes this collection a powerful tool to systematically study the bacteriophage ecology, evolution, and molecular mechanisms underlying phage-host interactions across all major viral groups infecting *E. coli*. Our extensive phenotypic and genomic analyses of these phages directly resulted in discoveries regarding the molecular basis of phage host range, receptor specificity, and their susceptibility or resistance to various bacterial immunity systems.
The BASEL (BActeriophage SElection for your Laboratory) collection has already been shared with multiple laboratories worldwide to promote an inclusive, collaborative, and systematic exploration of phage-host interactions beyond well-studied model phages. We envision that this research will uncover new fundamentals of phage biology leading to advancements in the rational selection and engineering of bacteriophages for applications in clinics and biotechnology.

*Student presentation

S-06*/P-142* Novel attachment inhibitors against human parainfluenza 3 virus with broad-spectrum activity

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Respiratory viruses can cause severe infections leading to bronchiolitis or pneumonia. Human parainfluenza viruses are human respiratory viruses causing severe infections, especially in young children, elderly people, and immunocompromised. Human parainfluenza 3 virus (PIV3) is the most common type and can cause lower respiratory infections leading to hospitalization. Unfortunately, we are lacking vaccines and antivirals against this virus. With the aim to develop new antivirals, we designed attachment inhibitors endowed with virucidal activity and broad-spectrum activity against respiratory viruses.
To identify the natural attachment receptors used by PIV3, we performed a glycan array with two clinical isolates from university hospitals and with a laboratory strain. LS tetrasaccharide d (LSTd), an α2,3 sialic acid-based glycan present in the human respiratory tract, and heparin octasacharide were the top common hits. We synthesized modified cyclodextrins harboring LSTd (CD-LSTd) or sulfonates (CD-MUS). The structure of the macromolecule was chosen from previous work in which it was shown to confer not only antiviral activity but also virucidal activity. Both molecules showed antiviral and virucidal activity in vitro, although the sulfonated had higher potency. Additionally, they retained activity in human-derived respiratory tissues. Combination experiments suggest different proximal binding sites. Therefore we designed and synthesized a unique modified cyclodextrin mimicking both attachment receptors (CD-LSTd/SO_3) with higher potency compared to CD-LSTd. The modified cyclodextrin mimicking sialic acid (CD-LSTd) showed as well antiviral activity against different subtypes of Influenza A virus such as H1N1 or H5N1 and Influenza B virus showing its broad-spectrum activity.
Current work is dedicated to the optimization of the synthesis to achieve better potency and virucidal activity for optimal therapeutical administration as well as *in silico* modeling of the mechanism of action and resistance studies. Altogether, we designed promising new antivirals active against clinical PIV3 with a broad-spectrum activity against other respiratory viruses.

*Student presentation

S-08/P-143 Towards Label-Free Recognition of Single Cell Bacterial Species Based on Phase Contrast Timelapses

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Understanding the spatial-temporal dynamics of single-cell bacteria is crucial for unravelling colony formations and bacterial interactions, making it a fundamental aspect of microbiome research. However, traditional approaches like bacterial labelling are laborious, time-consuming, and challenging to scale up, particularly in complex microbial ecosystems. In this study, we propose a machine-learning approach that leverages the morphology of single cells from phase-contrast images, added with information autofluorescence across the spectrum measured in bins.
Building upon the work of Helena Todorov, who developed Dimalis—a pipeline capable of identifying and tracking cells in timelapse images while enabling feature extraction of cell masks and their fluorescence—we apply this methodology to a subset of a synthetic soil microcosm. The community consists of seven distinct bacterial species (Burkholderia, Curtobacterium, Microbacterium, Mucigalinibacter, P. *putida*(GFP), and P. *veronii*(mCherry)) that are seeded onto agarose patches prior to imaging. Using a random forest model trained on data from a single timepoint [2 hours after seeding], we achieved a classification accuracy of 94% when applied to in silico mixed single-cell observations of monocultures. Our ongoing work involves adapting the model to incorporate temporal data. Furthermore, we are testing the model on triple cultures, where the labelled P. *putida* and P. *veronii* serve as ground truth, enabling the identification of a single remaining species.
The proposed label-free recognition approach holds significant potential for advancing our understanding of bacterial dynamics in diverse environments, overcoming the limitations of traditional methods and paving the way for broader applications in microbiome research.

S-14*/P-144* co-immobilisation of a defined gut consortium for continuous cultivation – a demonstration study

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Bacteria in the gut are found to grow in both planktonic as well as sessile lifestyle. Food particles and the mucosal layer provide a spatial dimension to which certain microbes can adhere. Recent studies have shown that these planktonic and sessile communities can widely differ from one another.
This study aimed to investigate the possibility to immobilise a defined gut consortium. We hypothesised that immobilisation can be used to mimic both growth lifestyles to enable stability and can result in different communities in one bioreactor allowing for microbial ecology studies.
We co-immobilised a previously established gut consortium, consisting of 9 species, into porous polymer beads that were used to inoculate bioreactors. After two consecutive batch fermentations for bacterial bead colonization, the bioreactors were operated further under continuous mode for 14-18 days. Operation parameters (retention time and CO_2-headspace gassing) were altered throughout the continuous fermentation to investigate changes in the sessile and planktonic bacterial communities. Bacterial communities were assessed by 16S amplicon sequencing. Effluent metabolites were quantified by HPLC-RI.
All 9 species established in both bioreactors. Main end metabolites and abundant taxa stabilised. However, species presence and abundance strongly depended on the community type (sessile or planktonic) and the fermentation operation. Three species (*L. rhamnosus*, *E. limosum* and *P. faecium*) were most abundant in the bead (sessile) community, while these were generally subdominant in the effluent (planktonic) community. Conversely, species that dominated the effluent community were either low in abundance or absent in the bead communities. Retention time switch and CO_2-limitation did only affect the effluent community composition and metabolites.
Taken together, we demonstrate that co-immobilisation of a defined gut consortium offers a novel tool to investigate biotic or abiotic interactions in both, sessile and planktonic defined gut communities in parallel.

*Student presentation

S-15*/P-145* Habitat fragmentation and single-cell heterogeneities create ecological opportunities for reversal of bacterial competition

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Far from growing in well-mixed suspended cultures, bacteria in nature often proliferate in highly structured and fragmented habitats (*e.g.* soil pores, plant roots, animal guts). Fragmentation leads to bacteria being constrained to smaller habitats, shared with potentially fewer other species. Microbial communities in such fragmented systems at a larger scale can therefore be considered as assemblies of micro-communities interacting within the boundaries of each microhabitat. The effects of micro-habitats on the emergence of bacterial interspecific interactions are poorly understood, but potentially important for the maintenance of diversity at a larger scale. To study this more precisely, we decided to cultivate species-pairs in picoliter droplets and study emerging interaction behaviour. We focus on four possible competition scenarios: imposed substrate competition, substrate indifference, tailocin killing and antibiotic inhibition. We used fluorescently tagged bacterial strains to track their growth in individual droplets with epifluorescence microscopy and flow cytometry, in contrast to their growth in liquid-suspended culture as non-fragmented habitat control. In contrast to liquid culturing, we observed that competition was partly reversed in a fragmented culture, which was a direct consequence of more variable founder cell physiologies and small founder population sizes. Mathematical simulations confirmed that a weaker competitor can take advantage of growth heterogeneity to outcompete a stronger competitor at a small population size, and suggested that a population which displays a wider range of growth kinetics among its cells will increase its chances at the micro-scale to establish in a fragmented habitat. Additionally, we observed that tailocin-producer bacteria could more efficiently kill tailocin-sensitive bacteria at small population sizes in micro-habitats, creating opportunities for a tailocin producer to dominate sensitive strains in rare cases. Our results thus demonstrate how habitat fragmentation can support alternative competitive outcomes and contribute to the maintenance of higher species diversity.

*Student presentation

S-21*/P-146* CRISPRI-Seq identifies genes enhancing phage endolysin Cpl-1 susceptibility in *S. pneumoniae*

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Streptococcus pneumoniae is a human pathogen killing above 1.6 million people per year. Moreover, antibiotic resistance remains a significant issue, which justifies the development of complementary strategies. Cpl-1 is a bacteriophage lytic protein (lysin) that has been shown to have high potential for clinical use. In this study, we aimed at using the novel CRISPRi-seq method to potentially identify genes interfering with Cpl-1 mechanism of action. Accordingly, we used an IPTG-inducible pooled CRISPRi composed of a 1928 sgRNAs library. Strain D39V was shotgun transformed with the library before being challenged with Cpl-1. Then, we compared the fitness of the sgRNAs to identify genes potentially involved in an increase of Cpl-1 susceptibility or tolerance. Identified genes were then further investigated using single CRISPRi clones or knockout mutants by kinetic growth assay. Eight genes were initially found to be linked to an increase in Cpl-1 susceptibility (*rodA, uppP, pbp2b, mreD, mreC, tacL, cps* and *mvk* genes) and seven to an increase in lysin tolerance (*divIB*, *ftsE*, *ftsX, divIVA*, *pgm*, *guaB* and a gene coding for a hypothetical protein). Further experiments confirmed the involvement of *rodA*, *uppP*, *pbp2B*, *mreD*, *mreC* and *mvk* genes as Cpl-1 susceptibility factors. However, no significant increase in Cpl-1 tolerance was observed for the candidate genes, which was not surprising given that natural selection of resistant clones has neither been reported in vitro nor in vivo. Although supplementary experiments are needed, we obtained promising preliminary results guiding the development of potential synergistic strategies for the treatment of pneumococcal infections

*Student presentation

S-24*/P-147* Single-gene functional interrogation of bacterial secondary bile acid dehydroxylation in *Clostridioides difficile* colonization resistance

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Clostridioides difficile is a common cause of nosocomial and antibiotic therapy associated intestinal infection. Research of the past ten years has identified strains belonging to the Lachnospiracea family that are able to 7α-dehydroxylate primary mammalian bile acids as major contributors to intestinal colonization resistance against *C. difficile*. A wealth of correlative data from *in vitro*, animal and human studies has since led to the hypothesis that bile acid 7α-dehydroxylation (7DH) is causally related to the protective effects of *Clostridium scindens* and related 7α-dehydroxylating organisms. Recent reports have begun to challenge this hypothesis, but unavailability of a genetic system in these organisms has so far precluded testing causality more specifically using isogenic 7DH deficient *bai* gene mutant strains. In the presented work, we have studied bile acid metabolism and *C. difficile* resistance functions of a newly available 7DH-deficient *baiH* mutant of *Faecalicatena contorta*, its 7DH-competent wild-type parent strain, and a well-characterized wild-type *C. scindens* strain in a gnotobiotic mouse *C. difficile* infection model. A comprehensive correlation analysis of 7DH-dependent bile acid metabolome and *C. difficile* infection resistance will be presented. To our knowledge, this is the first single-gene functional interrogation of bacterial 7DH in *C. difficile* infection resistance.

*Student presentation

S-41/P-148 Bacttles: a microbiology educational tool for general public and schools

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Serious games have gained special attention in the past years due to the increasing number of studies that report such strategies as beneficial among different levels of education. However, it is hard to find available educative tools that target very young audiences (primary school) in science-related topics. As part of my PhD thesis and motivated by my personal interest in scientific outreach, I present 'Bacttles', an educational tool for microbiology in the form of a strategic and easy-to-play card game that targets a wide range of ages thanks to its customizable degree of complexity. Bacttles addresses both the general public and teachers that wish to use it in their classrooms as a way of introducing microbiology concepts. Background information to teachers will be provided in form of an educator's booklet, which can also be used for informing interested lay people.
The lay out of the game and its mechanism evolved over time after multiple rounds of trial and re-design. The final version consists of a deck of cards, a 3D-printed board and tokens (with its paper-based alternative), and the educator's booklet, all digital content open source. The goal of the game is to be the most abundant species in a microbial community. Players start with a defined number of members in the community that may increase or decrease based on how they handle the environmental challenges that are presented.
In order to assess the impact of the game on the players' understanding of microbiological concepts, we targeted university open day fairs. Visitors playing the game were given a brief questionnaire before and after playing, in order to score differences in the acquisition of general concepts and concept extrapolation, and to score the general appreciation of the game. The game strategy and the results will be presented in this contribution.

POSTER PRESENTATION

S-58*/P-149* **Optical trapping of bacteria for ultrafast bacteriophage lysis detection at the single-cell level**

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Regarding their high bacterial strain specificity, rapid and accurate selection of therapeutic bacteriophages is crucial in phage therapy clinical protocols. Here, we report the use of photonic crystal cavities as on-chip optical nanotweezers for ultrafast phage susceptibility testing (PST) at the single bacterium level. On a silicon photonic chip, resonant photonic crystal cavities allow the trapping of a single *Escherichia coli* B cell and sensing its stressed-induced modifications. This is achieved by monitoring the transmitted optical power through the photonic chip that carries information about the bacterium's characteristics. *E. coli* cells were put in contact with T4 *Myoviridae* phages before being injected in the trapping device. We report direct observation of a bacterium-phage lytic event in the optical cavity. The cell's morphological changes caused by the phage activity prior and after lysis are detected via the transmitted power as well through a microscope imaging system. The lytic event leads to a sudden refractive index reduction, which is attested by an abrupt drop in transmission and a reduced imaging contrast of the cell. Accordingly, only 40min ± 5min after mixing phages and bacteria (t = 0), we observe an abrupt decrease in transmission signal correlating with the bursts of the trapped bacterium. This detection of the lysis event is much faster than current culture-based phagograms usually requiring 16h-24h incubation times. This innovative phagogram approach paves the way to ultrafast PST at the single bacterium level.

*Student presentation

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