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ABSTRACT BOOK

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SHORT TALKS

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MUCOVIB project: Intracellular bacteria and fastidious organisms among children with Cystic Fibrosis, an unknown association

Dr. Laure Pittet [1]***, Dr. Claire Bertelli*** [2]***, Dr. Isabelle Rochat*** [3]***, Dr. Chiara Mardegan*** [4]***, Dr. Katia Jaton*** [5]***, Dr. Anne Mornand*** [6]***, Prof. Laurent Kaiser*** [7]***, Prof. Klara Posfay-Barbe*** [4]***, Dr. Sandra Asner*** [8]***, Prof. Gilbert Greub*** [9]

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Background

Only little is known on the prevalence of intracellular/fastidious bacteria (*Chlamydia pneumoniae*and *Mycoplasma pneumoniae*) in patients with cystic fibrosis (CF) and their impact in pulmonary exacerbations (PE) and microbiota biodiversity.

Methods

This multicenter prospective cohort study followed all children with CF during routine visits and PE. Oropharyngeal (OP) swabs and sputa were investigated by *16S rRNA* amplicon sequencing (MiSeq). Nasopharyngeal (NP) swabs were used to detect respiratory viruses and intracellular/fastidious organisms by PCR.

Results

Fifty-eight children with CF were included, of whom 22% (13/58) had documented PEs; intracellular/fastidious organisms were detected in 3 patients (2 with PE and 1 asymptomatic). Metagenomics analyses were conducted on 37 sputa collected from 16 patients, of whom 6 presented with an exacerbation. Among them, the 3 patients with a *Staphylococcus aureus* PE and the one with *Pseudomonas aeruginosa* PE had a remarkable and persistent loss of biodiversity. In contrast, the patient who presented a *C. pneumoniae*PE kept a high species richness with
similar genus distribution overtime.

Conclusions

Intracellular/fastidious bacteria were detected in more than 5% (3/58) of all children with CF, and 15% (2/13) of those presenting with a PE. While *C. pneumoniae* resulted in a severe clinical PE, no direct effect on the commensal microbial flora was observed. While our sample size was too limited to infer any association between intracellular/fastidious organisms and PEs or changes in bacterial biodiversity over time, the observed high rate of positivity suggests that children with CF, specifically those with a PE, should also be routinely screened for it.

** Student paper*

CM 1 – Metagenomics / CM-02

Nasopharyngeal and middle-ear microbiota in children with acute otitis media

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Background
Acute bacterial otitis media is usually caused by otopathogens ascending to the middle ear from the nasopharynx (NP). However, it is unknown if the nasopharyngeal microbiota of children with acute otitis media (AOM) can serve as an age-dependent or independent proxy for the microbial communities of the middle ear fluid (MEF) as there is a lack of 16S rRNA amplicon sequencing studies simultaneously analyzing the microbial communities of the two sites.
Methods
Within this study, we performed 16S rRNA next generation sequencing on a total of 286 nasopharyngeal swabs (NPS) collected between 2004 and 2015 from Swiss children (0-6 years) with AOM. In addition, 42/286 children had spontaneous tympanic membrane perforation and, therefore, additional MEF could also be analyzed.
Results
Alpha (Richness, SDI and Evenness) and beta diversity measurements of the nasopharyngeal bacterial microbiota showed a clear dependency of the increasing age of the children. Bacterial richness and personalized profiles (measured by beta dispersion) were higher and more frequent in older children, respectively. Dissimilarity values based on the binary distance matrix of the microbiota patterns of NP and MEF also correlated with increasing age. In general, positive (PPV) and negative predictive values (NPV) of the most abundant operational taxonomic units (OTUs) in the NP were moderately and well predictive for their presence in the MEF, respectively.
Conclusions
As compared to culturing, sequencing-based microbiota studies are more complete and may detect less known bacteria related to AOM. This is crucial to better understand polymicrobial infections and therefore AOM pathogenesis.

CM 1 – Metagenomics / CM-03 *

16S rRNA metagenomics to investigate the death of immersed bodies: a preliminary study

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1. *Institute of Microbiology, Lausanne University Hospital and Lausanne University, Switzerland,* ***2.*** *CURML, University Center of Legal Medicine, Lausanne University Hospital and Lausanne University,* ***3.*** *Institute of Microbiology, Lausanne University Hospital and Lausanne University, Lausanne, Switzerland*

Background
Forensic pathologists assist the authorities in determining the cause of death for bodies found in water. Physical signs capable of discriminating whether immersion occurred *ante* or *postmortem*can be atypical or unspecific. Research for diatoms is one of the few paraclinical tests available, based on the assumption that microbes recovered at the periphery and in the organs are indicative of an active blood circulation when the water penetrated the lungs. However, the analytical performance of diatoms observation by microscopy remains poor. Selective bacterial cultures or targeted qPCR have been proposed as more sensitive alternatives due to the smaller size of bacteria that facilitates their penetration. Here we investigate the use of 16S amplicon-based metagenomics as an alternative approach to investigate immersed bodies.
Methods
DNA was extracted from lung, spleen and liver samples using the Qiagen Microbiome kit and from water collected around the body using the Macherey-NagelSoil Kit. A PCR targeting V3V4 regions of 16S rRNA was performed before amplicon sequencing on a MiSeq platform. Relative quantification of taxa and secondary metrics are compared to other forensic and judiciary evidence to validate the method.
Results
Preliminary results show a visible bacterial rDNA amplification in liver and spleen samples of one body that cluster with other expected positive samples (water, lungs and mock community). All negative samples from the four cases cluster with negative extraction controls.
Conclusions
The detection threshold and specificity of the method will have to be determined for the development of a standardized methodology applicable in practice.

**Student paper*

CM 1 – Metagenomics / CM-04

Comparison of wgMLST (BioNumerics) vs mapping and core-genome SNPs approach for the epidemiological investigations of vancomycin-resistant Enterococcus faecium

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1. *Applied Maths NV, Sint-Martens-Latem,* ***2.*** *Lausanne University Hospital, Lausanne*

Background
Whole Genome Sequencing is becoming frequently used for outbreak investigations. Mapping and core-SNPs based approaches are considered as the gold standard. The choice of a reference genome and the fact that results are dependant from the analyzed isolates limit standardization and influence resolution in an unknown fashion. Whole genome MLST (wgMLST) represents an attractive alternative. We compared wgMLST versus mapping methods of a 29 months surveillance of vancomycin-resistant *Enterococcus faecium* (VREfm) in a university hospital.
Methods
Previously, 187 VREfm isolates retrieved from January 2014 to May 2017 out of 157 patients were sequenced and analyzed using a mapping and core-SNPs approach. A wgMLST analysis was performed in BioNumerics using a scheme developed by Applied-Maths. Results of clustering with both methods were compared and confronted to epidemiological data.
Results
The comparison of the clustering of mapping vs wgMLST approaches showed only few differences. The topologies of both trees were visually highly similar. The 15 isolates considered as unique by SNPs analysis were also considered as unique by wgMLST. Core-SNPs analysis clustered the 171 isolates into 14 groups of highly similar genotypes. A similar clustering was obtained with wgMLST with the exception of 9 isolates considered as unique and one cluster of 9 isolates that was split into 2 groups and 2 unique genotypes.
Conclusions
The wgMLST scheme proposed in BioNumerics produces results that are highly similar and more discriminant than mapping based method of the core genome. Thus, it is a valuable alternative and user-friendly tool for epidemiological investigation of VREfm.

CM 2 – Mass spectrometry and other innovative diagnostic microbiology approaches / CM-05

Comparison of the BD Phoenix™ CPO Detect Test vs. Routine testing for detection of Carbapenemase Producers

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Background

The emergence of carbapenemase producing organisms (CPO) triggers the development of new reliable and rapid diagnostic tools. Becton-Dickinson recently developed the Phoenix™ CPO detect test, a growth based test embedded in gram-negative (GN) panels for detection and confirmation of class A, B and D carbapenemases. This study is aiming at determining the performance of the CPO tests and its adding value in routine diagnostic workflows.

Methods

The performance of the BD Phoenix™ CPO test was analyzed on a collection of 200 molecular-characterized strains of carbapenemase producing and non-producing GN strains. A prospective phase comparing the CPO test to routine testing is ongoing to determine differences in TAT, workloads and costs.

Results

The CPO test exhibited 97.9% sensitivity and 87.0 % specificity for carbapenemases detection. To confirm the specificity results, false positive strains are under molecular investigation to exclude the presence of carbapenemase encoding genes. The CPO test provided a classification for 83.8 % of the sample tested and 16.2% detection without classification. Among classified carbapenemases of class A, B and D, 94.4 % were correctly classified and 5.6 % misclassified. The results of the ongoing prospective study will be presented at the SSM in Lausanne.

Conclusions

With a high sensitivity of 97.9%, the CPO test is reliable for the detection of carbapenemase producing microorganisms. However, the 87% specificity and 83.8% classification performances will likely require the use of additional methods for definite confirmation of both positive detection and carbapenemase classification.

CM 2 – Mass spectrometry and other innovative diagnostic microbiology approaches / CM-06

Proteomics of antimicrobial tolerance modeling, predicting and understanding

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Background

The great therapeutic achievements of antibiotics have been dramatically undercut by the steady evolution of bacterial mechanisms to defend against drug action. While resistance represents the major cause of antibiotictreatment failure, it is long-known that bacteria use other mechanisms, such as tolerance, to protect a bacterial community or a subpopulation called persisters.

Methods

To explore the mechanisms of antibiotic tolerance in *Pseudomonas aeruginosa,*we sorted and collected the persistent fraction of bacterial populations that survived antimicrobial treatment and analyzed their proteomes using a machine learning approach. Besides, we further implemented this approach in order to quantitatively predict persisting fractions of bacterial untreated cultures.

Results

We identified of a core proteome signature of persisters that primarily includes proteins involved in energy metabolism and protein translation. With these protein indicators, predictive models achieved proper quantitative prediction of treatment survivals (figure abs.).

Conclusions

While clinical MS proteomics are still under represented, recent implementations significantly ameliorated their sensitivity, time and cost efficiencies and it seems now likely that these methods will eventually fulfill routine diagnostic purposes. Besides, exploration of proteomic features exploited by successful models would provide with potential molecular insights into any measurable microbial phenotype as we show here for antimicrobials tolerance and persistence.

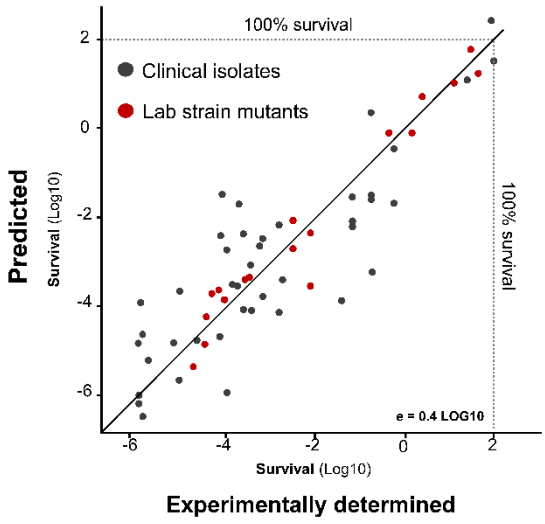

Figure abs. Antimicrobial treatment survival prediction vs. experimentally determination. Treatment survival predictions are based on a whole population proteome regression model. Dots indicate predicted (y-axis) or actual (x-axis) fractions of bacterial population that survived a three hours antimicrobial treatment. Chronic infection isolates (grey dots) and in vitro engineered isogenic mutants of a laboratory strain (red dots) are shown here. «e» indicates the overall average error.

Attachment #132

CM 2 – Mass spectrometry and other innovative diagnostic microbiology approaches / CM-07

Identification and characterization of the LysR-type transcription regulator FosR and its role in fosfomycin resistance in Klebsiella pneumoniae

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Background

Fosfomycin is a bacterial cell wall synthesis inhibitor increasingly used as last resort antibiotic for treating urinary tract infections. In Gram-negative bacteria, resistance can result from: i) reduced permeability, ii) MurA target mutations, iii) FosA enzyme production. In *Klebsiella pneumoniae*, FosA is chromosomally encoded and intrinsic to the species. The *fosA* gene is preceded by a divergently oriented gene coding for a putative LysR-type transcriptional regulator, hereafter FosR. The aim of this study was to characterize the role of FosR in *fosA* expression and subsequently in fosfomycin resistance.

Methods

Fosfomycin susceptibility testing was performed by agar dilution method and disk diffusion in presence and absence of sodium phosphonoformate (PPF). The *fosA* gene expression was quantified by RT-qPCR. The*lysRfosA* tandem was cloned in a low-copy plasmid and transformed in *E. coli*. Site-directed mutagenesis was used to introduce mutations in *lysR*.

Results

We analyzed 3 fosfomycin-resistant *K. pneumoniae* clinical isolates, whose susceptibility to fosfomycin was restored in presence of PPF. In these isolates, expression of the *fosA* gene was higher as compared to a fosfomycin susceptible strain. *fosA* expression was not inducible by subinhibitory concentrations of fosfomycin. Mutations within the *fosR* gene of the fosfomycin-resistant strains (*fosR*-R) were identified. Although cloning of*lysRfosA*tandem from a susceptible strain reduced the fosfomycin susceptibility of *E. coli*, introduction of *fosR*-R mutations significantly increased *E. coli* fosfomycin resistance. Moreover, RT-qPCR analysis showed that *fosR*-R mutations correlated with increased *fosA* mRNA levels.

Conclusions

Here we characterized FosR, a novel LysR-type transcriptional regulator controlling *fosA* expression in *K. pneumoniae*

CM 2 – Mass spectrometry and other innovative diagnostic microbiology approaches / CM-08

High mortality of non-Fournier necrotising fasciitis with Enterobacteriales: time to rethink classification?

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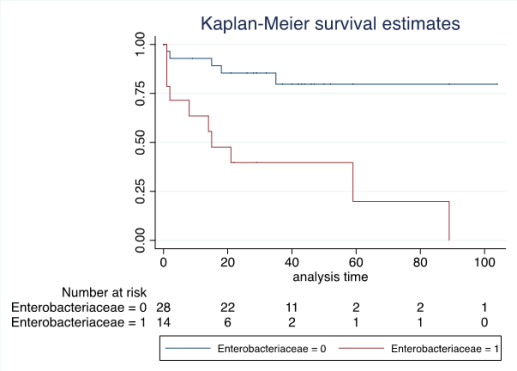
Background
Despite advances in critical care, necrotising fasciitis (NF) remains a severe life-threatening infection. The goal of our study was to determine predictors of mortality at the University Hospital Basel, Switzerland.
Methods
Retrospective single-centre study including all consecutive patients hospitalised with NF including Fournier NF from 01/2011-05/2017. Clinical, laboratory, microbiological and outcome data were assessed.
Results
Sixty-one patients were included. Median age was 66 years (IQR 51-75y). NF was polymicrobial (type 1) in 45.0%, monomicrobial with *Streptococcu*s spp. or *S. aureus* (type 2) in 40%, and monomicrobial with *Enterobacteriales* (n=8) or other pathogens (type 3) in 15.0%. Empirical antibiotic therapy was effective against the identified pathogens in 92% (56/61). Overall mortality rate was 28% (17/61 patients). Older age and renal insufficiency were risk factors for fatal outcome (both p<0.001). There was no difference in mortality in respect to time to operation (median 4.4h in non-survivors vs. 7.6h in survivors, p=0.084) or until initiation of antibiotic therapy (median 1.7h in non-survivors vs. 1.7h in survivors, p=0.69). Mortality was highest in monomicrobial type 3 NF (66.6%, 6/9) followed by type 1 (22.2%, 6/27) and type 2 NF (16.6%, 4/24). Patients with Fournier NF died in 9% (2/19) and with non-Fournier NF in 35% (15/42), p=0.06. Interestingly, the majority of deadly *Enterobacteriales* infections were related to non-Fournier NF with an associated mortality of 71% (10/14) and only 9% (1/11) in the Fournier group (p=0.004).
Conclusions
Our data indicate that non-Fournier NF with *Enterobacteriales* have a high mortality and seem to represent a distinct clinical entity.



Attachment #142

CM 3 – Molecular diagnosis & genomics / CM-09 + P-38

Up-to-date detection of Helicobacter pylori in gastric biopsies including resistance testing for macrolides and fluoroquinolons

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1. *lmz Dr Risch*

Background

Helicobacter pylori (HPYL) causes chronic infections of the gastric mucosa. Increasingly, eradication therapy is hampered by HPYL strains that acquired clarithromycin (CLA) or fluoroquinolone (FQ) resistance. In the former, drug resistance is conferred by mutations of the 23SrRNA gene (A2142G; A2142C; A2143G), in the latter by mutations of *gyr*A (codons at positions 87 and 91). Here, we evaluated two real-time (RT)-PCR for detecting both, HPYL and resistance conferring mutations in gastric biopsies.

Methods

DNA from gastric biopsies was extracted using Qiagen MiniKit. "LightMix Modular Helicobacter 23SrRNA" (Tib-Molbiol; Assay A) RT-PCR was used for detection of the specified mutations (by melting-curve-analysis after amplification). In addition we designed primers for realtime-detection of *gyrA* of HPYL followed by sequencing of the PCR-product (Assay B). Results were compared with GenoType HelicoDR (Hain lifescience, a hybridisation assay after end-point PCR: Assay C).

Results

With assay A, 31 clinical specimens were positive (32% CLA-resistance), 17 were negative. With assay B, 29 clinical specimens were positive (20.7% FQ-resistance), 19 were negative. 45 % of the pecimens (positive with assay A) contain either a CLA- or FQ-resistance (assay A and assay B). With assay C, 29 clinical specimens were positive (31% CLA-resistance, 14% FQ-resistance), 19 were negative.

Conclusions

Concordance of the tests were good. Exceptions were due to DNA concentrations at detection limit and missing band for *gyrA* (assay C) if Asn87Ile is present. Du to the high resistance level (CLA and FQ), mutations in 23SrDNA as well as *gyrA* should be tested in parallel.

CM 3 – Molecular diagnosis & genomics / CM-10

The MUCOVIB project : Concordance between upper and lower airway microbiota in children with Cystic Fibrosis

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Background

Metagenomics has revolutionized the study of bacterial diversity present in Cystic Fibrosis (CF) patients. Nasopharyngeal and oropharyngeal samples are commonly used to direct therapy for lower respiratory tract infections in non-expectorating children with CF, although a recent study suggested inconsistent intra-individual microbiota of upper and lower respiratory niches among infants with CF. We aimed to investigate the concordance between oropharyngeal (upper) and expectorations (lower) microbiota in children with CF.

Methods

As part of the ''Cystic Fibrosis, respiratory viruses, intracellular bacteria and fastidious organisms" (MUCOVIB) project, 58 children under 18 years of age with diagnosed CF were recruited during baseline follow-up visits or in the setting of a Pulmonary Exacerbation. Respiratory samples (n=40) included paired cough swabs and sputa collected during the same visit from 10 children able to expectorate. Amplicons of the 16S V3-V4 region were sequenced, clustered into OTUs and assigned taxonomical ranks from the EzBioCloud database.

Results

Equivalent species diversity, measured with Shannon index, was documented in both sample types (Pvalue=0.26). In most cases, hierarchical clustering based on OTU presence/absence clustered upper and lower samples from the same patient, during the same visit, suggesting the existence of a signature microbiota. A similar variance of bacterial composition was observed in both respiratory niches (P-value=0.9422), with some differences in species composition.

Conclusions

Our preliminary findings suggested a good intra-individual concordance of the microbiota in upper and lower respiratory niches, thus suggesting that oropharyngeal swabs can be used as proxy to measure bacterial biodiversity among children with CF unable to expectorate.

CM 3 – Molecular diagnosis & genomics / CM-11

First complete genome sequence of Actinobacillus porcitonsillarum using the Oxford Nanopore sequencing technology

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Background

Actinobacillus porcitonsillarum (APT) is a non-pathogenic Gram-negative *Pasteurellaceae*, which phenotypically resemble the pathogen *Actinobacillus pleuropneumoniae*(APP). Previous studies suggested that APT represents a new species, yet no full genome has been sequenced so far. The aim of the study was to provide the first full circular genome of an APT strain.

Methods

Total DNA was extracted from APT strain 9953L55 (proposed as type strain) and whole-genome sequencing was performed with the MinION (Oxford Nanopore) sequencer and on an Illumina Hiseq (Illumina) platform. After base-calling with Albacore, 1D Oxford Nanopore Technology pass reads were assembled with the Canu pipeline. The assembled scaffold was polished twice with paired-end (2X150 bp) Illumina reads with Pilon. Annotation was performed with Prokka.

Results

The 2263191 bp circular genome of APT 9953L55 consisted of 2072 coding sequences (CDS) and 2175 genes. Six copies of 16S rRNA, 23S rRNA and 5S rRNA encoding genes were found. An intact and complete *apxIICABD* operon encoding the RTX toxin was present, but no *apxI*, *apxIII* or *apxIVA* genes, consistent with previous studies. Interestingly, a CRISPR region with 37 repeat units preceded by CAS encoding genes was identified. Comparison of the full genomes of APT 9953L55 and APP reference strain S1536 showed high sequence similarity only in 1/3 of the APT genome (query cover 32%, dentity 93%).

Conclusions

Herein, we provided the first full genome of APT 9953L55. The high sequence divergence compared to APP confirms that APT is a new species.

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EM 1 – Water safety and wastewater / EM-01*

Microbial degradation of antibiotics: the necessity for micromolar concentration studies

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Background
Antibiotics are ubiquitously used and disseminated in the environment. They act on biological systems and can cause a wide range of side effects in non-target organisms; thus, necessitating their removal. However, very little is known about microbial antibiotics degradation, and most research has focused on antibiotic resistance. Antibiotic degradation may be more widespread than previous studies assume, but may preferentially occur at low, nontoxic, concentrations.
Methods
Our aim is to study the capacity of natural lake water microbial community to use antibiotics as substrates for growth. The bacteria are collected by filtration from Lake Geneva water and re-suspended in artificial lake water (ALW) to obtain very dilute starting biomass concentrations (105 cells per mL). Biodegradability of 14 antibiotics at concentration of 1 mg C per L was assessed from bacterial community growth over time by means of flow cytometry, in comparison to no-added carbon controls and ALW amended with phenol or 1-octanol.
Results
Five antibiotics, Bactrim, Chloramphenicol, Penicillin, Rifampicin and Vancomycin, yielded significant community growth. Others, such as Tetracycline and Sulfomethoxazole, caused significant growth inhibition, even at 1 and 0.1 mg C per L. Average cell carbon biomass estimations from 14C-mass balances (0.1 pg per cell) suggests community growth yields of ~10% (g C cell per g C substrate) for antibiotics. Biomass estimations of community samples by means of cell type classifiers developed in neural networks yielded up to 25% g C per g C.
Conclusions
Our results thus suggest that some antibiotics are used a growth substrates by bacteria in freshwater lakes.

** Student paper*

EM 1 – Water safety and wastewater / EM-02

Evolution of microbial communities in aerobic granular sludge sequencing batch reactor during changes of wastewater composition

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1. *École polytechnique fédérale de Lausanne (EPFL)*

Background

Aerobic granular sludge (AGS) is an emerging technology offering an alternative wastewater treatment with a reduced footprint compared to conventional activated sludge systems. This process is particularly suited for biological phosphorus removal.

Basic understanding of AGS has mainly been obtained in lab-scale studies with synthetic wastewaters containing volatile fatty acids (VFA). Yet, the aspect and performances of AGS cultivated in such model systems are very different from those obtained in reactor treating real astewater[1],. Furthermore, the microbial communities appears to be significantly different in these two systems.

The aim of this study is to understand the influence of hydrolysable organic matter on AGS microbial communities and sludge properties (settle ability and nutrient removal).

Methods

The wastewater composition of an AGS sequencing batch reactor was progressively changed, from simple (containing VFA) to a more complex mixture composed of VFA and fermentable compounds (glucose and amino acids). In a second step, half of glucose and amino acids added was replaced with their polymeric substances (starch and oligopeptides).

Results

Phosphorus and ammonium removal by the AGS were generally above 90% with all three synthetic wastewaters. But AGS settle ability and nitrogen removal capability decreased significantly with the introduction of polymeric substances.

The monitoring of microbial communities revealed a constant evolution in correlation with the wastewater composition. Betaproteobacteria,which were highly abundant with simple wastewater gave ground to Actinobacteria as the complexity of the wastewater increased.

Conclusions

Metagenomicanalysis will allow to assign specific roles to the differentbacterial populations in relation with the wastewater properties.

EM 1 – Water safety and wastewater / EM-03

Understanding the occurrence of antimicrobial resistance in water treatment systems and strategies for its reduction

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Background

The effect of chemical treatments and water pollution by antibiotics on the spread of antimicrobial resistance (AMR) is a growing concern. Understanding the occurrence of AMR in engineered water systems and defining the performances of treatment strategies is crucial to introduce effective intervention measures to reduce the selection for AMR in the environment. This study aimed at investigating the influence of engineered treatments on the occurrence of AMR in microbial communities in drinking water (DWTP) and waste water treatment plants (WWTP).

Methods

Sixty-eight water samples were collected along three DWTP and five WWTP before and after various treatments in several countries. V3-V4 16S amplicons were sequenced for all samples. Taxonomical assignment was performed with in-house pipelines using QIIME (Caporaso, 2010) and EzBioCloud (Yoon, 2017). Shotgun metagenomics was applied on a subset of 28 samples to identify ARGs by homology search to the CARD database (Jia, 2017).

Results

In DWTP, preliminary results suggest that sand filtration and carbon filters, but not ozonation, marginally reduce AMR prevalence in the microbial community. Disinfection and intermediate chlorination induced a reduction in bacterial diversity but increased AMR prevalence. DWTP without chlorination and with high chlorination levels showed a small decrease in overall AMR. In WWTP, secondary treatment and ultrafiltration/reverse osmosis largely decreased AMR prevalence in the microbial community. On the opposite, increased levels of AMR were observed after membrane reactors and UV-chlorination treatments.

Conclusions

ARG diversity and patterns were influenced by the type of treatment(s) rather than the initial ARG diversity in the water source.

EM 1 – Water safety and wastewater / EM-04 + P-72

Quantitative tracking of carbapenem-resistant bacteria and extended spectrum beta-lactamase producing bacteria from treated wastewater to Swiss river ecosystems

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Background
Understanding to what extent wastewater discharge contributes to antibiotic resistance dissemination in the receiving rivers is critical to enable preventative measures.
Methods
Water, biofilm, sediment and Gammarus microbiota samples were collected from nine wastewater-impacted Swiss river streams. Cultivation and sequencing methods were co-used to determine the abundance and diversity of all grown colonies on the Brilliance CRE and ESBL agar plates.
Results
The medium number of carbapenem-resistant bacteria are higher in the downstream (6500 and 19.5 CFU/ml) than upstream river (2600 and 14.0 CFU/ml), while the extended spectrum beta-lactamase producing (ESBL)-producing organisms were far more abundant (up to 255 times) in the river water than wastewater during considerable precipitation (2.3-14.9 mm within 60 hours). The 16S rRNA gene amplicon sequence variant (ASV) data show that cultivated bacteria were mostly not *Enterobacteriaceae*. While the resistant colonies are dominantly assigned to plant and/or soil associated *Pseudomonas*, *Stenotrophomonas*, and *Brevundimonas*, the resistant bacteria are, however, from at least 149 species including opportunistic human pathogens. Strikingly, unlike the ESBL-producing bacteria, the ASVs of wastewater cultivated carbapenem-resistant bacteria are highly abundant in the river biofilm (4.5% and up to 31% relative abundance), followed by water (2.4%), sediment (0.6%), and Gammarus gut (0.6%).
Conclusions
Treated wastewater harbor highly abundant carbapenem-resistant bacteria that can successfully colonize the river ecosystems, especially biofilms. The tested agar media do not appear well suited to trace the anthropogenic impact of wastewater on the environmental resistome. Metagenome-based genotype analysis is ongoing to further check their resistance mode (intrinsic or acquired) and clinical relevance.

EM 2 – Metagenomics / EM-05

A diversity of deep terrestrial subsurface metabolic processes revealed by metaproteogenomics

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***1.** Envi, **2.** École polytechnique fédérale de Lausanne (EPFL), **3.** Posiva Oy, **4.** KTH, **5.** Oak Ridge National Laboratory*

Background
Deep geological repositories are the preferred method for final storage of spent nuclear fuel. In Finland, the repository is being built in the Olkiluoto Island. Safe storage of the waste is predicated on an understanding of the underlying geochemical processes underway at the site. Of particular concern is the generation of sulfide, as it can induce corrosion of the waste-bearing copper canisters.

Methods
Here, we use proteogenomic approaches coupled with geochemical tools to identify the electron donors fueling sulfidogenesis.

Results
We investigated three groundwaters from varying depths and with varying geochemical characteristics. Geochemical and isotopic data indicated that sulfate reduction was ongoing in two of the three groundwaters. However, combining these analyses with genome-resolved metaproteogenomics revealed that sulfate reduction was active in all three groundwaters. In the groundwater with no detectable sulfide, a community of sulfatereducing and sulfide-oxidising bacteria mediate a cryptic sulfur cycle. At the transition between sulfate-rich and methane-rich groundwaters, the presence of anaerobic methane oxidising archaea and isotopically light dissolved inorganic carbon suggest that anaerobic methane oxidation as well as hydrogen may contribute to sulfate reduction. Finally, in the deepest groundwater, harboring the highest sulfide concentration, H2-fuelled primary production generates small organic compounds that drive sulfidogenesis.

Conclusions
This study sheds unprecedented light on the drivers for sulfidogenesis and provides key information for the repository safety case. It also highlights that cryptic biogeochemical processes are not always revealed by sole consideration of geochemical parameters.

EM 2 – Metagenomics / EM-06

The small RNA RyhB is a regulator of cytochrome expression in Shewanella oneidensis

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Background
Shewanella oneidensis is a facultative anaerobe that is remarkable for its metabolic versatility. In the absence of dissolved oxygen, it can utilize a large number of soluble compounds as terminal electron acceptors, and is furthermore capable of transferring electrons to reduce insoluble metal oxides in the extracellular environment. This respiratory flexibility has been attributed to the large number of *c*-type cytochromes encoded in the genome of *S. oneidensis*MR-1 and serving as fundamental electron transport chain proteins. However, it is not clear how *S. oneidensis* controls expression of the *c*-type cytochromes in response to environmental changes.
Methods
We studied the role played by the ferric uptake regulator Fur and the iron-responsive small RNA RyhB. We characterized mutant strains using RNA sequencing, quantitative RT-PCR and various physiological assays.
Results
We demonstrate that loss of Fur leads to diminished growth and an apparent decrease in heme-containing proteins. Remarkably, deleting the Fur-repressed *ryhB* gene almost reverses these physiological changes. RNA sequencing identified a number of putative RyhB repressed genes and a large fraction of these encode *c*-type cytochromes. We show that RyhB destabilizes the mRNA of four of its target genes and this requires the presence of the RNA chaperone Hfq. Iron limitation decreases the expression of the studied RyhB target genes and this regulation relies on the presence of both Fur and RyhB.
Conclusions
This study suggests that controlling cytochrome expression is of importance to maintain iron homeostasis and that sRNAs molecules are important players in the regulation of fundamental processes in *S. oneidensis*.

EM 2 – Metagenomics / EM-07*

Arsenic methylation across microbial phyla

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Background
Arsenic (As) undergoes extensive microbial cycling in the environment, including methylation that results in the addition of one or several methyl groups to inorganic arsenic. The reaction is catalysed by the enzyme arsenite methyltransferase (ArsM), generating volatile and non-volatile arsenicals. It is unclear whether this process is an arsenic detoxification mechanism or a strategy to inhibit the growth of competitors. As methylation has been demonstrated in several microorganisms, including the Bacteroidete *Arsenicibacter rosenii* SM-1 and the Firmicute *Clostridium* sp. BXM. However, members of numerous other phyla harbor this gene and it is unclear whether all represent functional proteins and active methylating organisms. Thus, the goal of this study was to systematically probe the functionality and the *in vivo* activity of ArsM across phyla.
Methods
We assessed the capacity for As(III) methylation and volatilization across seven microbial strains encoding the *arsM* gene: two archaea (*Methanosarcina mazei* Gö1, *Methanosarcina acetivorans* C2A); two Firmicutes (*Anaeromusa acidaminophila* DSM 3853, *Clostridium pasteurianum* DSM 525); a Streptomycete (*Streptomyces vietnamensis* DSM 41927); a Deltaproteobacterium (*Geobacter metallireducens* GS-15); and a Bacteroidete (*Arsenicibacter rosenii* SM-1). Furthermore, all *arsM* genes were cloned into the arsenic-sensitive *Escherichia coli* AW3110(DE3) and methylation measured by HPLC-ICP-MS.
Results
The results show that most of the strains were not able to methylate As despite harboring *arsM* genes that encode functional ArsM proteins.
Conclusions
We hypothesize that more efficient As detoxification pathways might prevail, precluding methylation. We conclude that the presence of *arsM* does not equate As methylation activity and that more work is warranted to deconvolute *arsM* regulation.

** Student paper*

EM 2 – Metagenomics / EM-08

Honeybees from the same colony harbour individualized gut bacterial communities

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Background
The honeybee gut is colonized by a remarkably simple bacterial community, which is dominated by only 8 bacterial phylotypes (> 97% 16S rRNA identity). However, these phylotypes are composed of highly diverse strains, for which the population structure, distribution and functional relevance is not known. In the current study, we investigated the strain-level diversity of the gut microbiota, focusing on the question: Do individual honeybees harbor distinct bacterial communities?
Methods
We collected 54 honeybees representing different colonies and life-stages, over a time-period of 2 years. All bees were sampled individually and subjected to whole-genome shotgun sequencing, resulting in deep coverage sequence data of the complete gut microbial community for each bee. Based on mapping to a genomic database, SNPs were quantified for all samples, and the extent of shared alleles among bees was investigated.
Results
Overall, we found a high level of strain diversity for all core members, with a mean of 11% polymorphic sites within core genes. In contrast, within individual bees, the mean fraction of polymorphic sites was only 3.5%, indicative of competitive exclusion among strains. Interestingly, bees sampled at the same time and hive did not harbor more similar communities than other pairs of bees, suggesting that most of the diversity represents standing variation.
Conclusions
Taken together, our results indicate that the honeybee gut microbiota contains extensive strain-diversity within colonies, which distributes differentially among bees. Our current work therefore aims to elucidate how individualized gut microbiota profiles are generated, and the possible impact of diversity on honeybee fitness.

EM 3 – Ecology & evolution / EM-09*

Understanding the activation of integrative and conjugative element transfer to the recipient cell by investigating the expression of the element's core genes inside the donor cell

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Background

Pseudomonas knackmussii B13 carries two copies of a mobile Integrative and Conjugative Element named ICE*clc*, which has served as model to understand ICE ecology and behavior. One of the particularities of ICE*clc* is its bistable activation in stationary phase, leading to 3-5% of individual cells in a population to develop transfer competence and becoming able to transfer the ICE*clc* by conjugation to new recipient cells.

Methods

The aim of our project is to unravel the network of regulatory decisions that leads to activation of the ICEclc transfer competence state in a subset of cells. Based on previous studies, all potential ICE*clc* promoters were identified and individually tested for bistable expression. This was done by fusing a promoterless *gfp*gene to each of the ICE*clc*promoters and integrating this construct in single copy in the chromosome of wild-type or mutant P. knackmussii B13 strains.

Results

We found 10 bistable promoters, situated in a strongly conserved region of ICE*clc*. Dual testing of each of those 10 promoters in conjunction with the *intB13*integrase promoter indicated that they are expressed in the same individual cell. From time-lapse microscopy we observed the onset of ICE*clc* promoter activation, suggesting a hierarchical network in expression of the ICE integrase and its conjugative system. We further show that most of the bistable promoters are dependent on *inr*R, a gene coding for a regulatory factor on ICE*clc*.

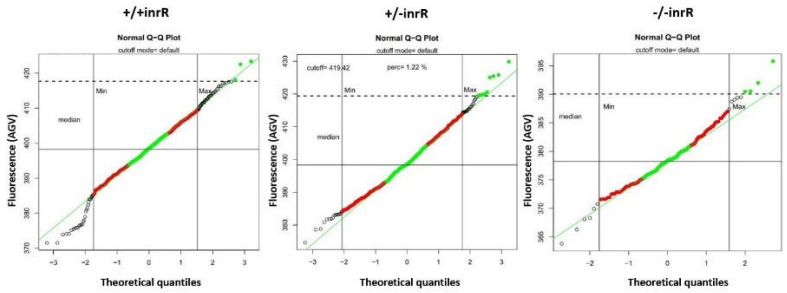
Conclusions

The characterization of the various bistable promoters will help to further understand how bistability is exerted on promoter sequences.

**Student paper*

Dependency of bistable promoter ***PinrR*-gfp** on its own presence or absence in the ICE*clc*

Time point: **24h**



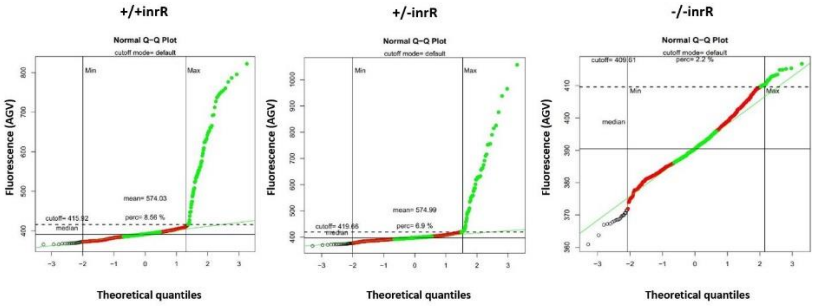
Fluorescence intensity range: 370-420
Subpopulation size: 0%

Fluorescence intensity range: 380-430
Subpopulation size: 1.22%

Fluorescence intensity range: 365-395
Subpopulation size: 0%

Dependency of bistable promoter ***PinrR*-gfp** on its own presence or absence in the ICE*clc*

Time point: **48h**



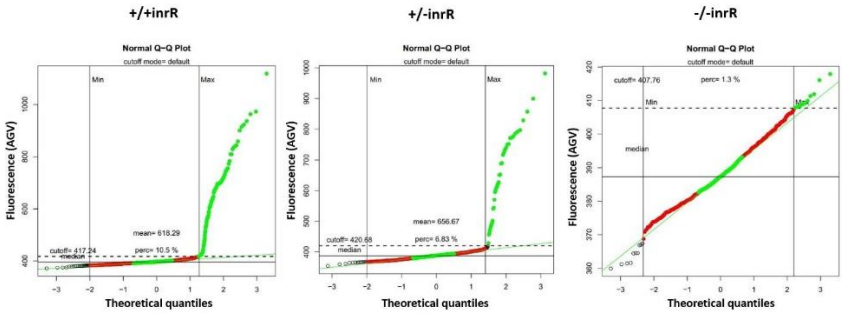
Fluorescence intensity range: 400-800
Subpopulation size: 8.56%

Fluorescence intensity range: 400-1000
Subpopulation size: 6.9%

Fluorescence intensity range: 360-410
Subpopulation size: 2.2%

Dependency of bistable promoter ***PinrR*-gfp** on its own presence or absence in the ICE*clc*

Time point: **72h**



Fluorescence intensity range: 400-1000
Subpopulation size: 10.5%

Fluorescence intensity range: 400-1000
Subpopulation size: 6.83%

Fluorescence intensity range: 360-420
Subpopulation size: 1.3%

Attachments #48

EM 3 – Ecology & evolution / EM-10*

Transfer of an integrative and conjugative element in Pseudomonas may be triggered by oxidative stress

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Background
The integrative and conjugative element (ICE) ICE*clc* from *Pseudomonas knackmussii* B13 confers its host the ability to degrade 3-chlorobenzoate (CBA). ICE*clc* is stably integrated in the genome, but can be transferred to new recipient cells by conjugation. ICE transfer exclusively occurs from a small subpopulation of transfer competent cells and is initiated during stationary phase after growth with CBA.
Methods
The aim of this work is to better understand the possible trigger(s) and the role of CBA that favor development of the transfer competence state. Our working hypothesis is that some cells accumulate biochemical damage during growth, which is sensed by the ICE and triggers its activation mechanism. As a measure for cellular damage, we tested whether oxidative stress correlates to ICE activation.
Results
An oxidative stress bioreporter was constructed placing the *gfp* gene under the control of the promoter P_{AHPC} in *Pseudomonas putida* and its functionality was successfully calibrated using UV-A, paraquat and hydrogen peroxide. Next we tested whether growth on CBA is more stressful for cells than succinate. Indeed, exponentially growing cells on CBA display more fluorescence from P_{AHPC} than succinate-growing cells. We will describe ongoing time-lapse microscopy experiments to record the life history of individual cells in order to determine whether higher oxidative stress during exponential phase correlates to later ICE activation.
Conclusions
The ecological relevance of this may be that the ICE tries to escape cells that are in bad shape in order to survive in a hopefully healthier cell.

**Student paper*

EM 3 – Ecology & evolution / EM-11*

Origin of sediment bacteria: water versus soil

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Background

Nowadays a thorough understanding of the environments' reaction in response to climatic change seems unavoidable. One way to do this is by studying past events of change and the response of biological communities to it. Several paleoecological studies have already used endospore-forming bacteria in order to reconstruct environmental history. Since endospores are able to survive for extended periods of time, the endospore seed bank community might reflect the evolution of these sites. However, for this approach to be validly used, the autochthonous origin of the endospore-forming community needs to be demonstrated.

Methods

In this study, we explored the origin of endospores in two alpine lakes located in Grisons, Switzerland (Jöri Lakes I and XIII). We sequenced microbial communities from samples representative of the lakes (sediments cores and water column) and of the surrounding environments (soils and river inlets).

Results

By sequencing the 16S rDNA and *spo0A* gene markers, we have demonstrated that endospores preserved in sediment cores are more similar to the community in the water column, compared to communities found in samples external to the lake.

Conclusions

This confirms an autochthonous origin of this fraction of the microbial community and shows that endospores evolve in the water column and reflect environmental lake history. This is an essential requirement for further paleoecological studies using endospores as a proxy.

**Student paper*

EM 3 – Ecology & evolution / EM-12* + P-73*

Phylogenetics of bacteria and their plasmids: comparing bioinformatic approaches

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Background

To combat the ever-growing problem of antibiotic resistant bacteria, it is important to quantify the transmission rates of bacteria and their plasmid at large epidemiological scales. A first step in this direction is to determine the phylogenetic relationship of diverse, widely sampled, bacteria and their plasmids directly from WGS data. We aimed to find the optimal way to sequence, assemble, and analyse WGS data to construct robust chromosomal and plasmid phylogenies.

Methods

We sequenced 24 ESBL producing Escherichia coli strains using Illumina paired end, Pacbio Sequel, and Oxford Nanopore MinION technologies. After trimming and quality control, the reads were assembled using (Hybrid) SPAdes, Canu and UniCycler. Putative plasmid sequences and resistance genes were located using BLAST against Plasmidfinder and Resfinder databases. Phylogenies for the chromosomal core genes and plasmid sequences were created in Beast2.

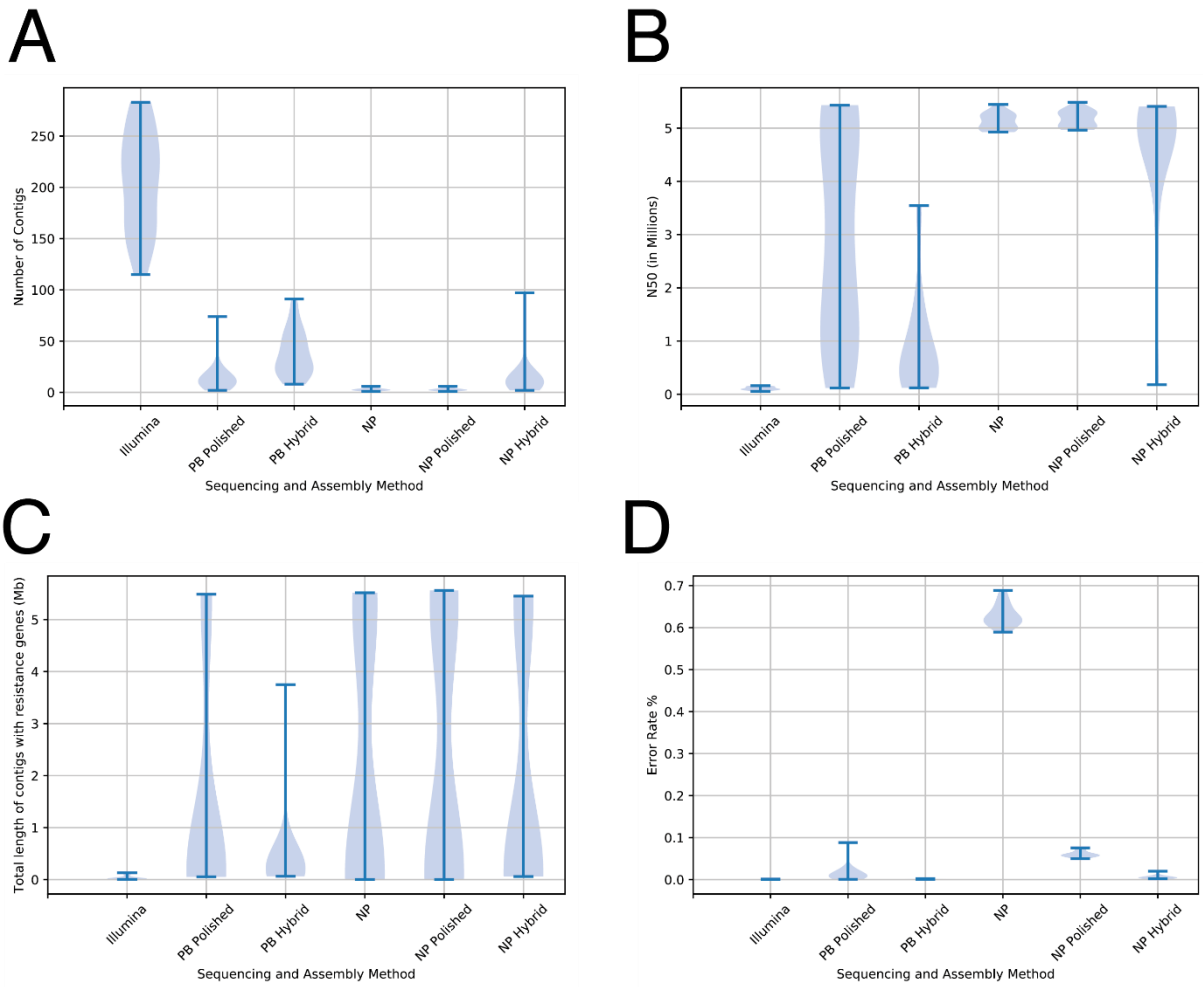
Results

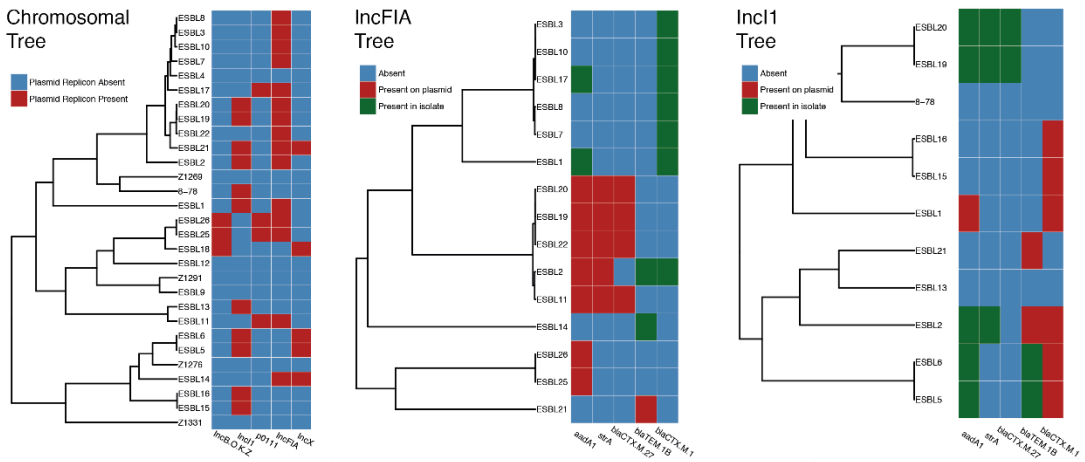
The resistance genes were flanked by IS elements with repeat regions that confuse Illumina-based assemblies. Long reads were needed to correctly resolve the location of these genes. Similarly, closed plasmids were reconstructed only with long read information. Known transmission pairs carry highly similar plasmids. However, highly similar resistance plasmids are also carried by diverse E. coli strains.

Conclusions

We can construct robust chromosomal phylogenies from whole genome sequencing data. When long read data is available, plasmid phylogenies can be created.

**Student paper*





Attachments #202

PB 1 – Cell microbiology / PB-01*

Explore RdhK based regulatory network of organohalide respiration using a hybrid proteins strategy

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1. *École polytechnique fédérale de Lausanne (EPFL)*

Background
Reductive dehalogenase (*rdh*) gene clusters encode for proteins involved in organohalide respiration (OHR), a bacterial process where organohalide compounds are used as terminal electron acceptors. RdhK proteins, members of the CRP/FNR family of transcription regulator, are dedicated to the regulation of *rdh*gene clusters. To date only a few RdhK proteins have been characterized in *Desulfitobacterium hafniense*while many copies of *rdhK* genes have been identified within OHR bacteria genomes. RdhK, when bound to a specific organohalide compound, recognizes a palindromic sequence located in the promoter region of *rdh*genes. Generally, the protein activates the cluster of genes responsible for the respiration of the recognized effector. Therefore, the identification of both binding partners may represent an indirect way to identify new substrates for yet undescribed *rdh*gene clusters.
Methods
Visualization of the tripartite complex requires the presence of the interdependent effector and dehalobox partners which makes *in vitro*screening not applicable. To circumvent this problem, the design of hybrid RdhK proteins is proposed here. The idea is to allow the decoupling of the effector screening from the screening of the DNA targets.
Results
According to RdhK6 structure, two alternative hybrids were designed. The corresponding proteins were purified and tested for *in vitro*interactions. A comparison of their interactions specificity and their potential use to fit the objectives will be presented.
Conclusions
Moreover, results obtained will be confronted to the described RdhK mechanism. Finally, preliminary results on the application of this method for the characterization of new RdhK proteins will be discussed.

**Student paper*

PB 1 – Cell microbiology / PB-02

Was chlamydial adaptation to hypoxia an early determinant of plastid endosymbiosis?

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Background
Menaquinone (vitamin K2) shuttles electrons between membrane-bound respiratory complexes under microaerophilic conditions. In photosynthetic eukaryotes and cyanobacteria, phylloquinone (vitamin K1) participates in photosystem I function. Here we elucidate the evolutionary history of vitamin K metabolism in algae and plants.
Methods
Phylogenetic analysis was performed to ascertain the gene origin of all enzymes of the core menaquinone and isoprenoid side chain of vitamin K.
Results
We show that Chlamydiales intracellular pathogens made major genetic contributions to the synthesis of the naphthoyl ring core and the isoprenoid side-chain of these quinones. Production of the core in extremophilic red algae is under control of a menaquinone (Men) gene cluster consisting of 7 genes that putatively originated via lateral gene transfer (LGT) from a chlamydial donor to the plastid genome. In other green and red algae, functionally related nuclear genes also originated via LGT from a non-cyanobacterial, albeit unidentified source. In addition, we show that 3-4 of the 9 required steps for synthesis of the isoprenoid side chains are under control of genes of chlamydial origin.
Conclusions
These results are discussed in the light of the hypoxic response experienced by the cyanobacterial endosymbiont when it gained access to the eukaryotic cytosol. They suggest a direct role of Chlamydiales during plastid endosymbiosis which consisted of induction of menaquinone synthesis in the incipient plastid.

PB 1 – Cell microbiology / PB-03

QstR-dependent regulation of competence and type VI secretion in Vibrio cholerae

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1. *École polytechnique fédérale de Lausanne (EPFL)*

Background
During growth on chitinous surfaces in its natural aquatic environment *Vibrio cholerae* develops natural competence for transformation and kills neighbouring non-kin bacteria using a type VI secretion system (T6SS). Activation of these two phenotypes requires the chitin-induced regulator TfoX. In addition, integrated signals from TfoX and the quorum sensing circuit lead to the production of the intermediate regulator QstR. However, despite belonging to the LuxR-type family of regulators, the exact mechanism on how QstR regulates these processes was unknown.
Methods
We used RNA sequencing, chromatin immunoprecipitation coupled with deep sequencing (ChIP-seq), and a range of genetic and biochemical methods to characterise QstR's function.
Results
In this work, we first defined the QstR regulon using an RNA-seq approach. Furthermore, by identifying QstR binding sites we demonstrated that QstR is likely a dual transcription factor leading to the induction or repression of specific genes. We further showed that QstR's function is dependent on dimerization. However, in contrast to the well-studied LuxR-type biofilm regulator VpsT, which requires the co-factor c-di-GMP, QstR dimerisation and function occurred independently of c-di-GMP. Surprisingly, although the gene encoding the important DNA-binding protein ComEA is expressed in a QstR-dependent manner, no direct binding of QstR was detected upstream of *comEA* suggesting the existence of additional regulatory intermediates.
Conclusions
Overall these results provide detailed insights into the function of a key regulatory protein that is involved in natural transformation and T6SS-dependent interbacterial predation.

PB 1 – Cell microbiology / PB-04

Molecular mechanisms of host-pathogen interaction between Vibrio cholerae and amoeba

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1. *Global Health Institute, Ecole Polytechnique Fédérale de Lausanne, Lausanne*

Background

V. cholerae is the causative agent of the disease cholera and therefore a pathogen of major importance. However, apart from its potential to infect humans, we previously showed that the bacterium could also interact with the aquatic amoeba *Acanthamoeba castellanii.* Precisely, we showed that *V. cholerae*(i) resists intracellular killing by *A. castellanii*; (ii) is released from trophozoites by exocytosis; (iii) establishes an intracellular proliferation niche within the osmoregulatory organelle of the host (e.g., the contractile vacuole); (iv) maintains its niche upon amoebal encystment; and (v) promotes the final lysis of the *A. castellanii*cysts (Van der Henst, 2016). While this infection cycle was previously established, the molecular mechanisms leading to the amoebal cyst lysis remained unknown.

Methods

The experimental approaches were based on bacterial genetics and live cell time-lapse microscopy using a confocal laser-scanning microscope.

Results

We identified several enzymes that are secreted by *V. cholerae* and interfere with its interaction capability with amoebae. Precisely, we showed that the pore-forming toxin hemolysin severely intoxicates *V. cholerae* if not properly regulated at the post-translation level. Interestingly, we observed that environmental isolates of *V. cholerae* were hyper-hemolytic and resulted in a premature intoxication of the host. Moreover, we identified a lecithinase that fosters the lysis of the infected cysts.

Conclusions

In aquatic environments, *V. cholerae* cells defend themselves against predatory protozoan grazers. We hypothesize that pandemic strains of *V. cholerae* have evolved their regulation of extracellular enzymes to avoid a premature death of their host while maximizing their growth inside the amoebal osmoregulatory organelle.

PB 2 – Cell division / PB-05*

Investigation of the potential role of ParB in chromosome and plasmid segregation among Chlamydiales order

Ms. Aurélie Scherler [1], Dr. Marie de Barsy [2], Prof. Gilbert Greub [1]

1. *University of Lausanne and University Hospital Center,* ***2.*** *Université catholique de Louvain, Cliniques Universitaires Saint-Luc, Brussels*

Background
To allow life continuity, all living organisms have developed mechanisms to ensure equal DNA distribution to daughter cells. While the mitosis is well described in eukaryotes, chromosome and plasmid segregation remains unclear in prokaryotes. Studies on low-copy number plasmids showed that this mechanism principally relies on the partitioning system ParAB-*parS*, composed of two proteins, ParA (ATPase) and ParB (DNA-binding protein), and a cis-acting centromere-like sequence, *parS*. ParB binds to *parS*-sites with high affinity, and the ParB-*parS* complexes interact with ParA, required for segregation. While ParB function has been investigated in different bacteria, its role remains poorly understood in *Chlamydiales*, an order of obligate intracellular bacteria.
Methods
To clarify the role of ParB among the *Chlamydiales* order, we used *Waddlia chondrophila*, potentially responsible for miscarriage in human, as model organism.
Results
Chromosomal regions bound by ParB were identified by chromatin immunoprecipitation followed by deepsequencing (ChIP-seq) and binding sites were confirmed by ChIP-qPCR. The expression of *parB* was assessed by qRT-PCR with a peak of expression at 8h post-infection. Furthermore, ParB was localized in *C. trachomatis* expressing *parBWc* and in the cytoplasm with colocalization with DNA in *W. chondrophila*. Finally, we will investigate the link between ParB and aberrant bodies since ParB might be implicated in the formation of these persistent forms characterized by continuous DNA replication without division. Therefore, *parB* expression and its localization will be assessed in *W. chondrophila* persistent forms by qRT-PCR and immunofluorescence, respectively.
Conclusions
This work will improve our knowledge on the role of ParB in chromosome segregation in *Chlamydiales*.

** Student paper*

PB 2 – Cell division / PB-06*

Harnessing CRISPR system for genome editing in human pathogen S. pneumoniae

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Background
CRISPR systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by detection and cleavage of invading foreign DNA. Modified versions of this system can be exploited as a biotechnological tool for precise genome editing at a targeted locus. In this study, we develop the CRISPR system for RNA-programmable, high throughput genome editing in *Streptococcus pneumoniae*, an opportunistic human pathogen and the causative agent of pneumonia, meningitis, and acute otitis media.
Methods
After a precise double-stranded break (DSB) is introduced, the cells' DNA repair mechanism of Homologydirected repair (HDR) pathway is being exploited. This is achieved through the transformation of a template DNA fragment that will recombine within the genome and eliminate recognition of the endonuclease target
Results
Nearly 90% of cells that were recovered using our approach contained the desired deletion.
Conclusions
Hereby, we developed a genetic system for making targeted, marker-less gene knockouts and large genome deletions. Hence, our findings demonstrate successful genome engineering with a robust and versatile technique.

** Student paper*

PB 2 – Cell division / PB-07

Multilayered control of the initiation of DNA replication in Caulobacter crescentus

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Background
In bacteria, the activity of the conserved replication initiator protein DnaA, which binds on the origin of replication, is required for chromosome replication. In the model alpha-proteobacterium *Caulobacter crescentus*, DNA replication occurs only once per cell cycle in stalked cells. Precise temporal control of DNA replication and its coordination with cell cycle progression need a tight regulation of DnaA activity. The inactivation of DnaA by the DnaA homolog protein HdaA, fine-tunes DnaA activity and its degradation to prevent over-initiation events.
Methods
A combination of forward genetics, biochemistry and cell biology approaches were used to identify and characterize a novel regulator of DNA replication.
Results
We identified HdaB which has similarities with the DNA binding domain of DnaA and is conserved in most alpha-proteobacteria. Excess of HdaB leads to cell division defects and to cell death similar to Thymine-Less-Death (TLD). Moreover, akin to HdaA depleted cells, HdaB over-producing cells over-initiate DNA replication as a consequence of elevated active DnaA levels. To better understand why HdaB overproducing cells die we conducted a genetic screen to identify mutations that could bypass the TLD-like lethality. Notably, insertions in *dnaK* and *recQ* were found to ameliorate cell division defects and to abolish cell death of HdaB overproducing cells by reducing DnaA levels. Since, HdaB interacts with HdaA *in vivo*, it supports a model according to which HdaB restricts DnaA inactivation by HdaA.
Conclusions
Our work uncovered the existence of a conserved regulator that is sufficient to induce a TLD-related cytotoxicity as many antibiotics do to kill bacteria.

PB 2 – Cell division / PB-08

Meiotic cell cycle and re-fertilization blocks are coordinately regulated in fission yeast

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Background

To ensure genome integrity throughout the sexual lifecycle, a strict alternation between fertilization and meiosis is required, such that fertilization occurs only between two haploid cells and meiosis only in diploid ones. Nitrogen starved fission yeast *Schizosaccharomyces pombe* induces a G1 cell cycle arrest and commences sexual differentiation that culminates in partner fusion, which is immediately followed by meiosis. We recently showed that partner fusion also initiates an active mating block in zygotes and that zygotes lacking, or merely delaying, this re-fertilization block fuse with additional partners resulting in ploidy changes.

Methods

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Results

We now show that prevention of re-fertilization requires upstream meiotic inducers but bifurcates from meiotic signaling. Recurring fertilization correlates with the zygotic cell cycle arrest and forced cell cycle progression prevents zygotic mating in absence of re-fertilization blocks. We find that the previously presumed linear signaling, from Mei3 to Pat1 to Mei2, considered to specifically induce meiosis is in fact branching out and functions to push cells through mitosis if meiosis is impaired. Mei2 and Mei3 independently exert distinct regulation on the cyclin dependent kinase: While Mei2 activates G1-cyclin Cig2, Mei3-induced G1-S transition is Cig2-independent and, according to preliminary results, does not rely on G1 cyclins.

Conclusions

Our work dissects the regulation of the first reported re-fertilization block in fungi. The signaling to block mating shares components with, but bifurcates from, meiotic induction. Importantly, we redefine the meiotic signaling cascade to account for its coordinate regulation of re-fertilization blocks, promoting the cell cycle and changing cell fate.

PB 3 – Bacterial pathogenesis / PB-09

Development of a novel vaccine for contagious caprine pleuropneumonia based on a fast-growing Mycoplasma feriruminatoris chassis

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Background

Contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma mycoides*subsp. *capripneumoniae*(*Mccp*), is a disease of utmost importance in Africa and Asia. A sustainable CCPP control program would benefit from novel and more effective vaccines. The fastest-growing *Mycoplasma*species to date, *Mycoplasma feriruminatoris* (*Mferi*, generation time ~30 min.), appears as the ideal candidate organism towards the construction of an avirulent chassis that will be used to express antigens of *Mccp* on its surface.

Methods

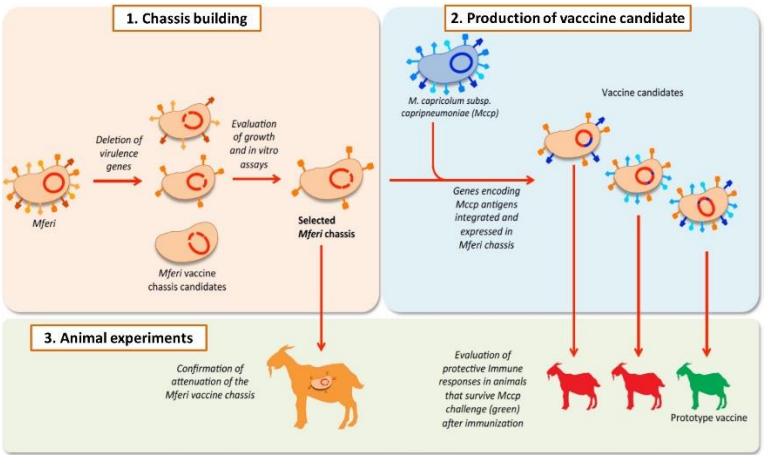
The construction of this *Mferi*-based chassis will be performed using synthetic biology methods, including (1) bacterial genome cloning and reconstruction in yeast,(2) seamless deletions of virulence genes and (3) back transplantation of the genome into a recipient mycoplasma. Selected *Mccp* antigens will then be expressed at the surface of this *Mferi*-derived chassis using similar techniques. The different mutants derived from this strategy will be characterized using *in vitro* and cell assays before animal trials.

Results

Two complementary approaches are now used for the deletion of five genomic regions encoding *M.feri* candidate virulence genes. One uses the CRISPR/Cas9 system for the systematic and successive deletion of each region.The second approach relies on the transformation-assisted recombination (TAR) cloning strategy and aims at reassembling the *Mferi* chassis genome in yeast without these regions.

Conclusions

The establishment of SB technologies on the *Mferi* genome, enhanced by its fast-growing, offers a very powerful system to boost vaccine production for mycoplasma diseases. In addition, because of its exceptional growth properties, *Mferi* could give insights on fundamental research questions related to other mycoplasmas.



Attachment #190

PB 3 – Bacterial pathogenesis / PB-10*

Hyorhinis mutant libraries reveals potential virulence-associated, non-essential genes

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Background

*Mycoplasma hyopneumoniae*and *Mycoplasma hyorhinis*are two genetically closely related species inhabiting the respiratory tract of pigs. *M. hyopneumoniae* is the etiological agent of enzootic pneumonia, while *M. hyorhinis* is considered a commensal of the respiratory tract of pigs potentially acting as a pathogen in polyserositis and arthritis. We hypothesize that genes that are non-essential under lab condition and are unique to one species could account for these differences in pathogenicity.

Methods

To identify such genes, we refined a transposon (Tn) mutagenesis approach for both species. A Tn-sequencing technique for mycoplasma has been established and successfully applied to sequence pools of mutants in parallel. Tn-sequencing data of mutant libraries were analyzed using the software Tn-seq and genes were classified as “essential” and “non-essential”.

Results

Out of 684 coding sequences (CDS) of the *M. hyopneumoniae* strain F7.2C, 292 CDS were classified as nonessential. Besides unique hypothetical proteins, we also found genes involved in the inositol catabolism are non-essential for *M. hyopneumoniae*grown under lab conditions. Furthermore, genes coding for proteins involved in the binding of plasma proteins were found to be non-essential. On the other hand, *M. hyorhinis* strain JF5820 contains a total of 758 CDS, of which 322 have been classified as non-essential. Besides several unique CDS for hypothetical proteins, we found non-essential genes unique to *M. hyorhinis* being involved in DNA modification.

Conclusions

The essentiality patterns of genes of two different porcine mycoplasma species were investigated. A high proportion of unique and non-essential CDS are hypothetical proteins, possibly explaining differences in pathogenicity.

** Student paper*

PB 3 – Bacterial pathogenesis / PB-11* + P-59*

An interbacterial killing device influences bacterial evolution in Vibrio cholerae

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Background

As a mode of horizontal gene transfer (HGT), natural competence for transformation, which involves the direct uptake and integration of exogenous DNA, represents an important driving force for bacterial evolution. In the aquatic environment, *Vibrio cholerae*becomes naturally competent due to its association with zooplanktons, which harbor an exoskeleton composed of chitin. In pandemic *V.cholerae*the type VI secretion system (T6SS), which is a molecular killing device delivering toxic effector proteins into adjacent cells, is simultaneously induced on chitinous surfaces [1]. The genetic material released by such lysed neighboring prey is then available for absorption by the attacking naturally competent predator. In this study, we hypothesized that the T6SS enhances bacterial evolution in *V. cholerae*by 1) increasing the pool of exogenous transforming DNA material and 2) fostering the transfer of large stretches of genomic DNA.

Methods

Genetically modified *V. cholerae*predator and prey strains were grown on chitinous surface to induce natural competence. Transformants were isolated and subject to whole-genome sequencing to determine the extent of the transferred genetic material.

Results

The length as well as the number of horizontally acquired DNA events is increased for T6SS-positive compared to T6SS-negative predator cells. T6SS-positive predators horizontally acquired DNA fragments with a total length of 60kbp on average.

Conclusions

Our data shows that T6SS-mediated neighbor predation leads to the horizontal spread of large genomic stretches and therefore results in the shuffling of new functions between *V. cholerae*strains.

[1]Borgeaud S, Metzger LC, Scrignari T, Blokesch M. (2015) Science 347: 63-67.

** Student paper*

PB 3 – Bacterial pathogenesis / PB-12

A new lethality-based assay to screen anti-virulence compounds against Staphylococcus aureus

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Background

Emergence of antimicrobial resistance (AMR) represents a major threat for public heath and requires urgently the development of new antimicrobial drugs. One promising strategy to efficiently tackle this issue is to target bacterial virulence rather than cell viability. The exceptional ability of *Staphylococcus aureus* to cause infection is due to the wide array of virulence factors produced by this bacterium, recently classified as a high priority pathogen. Pathogenicity of *S. aureus* is regulated by the quorum-sensing mechanisms via the accessory gene regulator (Agr) system.

Methods

We developed an assay to identify compounds with anti-quorum sensing activity. Basically, we setup a reporter system in which the activation of the Agr system induces lethality. To do so, we fused the promoter of the Agr operon, P2, to the *pyrFE* genes, encoding two enzymes that can convert non-toxic 5-fluoroorotic acid (5-FOA) to toxic 5-fluoro-UMP.

Results

We showed that in the presence of 5-FOA, the wild type *S. aureus* was not able to grow. On the other side, growth of the mutant *S. aureus Δagr* carrying the reporter fusion was not affected in the present of 5-FOA. As a positive control for Agr system inhibition, we used autoinducing peptides (AIP) produced by*S. caprae*, and observed restoration of bacterial growth in presence of 5-FOA. We are currently using this experimental approach to screen library compounds coming form fungal extracts.

Conclusions

Our new screening procedure allows the discovery of novel antivirulence molecules against *S. aureus* that could lead to the development of innovative and alternative therapies.

MY 1 – Clinical mycology / MY-01

Comparative genomics for understanding the development of multidrug resistance in Candida lusitaniae

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Background
Multidrug resistance (MDR) has emerged in hospitals due to the use of several agents administered in combination or sequentially to the same individual. We investigated here MDR in *Candida lusitaniae* during therapy of a patient with amphotericin B (amB), azoles and candins over a 3 months time lapse and resolved MDR mechanisms using genome sequencing approaches.
Methods
Different isolates were obtained during therapy and they were assigned to 5 different antifungal susceptibility profiles (P1-P5). The genomes of isolates P1 to P5 were subjected to whole genome sequencing in order to resolve mutations associated with antifungal resistance.
Results
P1 was the earliest isolate obtained at initiation therapy and was susceptible to all antifungals. P2, P3, P4 and P5 were MDR isolates since they exhibited resistance to at least 2 drug classes. P1 to P5 genomes did not undergo significant rearrangements and were related to P1. The PacBio assembled genomes were next compared to each other and with P1. From a total of 13 non-synonymous SNPs (NSS) in P1 to P5 comparisons, 6 were associated with MDR. These NSS were found in genes belonging to a transcription factor, to the target of candins and to genes involved in ergosterol biosynthesis.
Conclusions
In conclusion, genome analysis could resolve the resistance profiles identified in this clinical case. MDR isolates P2 to P5 accumulated 6 different mutations conferring resistance to all known antifungal agents. This study illustrates the capacity of *C. lusitaniae* to rapidly adapt to drugs within the host. Genome analysis helped to decipher drug resistance mechanisms

MY 1 – Clinical mycology / MY-02

Functional and expression analyses of the Pneumocystis MAT genes suggest obligate sexuality through primary homothallism within host lungs

Ms. Sophie Richard [1], Dr. Joao Almeida [2], Dr. Ousmane Cissé [3], Ms. Amanda Luraschi [1], Prof. Olaf Nielsen [4], Dr. Marco Pagni [5], Dr. Philippe Hauser [1]

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Background

Fungi of the genus *Pneumocystis*are obligate parasites colonizing mammals' lungs which are host-species specific. *Pneumocystis jirovecii* and *Pneumocystis carinii* infect respectively humans and rats. They can turn into opportunistic pathogens in immuno-suppressed hosts causing pneumonia. Their cell cycle is poorly known, mainly because of the absence of a method of culture *in vitro*. Comparative genomics suggested that their mode of sexual reproduction is primary homothallism involving a single mating type (*MAT*) locus encompassing plus and minus genes (*matMc*, *matMi*, and *matPi*). Here, we tested this hypothesis in the laboratory.

Methods

The function of genes was studied by restoration of sporulation in the corresponding mutant of fission yeast. Standard and reverse-transcriptase PCR was used to assess the structure and expression of the *P. jirovecii MAT* locus from bronchoalveolar lavages of patients. Transcription factors were searched by matching *Pneumocystis* genomes against representative bait sequences using tBLASTn. Cis-acting sequences were searched using local and global alignments tools.

Results

The function of the *P. jirovecii* and *P. carinii matMc* genes was ascertained. The same single *MAT* locus was evidenced in all *P. jirovecii* isolates, and all three *MAT* genes were often concomitantly expressed during *Pneumocystis* pneumonia. Other types of *MAT* transcription factors were not identified, nor cis-acting motifs flanking the *MAT* locus that could have been involved in *MAT* switching.

Conclusions

Pneumocystis sexuality through primary homothallism appears obligate within host's lungs in order to complete the cell cycle, *i.e.* produce asci necessary for airborne transmission to new hosts.

MY 1 – Clinical mycology / MY-03*

Rapid induction of reversible azole resistance in Aspergillus fumigatus

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Background

*Aspergillus fumigatus*is an opportunistic pathogenic mold responsible of invasive aspergillosis in immunosuppressed patients. Voriconazole is the first-line treatment, but emergence of resistance is a concern. While azole resistance is usually due to mutations in the *cyp51A* gene encoding the target enzyme other mechanisms may be involved.

We investigated the mechanisms of stress adaptation and resistance to azoles in *A. fumigatus*, which are not related to *cyp51A* mutations.

Methods

A. fumigatus wild-type strain (Ku80) was exposed to sub-inhibitory concentrations of voriconazole on 10 successive agar plates generating the azole-resistant strain Ku80R. We performed successive cultures of Ku80R on azole-free agar plates to see if the resistance is reversible. Voriconazole minimal inhibitory concentration (MIC) and RNAseq (Ku80 and Ku80R exposed/not exposed to Voriconazole) were performed.

Results

Ku80R voriconazole and posaconazole MICs was significantly increased compared to Ku80. Partial reversion of this resistance was noticed and sequencing the entire cyp51A gene and promoter did not show any mutation. Seven transporters of the ATP Binding Cassette and Major Facilitator Superfamily were significantly overexpressed in the presence of voriconazole in wild-type Ku80. In Ku80R, several transporters exhibited significant higher expression compared to Ku80 under basal conditions (no treatment) and/or in the presence of voriconazole. Basal expression of cyp51A and other genes of ergosterol biosynthesis was also increased in Ku80R compared to Ku80.

Conclusions

We rapidly induce reversible pan-azole resistance in *A. fumigatus* and this adaptation to azole stress was probably due to overexpression of the target gene *cyp51A* and of some ABC and MFS transporters.

**Student paper*

MY 1 – Clinical mycology / MY-04

Detection of Candida auris by MALDI-TOF MS

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Background

C*andida*spp. are a major cause of bloodstream infections associated with high mortality. Recently *Candida auris* emerged all over the world as a previously unrecognized *Candida* species. *C. auris*could be classified in 5 clades. All of them are characterised by intrinsic resistance to fluconazole and rapid acquisition of resistance to all antifungals. Remarkable, this yeast is a challenge for hospital hygiene due to its potential for nosocomial transmission leading to hospital outbreaks.

In Switzerland, the first case of *C. auris*was documented in Geneva in October 2017. Thus, accurate and rapid identification of *C. auris*is crucial for an efficient antifungal stewardship and adapted epidemiological measures. *C. auris*is misidentified by classical biochemical methods but efficiently identified by MALDI-TOF MS using "research use only" libraries. However, some difficulties in obtaining a high score with direct deposit of *C. auris*on the MALDI plate when using the Biotyper have been reported. Both protein extraction procedure and addition of new *C. auris* spectra in the MALDI-TOF MS databases seem to improve identification.

Methods

In this study we compared 5 methods of extraction/deposit ranging from simple colony deposit to full mechanical protein extraction for several *C. auris*strains of different clades. Identification was done using a Biotyper system.

Results

It appeared clearly that full protein extraction is needed to obtain accurate identification using a biotyper system.

Conclusions

We are currently adding new *C. auris*spectra from different clades in our database to evaluate the identification improvement using again different types of extraction/deposit methods.

MY 2 – Environmental mycology / MY-05

Assessment the potentiality of some soil fungi in Battery Myco-remediation

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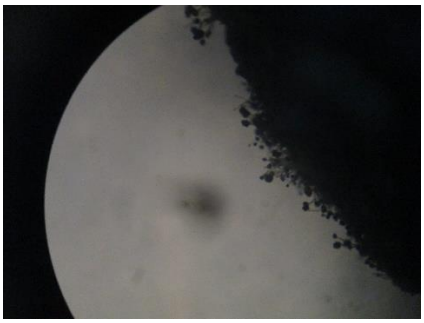
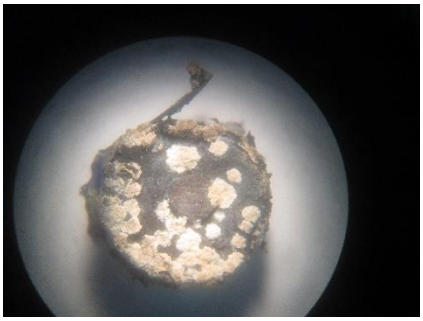
1. *Suez Canal University*

Background
Heavy metals have been reported as hazard pollutants, due to their mobility in natural ecosystems and their toxicity to living
organisms. Some electronic wastes like batteries contain high amount of Heavy metals (i.e.zinc, nickel, Manganese etc).
Methods
The presence of fungi has allowed absorption most types of wastes, it called mycoremediation. In this study we aiming to find fungal isolates able to biodegrade batteries. Several soil fungi were isolated and screened to select the most promising isolates able to accumulate heavy metals.
Results
Aspergillus niger showed the highest capability to absorb zinc upto 96 % based on atomic absorption analysis, after that 4
fungal species were applied in disc of batteries to test its degradation potentiality. Our results indicted that Eveready batteries degradation were more than Panasonic batteries this could be attributed to alkaline pH in Panasonic batteries that reached to 9. Several parameters used to measure the ability of fungi in degradation of batteries like disc weight loss, reduction in medium pH. *Aspergillus niger* showed the highest weight loss in Eveready batteries discs reached to 25% and reduction of pH upto 6.5. Fungi like *Aspergillus niger* represented the best remediation agents for different heavy metals existed in used batteries. This research introduce safe and cheap remediation method based on soil fungi with high potentiality to accumulate toxic heavy metals.
Conclusions
Fungi like *Aspergillus niger* represented the best remediation agents for different heavy metals existed in used batteries. This research introduce safe and cheap remediation method based on soil fungi.





Attachments #006

MY 2 – Environmental mycology / MY-06*

IMPLICATIONS OF TYPHOON ON COMMUNITY ASSEMBLAGES OF MYXOMYCETES: A CASE STUDY IN BEACH AND INLAND FORESTS OF AURORA AND QUEZON PROVINCE, PHILIPPINES

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Background

Typhoons are one of the most devastating natural catastrophes and could cause modification of plant communities in a forest habitat. Can such modification in community assemblages be also true for myxomycetes?

Methods

In this research, community assemblages of myxomycetes were studied in beach and inland forests in Aurora and Quezon Province, Philippines following a typhoon. Substrates, i.e. aerial leaf litter (AL), ground leaf litter (GL) and twigs (TW), including field specimens were collected for the preparation of moist chambers and assessment of diversity.

Results

Myxomycete records from the field and moist chambers led to the identification of 63 species belonging to 21 genera in two forest habitats in both provinces. Comparing now the two forest types using different diversity indices, inland forest had higher species diversity, richness and evenness than beach forest, both observed in the two provinces. However, higher taxonomic diversity was obtained from beach forest than inland forest. Community analysis revealed that there were more than 60% and 50% similarities in myxomycetes species composition between beach and inland forests in Aurora and Quezon province, respectively. Moreover, higher species diversity were observed in both forest types when field and substrate collections were conducted a month after a typhoon.

Conclusions

The results suggested that natural disturbance such as typhoon could alter the assemblages and diversity of myxomycetes in beach and inland forests.

**Student paper*

Community Analysis between forest type

Aurora

Forest type	Beach Forest	Inland Forest
Beach Forest		PS=0.66
Inland Forest	CC=0.65	

Quezon

Forest type	Beach Forest	Inland Forest
Beach Forest		PS=0.54
Inland Forest	CC=0.65	

Beach Forest: 29 species
Inland Forest: 33 species
Common Species: 20 species

Beach Forest: 33 species
Inland Forest: 41 species
Common Species: 23 species

Myxomycetes Diversity in Aurora and Quezon

Site	Date of last typhoon	Time of Collection	Gap
Aurora	October, 2016	May, 2017	8 months
Quezon	September, 2017	October, 2017	1 month

Site	No. of Genera	No. of Species	S/G	HS	HG	E	FAI	SID
Aurora	18	42	2.33	1.17	6.88	0.45	11.98	0.15
Quezon	19	48	2.53	1.28	7.92	0.50	14.57	0.11

Taxonomic Diversity: Aurora

Species Diversity: Quezon

Taxonomic and Species Diversity between forest type

Aurora

Forest Type	No. of Genera	No. of Species	S/G	HS	HG	E	FAI	SID
Beach Forest	15	29	1.93	1.08	5.15	0.46	8.78	0.17
Inland Forest	15	33	2.20	1.17	6.33	0.53	12.75	0.13

Quezon

Forest Type	No. of Genera	No. of Species	S/G	HS	HG	E	FAI	SID
Beach Forest	16	30	1.88	1.10	5.36	0.47	9.31	0.15
Inland Forest	17	41	2.41	1.35	7.94	0.62	18.27	0.08

Taxonomic Diversity: Beach Forest

Species Diversity: Inland Forest

Attachments #016

MY 2 – Environmental mycology / MY-07*

New insights in bacteria-fungi interactions in nutritional interference: the case of oxalotrophy as a biocontrol strategy against phytophatogenic fungi

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Background

Phytopathogenic fungi have a wide host range and cause economically important damage in crops worldwide. Some of these pathogens use organic acids, and in particular oxalic acid, as a virulence factor. Degradation of this organic acid could be a new approach of biological control of fungal pathogens. Oxalotrophic bacteria are found in various environments and use oxalate as carbon and energy source. Thus, they could provide a protection to the host against the pathogen through nutritional interference.

Methods

Confrontation experiments in Petri dishes were conducted with different media (MA1/10, MS, R2A) using*Botrytis cinerea*,*Rizoctonia solani* and*Zymoseptoria tritici* (phytopathogenic fungi), *Cupriavidus necator*and*C. Oxalaticus* (oxalotrophic bacteria).*Trichoderma rossicum*and*Pseudomonas putida* (a non-pathogenic fungus and a nonoxalotrophic bacterium) were used as controls.

Results

C. necator and *C. oxalaticus* – two oxalotrophic bacteria – control fungal growth of *B. cinerea* and *Z. tritici* on different media, but the control is less pronounced with *R. solani*. In contrast, *P. putida* – a non-oxalotrophic bacterium – exerts an intense pressure on *R. solani* and *Z. tritici* on R2A medium. All three bacteria are attracted by *Z. triciti* on different media.

Conclusions

Further investigations need to be done on the production of oxalic acid by the different fungi and its consumption by the bacteria. As *P. putida* seems to be a promising biocontrol agent against *Z. tritici*, further experiments are planned to decipher the biocontrol mechanism and its potential application for the control of other phytopathogenic fungi.

**Student paper*

MY 2 – Environmental mycology / MY-08*

A chromosome-scale assembly of the wheat powdery mildew genome provides insight into origin and evolution of genes associated with host-pathogen interactions

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Background

*Blumera graminis forma specialis (fsp) tritici*causes wheat powdery mildew, one of the most important wheat diseases. To improve disease management, it is essential to understand the evolution of pathogen adaptation to host resistance. The highly repetitive *B.g. tritici* genome has so far prevented a systematic analysis of the diversity and evolution of effector genes which are known to be involved in host adaptation. Here, we present the analysis of a high-quality genome sequence of *B.g. tritici* isolate 96224.

Methods

The chromosome-scale scaffolds were assembled by combining information from PacBio sequencing, BAC analysis and a high-resolution genetic map.

Results

With 1,017 genes, the arsenal of candidate effectors genes is nearly twice as large as previously estimated. Candidate effector genes are mostly present in distinct clusters in which individual genes show a wide range of expression levels. Additionally, candidate effector genes are located in regions with increased genetic recombination. We found that hundreds of candidate effector genes are derived from transposable element (TE) sequences, indicating that novel genes evolve stepwise from TEs.

Conclusions

Our data suggest that high recombination rates and high repeat content result in a genomic environment that drives the evolution of genes that are putatively involved in host-pathogen interactions.

**Student paper*

MY 3 – Basic mycology / MY-09*

Investigation of the parameters of Pom1 gradient formation

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Background

Concentration gradients are critical for providing positional information in various cellular and developmental contexts. In fission yeast, the DYRK-family kinase Pom1 forms membrane-associated gradients from the poles of the cell and provides cues for the spatio-temporal regulation of cell division. *In vivo* experiments have shown that gradients are nucleated by a type I phosphatase complex delivered to cell poles on microtubule plus-ends. This dephosphorylation reaction promotes Pom1 membrane binding, while detachment is ensured by the protein‘ s auto-phosphorylation properties. The study addresses what parameters define gradient shape and what are the minimal elements sufficient for gradient formation.

Methods

Classical molecular and genetic tools coupled with advanced imaging techniques as super-resolution PALM microscopy. Created ‘shortcut‘ proteins for successful gradient reconstitution *in vivo* in systems lacking the gradient, as well as tests in *in vitro*microtubule reconstitution experiments.

Results

We describe that gradient shape depends on a multi-phosphorylation reaction, suggesting that multiple reactions encode a timer function for Pom1 diffusion along the membrane. We altered gradient distribution by modifying the protein‘s phosphorylation state with a biologically relevant result - an increase of cell length. Previously proposed model suggested that differential diffusion of Pom1 clusters is the driving force of gradient formation, however, we observe no differences in the diffusion constants of individual molecules via PALM. We also find that microtubule delivery of the phosphatase complex is not sufficient for robust gradient patterning, indicating that clustering and/or membrane retention mechanism are necessary for gradient nucleation.

Conclusions

The study suggests an alternative model for Pom1 gradient formation.

**Student paper*

MY 3 – Basic mycology / MY-10

Comparative study of genetically similar but phenotypically distinct Candida albicans isolates

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Background

Candida albicans is an opportunistic fungal pathogen with a highly diverse population structure. Recently it has been proposed that the genetic background of the fungus influences the outcome of the disease. We have previously shown that different *C. albicans* isolates differ in their capacity to persist in the host, which is related to the degree of immune activation by the host. In this study, we investigate the phenotypes and functions of pairs of fully genome-sequenced *C. albicans* isolates that differ in their ability to interact with the host, while they are genetically highly similar.

Methods

The interaction of *C. albicans* isolates with the host was assessed *in vitro* using epithelial cell cultures and *in vivo* using an experimental model of mucosal infection in mice.

Results

We found that the selected strains vary greatly in their ability to induce cellular damage and to trigger the release of pro-inflammatory cytokines such as IL-1α from keratinocytes. The capacity of the isolates to induce host damage and inflammation was inversely linked to their ability to persist for longer periods of time in the oral mucosa *in vivo*. Using phenotypic and transcriptomic analysis together with computational approaches, we aim at identifying genetic factors associated with fungal-host interactions by taking advantage of the genetic relatedness of the strains.

Conclusions

This study shall reveal novel fungal determinants of *C. albicans* pathogenicity at mucosal host surfaces.

MY 3 – Basic mycology / MY-11*

What is a fungal barcode?

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Background
Metabarcoding is widely used to describe fungal diversity and community structures. The internal transcribed spacer (ITS) region, covering ITS1, 5.8S, and ITS2 is the primary fungal DNA barcode. Universal primers ITS3/ITS4, which are routinely used for soil fungal diversity surveys, amplify an approximately 100bp long stretch of 5.8S, and the ITS2 region. Taking advantage of the divergent evolutionary rate of 5.8S and ITS2, we hypothesized that classification accuracy increases when using the conserved 5.8S for kingdom, and the variable ITS2 for genus- or species-level identification.
Methods
To test the accuracy of fungal identifications 200'000 random ITS sequences from Genbank were classified with 5.8S and ITS2. Consequences of these different target verifications on diversity analyses from community- to OTU-levels were assessed using a metabarcoding dataset with 32 million sequences, grouped into 30'843 OTUs.
Results
The accuracy of fungal identification attained 99.2% based on 5.8S and 95.7% based on ITS2. Based on 5.8S, 24'284 OTUs were classified as fungi, while only 16'191 OTUs were assigned as such based on ITS2.This decrease of 33% of OTUs when using ITS2 for fungal identification was mainly caused by OTUs probably belonging to ascomycetes, early diverging lineages, and unknown fungi. Nonetheless, almost no consequences were found for dissimilarity-based community structure analyses (Mantel test,r=0.98).
Conclusions
We underline the necessity of accurate sequence analysis for fungal biodiversity surveys,e.g. fungal richness, taking into account molecular characteristics of the markers applied and suggest using conserved regions such as the 5.8S-barcode for higher taxonomic target verification, while considering variable regions for lower-level taxonomic classification.

*Student paper

MY 3 – Basic mycology / MY-12

Two small GTPases orchestrate cell polarity in fission yeast

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Background
The establishment and maintenance of cell polarity is a critical feature of many cell types. In eukaryotes, the Rho-family GTPase Cdc42 in its active GTP-bound form plays a central role for polarization. Single cell eukaryotic organisms, like yeasts, are useful model systems to investigate the basis of cell polarity regulation. In these cells, Cdc42 activation at presumptive polarity sites promotes a positive feedback mechanism that further support Cdc42-GTP accumulation to sustain polarized growth. Several studies in budding yeast have shown that this positive feedback involves the assembly of a complex containing Cdc42-GTP, its activator, a Guanine Nucleotide Exchange Factor (GEF), its effector, a p21 Activated Kinase (PAK) and a scaffold protein. Fission yeast (*Schizosaccharomyces pombe*) cells are rod-shaped and grow in a bipolar fashion from the cell ends, where active Cdc42 accumulates.
Methods
Through a genetic approach we have dissected the main cell polarity players in *pombe*and shown the existence of a complex as in budding yeast, sufficient to promote polarization and to support bipolar growth. Interestingly, in fission yeast, a second small GTPase, Ras1 that gets also activated at the cell tips, regulates Cdc42 activity. We are in the process of investigating the relationship between these two small GTPase modules and their requirements for cell polarity establishment.
Results
Ras1 is required for proper Cdc42 activation through recruitment of its GEF. GEF-Cdc42-GTP-PAK complex exists in fission yeast and form a positive feedback.
Conclusions
We are currently investigating the relationship between Cdc42 and Ras1 small GTPases and their role in cell polarity regulation.

VI 1 – Virus-host interaction / VI-01*

Characterization of the epitranscriptomic landscape of HIV-infected cells

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1. *CHUV-Microbiology Institute*

Background

Epitranscriptomics, *i.e.* post-transcriptional modifications, affect cellular processes, such as RNA splicing, export, stability and translation, and may thus affect HIV expression. To investigate the role of epitranscriptomic marks on HIV expression, we explored the dynamics of N6-methyladenosine (m6A) and 5 -methylcytosine (m5C) RNA modifications in a productive HIV infection model.

Methods

The SupT1 T cell line was infected with a VSV-G pseudotyped HIVeGFP-based vector to ensure ~70% efficiency of transduction. Cells were collected at 12, 24 and 36h post-infection for mRNA extraction and FACS analysis. M6A RNA modifications were investigated by methylated RNA immunoprecipitation followed by sequencing (MeRIP-Seq). Briefly, MeRIP-Seq started with fragmentation of mRNA, which is subsequently, either left untreated (input control), or immunoprecipitated with a murine IgG antibody (IP control), or immunoprecipitated with a specific anti-m6A antibody (test condition). M5C RNA modifications were investigated using a bisulfite conversion approach, followed by sequencing. Libraries from immunoprecipitated or bisulfite converted samples and respective controls were prepared for high throughput sequencing using the Illumina platform.

Results

Preliminary experiments allow successful optimization of both MeRIP-Seq and Bisulfite conversion techniques to study the epitranscriptomic landscape of HIV-infected cells.

Conclusions

This study should highlight the presence of RNA modifications on HIV transcripts, as well as their pattern over time, in the context of a productive infection. This should constitute a reference for the epitranscriptomic landscape during HIV replication and provide a valuable resource for comparative studies in the context of other conditions or viral infections.

**Student paper*

VI 1 – Virus-host interaction / VI-02*

Hantavirus entry into human respiratory epithelial cells involves macropinocytosis

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Background

Hantaviridae are emerging rodent-borne viruses associated with severe human diseases with high mortality. The limited therapeutic options make the development of novel efficacious anti-viral agents to combat hantaviruses an urgent need. The identification of cellular factors hijacked by hantaviruses in order to enter host cells is a promising approach for the development of novel strategies to combat pathogenic hantaviruses.

Methods

Considering the biosafety restrictions linked to work with live pathogenic hantaviruses and that virus cell attachment and entry are mediated exclusively by the viral envelope, we established a pseudotype platform based on recombinant Vesicular stomatitis reporter virus (VSV) bearing the glycoprotein of hantavirus Hantaan virus (HTNV) and Andes virus (ANDV). We first screened a library of 90 small molecule kinase inhibitors in our hantavirus pseudotype platform using a semi-high-throughput assay. Then, we complemented our screen with a targeted approach including a panel of well-defined "diagnostic" inhibitors against cellular factors involved in endocytosis.

Results

Our screen identified specific sets of cellular kinases required for cell entry of HTNV and ANDV that only partially overlapped, indicating important virus-specificdifferences. Entry of both, HTNV and ANDV critically depended on sodium-proton exchangers and actin, which are major hallmarks of micropinocytosis. HTNV and ANDV further showed differential dependence on known regulatory factors of macropinocytosis.

Conclusions

Our results using pseudoviruses provide first evidence for a role of macropinocytosis-related pathways in hantavirus entry into human respiratory epithelial cells with important virus-specific differences. Promising candidate anti-viral drugs are currently validated using live pathogenic hantaviruses in the high containment facilities at Spiez Laboratory.

**Student paper*

VI 1 – Virus-host interaction / VI-03*

Epidemiological analysis of enterovirus cases in Bern since 1998

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1. *Institute for Infectious Diseases, University of Bern, Bern*

Background
Human enteroviruses are small RNA viruses associated with a variety of symptoms ranging from mild respiratory diseases to severe neurological infections. Using the bio bank available at the Institute of Infectious Diseases (IFIK), University of Bern, an epidemiological analysis of enterovirus cases was conducted from patient samples collected since 1998.
Methods
The bio bank consists of samples screened for enterovirus presence by the IFIK diagnostic department. Number of cases was related to specimen types, associated symptoms and demographic information, and analysed across time and seasons. Additionally, for more severe cases, genotyping directly from patient samples was performed by sequencing the surface protein VP1 encoding gene using nanopore sequencing and classical methods (Sanger, Illumina MiSeq).
Results
In the analysed period from 1998 to 2016, almost 2000 samples were found to be enterovirus positive, from which nearly 700 produced isolates in cell cultures. Enteroviruses were most commonly detected in cerebrospinal fluid (52%) and stool (30%) samples. The majority of patients with enterovirus positive samples were under the age of 15 (80%), with 30% under the age of 1. A seasonal trend with peaks of enterovirus infections in the summer months was observed. Further phylogenetic comparison of genotypes commonly found in Bern will be presented and compared to the genotypes identified in outbreaks outside of Switzerland.
Conclusions
This study gives insights on the extent and types of enterovirus infections, and epidemiological information in one of the largest Swiss hospitals over the last 20 years.

**Student paper*

VI 2 – Emerging viruses / VI-04

Receptor tyrosine kinases are promising targets in Lassa virus antiviral therapy

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Background

Lassa Virus (LASV) causes a severe viral hemorrhagic fever with high mortality in humans. The main LASV receptor is dystroglycan (DG) in its functional O-glycosilated form. However, glycosylation of DG does not always correlate with LASV tropism in vivo, suggesting alternative receptors. In absence of functional DG, LASV can hijack phosphatidylserine receptors of the Tyro3/Axl/Mer (TAM) family and enter cells via “apoptotic mimicry”. Productive LASV entry via the receptor tyrosine kinase (RTK) Axl involves virus-induced activation of Axl signalling and macropinocytosis.

Methods

Here we investigated the role of Axl RTK activity for LASV entry as a function of DG modification, using recombinant lymphocytic choriomeningitis virus expressing LASV glycoprotein (rLCMV-LASVGP) as a BSL2 surrogate.

Results

Rather unexpected, we found that the specific Axl RTK inhibitor R428 potently inhibits LASV entry in different cell types with EC50 in the mid-nanomolar range, independently of the functional glycosylation of DG. Notably, LASV entry via functionally glycosylated DG into human epithelial cells did not induce Axl activation but was still highly sensitive to R428, suggesting a role of Axl RTK activity as an essential “permissive” signal (Fig. 1ab). Combination of R428 with the hepatocyte growth factor receptor (HGFR) inhibitor EMD1214063 resulted in a pronounced synergistic anti-viral effect, indicating nonredundant roles of these RTKs in LASV entry. Used in combination with ribavirin, a nucleoside analogue used to treat human Lassa fever in the clinic, the RTK inhibitors showed additive anti-viral effects (Fig. 2).

Conclusions

Our studies provide a rationale to target RTKs in combinatorial therapy against human Lassa fever.

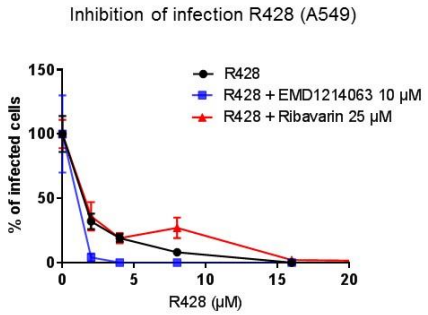
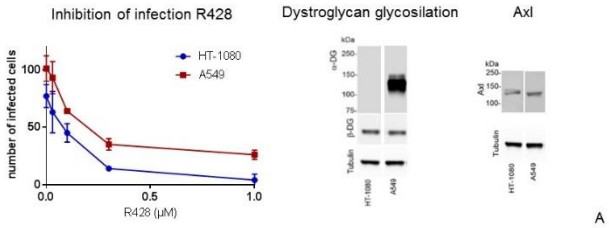


Fig.2



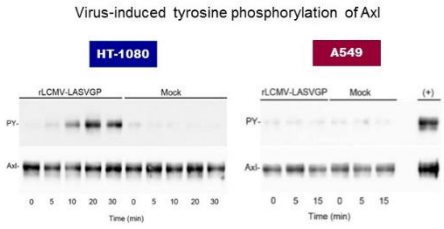


Fig.1

Attachments #080

VI 2 – Emerging viruses / VI-05

Identification of small-molecule viral inhibitors targeting various stages of the life-cycle of hantaviruses

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Background
Hantaviruses are rodent-borne viruses associated with severe human diseases. There is currently no licensed vaccine against hantaviruses and therapeutic options are limited to supportive care. Our research projects aim at the development of novel strategies for antiviral therapeutic intervention.
Methods
In order to target 1) host cell entry, 2) transcription and 3) replication, we have developed cell-based assays suitable for high-throughput screening of small synthetic molecules to identify novel candidate inhibitors. First we have established and validated an efficient production system for VSV pseudotypes bearing the glycoproteins of the prototypic highly pathogenic Old World hantavirus Hantaan and the New World hantavirus Andes. Second, to target the viral RNA-dependent RNA polymerase L, we have developed a robust cell-based functional assay of for the HTNV and Puumala virus endonuclease function of L. Finally, as a starting point to develop a minireplicon system for hantaviruses, we established a minireplicon system for the Bunyavirus Uukuniemi that can be implemented for drug screening.
Results
In our screens, we have identified several inhibitory compounds interfering with viral entry that are currently tested against infectious hantaviruses in the high containment laboratories at Spiez Laboratory.
Conclusions
Taken together, the inhibitors identified by the cell–based assays may pave avenue for the development of new class of anti-hantavirus drugs for prophylaxis and therapy and are useful “molecular probes” to gain novel insights into the fundamental virology of these important pathogens.

VI 2 – Emerging viruses / VI-06

Combination of second and third generation sequencing technologies for genome sequencing of RNA viruses in clinical samples

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1. *Institute for Infectious Diseases, University of Bern, Bern*

Background
RNA viruses affect millions of people every year and lead to a large range of symptoms, encompassing mild illnesses to severe neurological disorders. Whole-genome sequencing would often be needed to characterize fast evolving viruses, but the approach is more expensive and time consuming than classical genotyping methods. In this study, we used the unique possibility of nanopore sequencing to sequence RNA natively, and applied direct RNA sequencing to a stool sample from an enterovirus-positive patient.
Methods
RNA from stool sample, extracted either by automatic extraction system or manually, was directly sequenced using a MinION nanopore sequencer (Oxford Nanopore Technologies), a small portable device allowing long reads and real-time acquisition of RNA sequences. Results of direct RNA sequencing were compared to those obtained by sequencing the corresponding viral cDNA molecules with or without amplification on an Illumina MiSeq sequencer.
Results
Nanopore direct RNA sequencing of polyadenylated RNA extracted from stool sample produced several kb long RNA fragments, representing the near-complete RNA genome of the target virus. The approach was found to be compatible with automated extraction procedure, but requires high RNA quantities, which may impair the sensitivity of the approach. Illumina sequencing confirmed the finding of direct RNA sequencing and allowed the identification of potential genomic variants in the stool sample.
Conclusions
We show that third generation sequencing (nanopore sequencing) combined with second generation sequencing (e.g. Illumina MiSeq) technologies offers long and accurate genomic sequences, which will allow significant advances for pathogen identification and characterization in clinical diagnostic laboratories.

VI 3 – Viral tropism and virulence / VI-07

Rapid and accurate diagnosis of Influenza A/B and respiratory syncytial virus in adults using FilmArray, Liaison MDX and GeneXpert systems

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Background

Rapid and accurate diagnosis of respiratory viral infections is essential for effective patient management. In most clinical laboratories diagnostic is performed using antigen immunoassays or nucleic acid amplification tests (NAATs). We compared the performance of three NAATs, FilmArray Respiratory Panel 2*plus* (BioFire Diagnostics), Liaison MDX Simplexa Flu A/B & RSV (DiaSorin) and GeneXpert Xpert Xpress Flu/RSV (Cepheid), for simultaneous detection of Influenza A/B and respiratory syncytial virus (RSV) in adults. Furthermore, RSV Respi-Strip (Coris) antigen assay, routinely performed in our laboratory, was included in the study.

Methods

Performance characteristics were obtained using 149 prospective nasopharyngeal swabs (eSwab, Copan) collected during peak influenza season 2017/2018. Additionally, 23 retrospective specimens previously analysed RSV-positive with FilmArray RP1.7 were included in order to determine RSV sensitivity. Specimens were considered positive if 2/3 (Influenza A/B) or 2/4 (RSV) tests showed a positive result.

Results

All three NAATs resulted in sensitivities/specificities of 100% for Influenza A and high agreement rates for Influenza B (Table 1). In contrast to NAATs, Respi-Strip antigen assay for diagnosis of RSV in adults exhibited lower sensitivity (63%) in prospective specimens. Sensitivities for RSV using FilmArray, Simplexa, Xpert and antigen assays on retrospective specimens were 100%/83%/100%/44%, respectively.

Conclusions

Overall, all NAATs showed accurate results for all viral targets in clinical specimens allowing quick time-toresult. Respi-Strip antigen assays showed lower sensitivity compared to NAATs thus providing limited diagnostic reliability in adults. eSwab (Copan) specimen collection and transport system for detection of Influenza and RSV showed full compatibility with all performed assays.

Table 1. Performance characteristics obtained in the prospective study (n=149).

FA = FilmArray, MDX = Liaison MDX, GX = GeneXpert, Coris AG = antigen assay, PPV/NPV = positive/negative predictive value, n = number of specimens

	Influenza A 21 pos / 128 neg			Influenza B 51 pos / 98 neg			RSV 8 pos / 141 neg			
	FA	MDX	GX	FA	MDX	GX	FA	MDX	GX	Coris AG
Sensitivity %	100	100	100	100	94	100	100	100	100	63
Specificity %	100	100	100	99	100	98	99	100	100	100
PPV %	100	100	100	98	100	96	89	100	100	100
NPV %	100	100	100	100	97	100	100	100	100	98

Attachment #77

VI 3 – Viral tropism and virulence / VI-08*

The prevalence and epidemiology of human adenovirus infections in Bern; 1998 –Jan 2018

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1. *Institute for Infectious Diseases, University of Bern. Biology Division, Spiez Laboratory, Swiss Federal Office for Civil Protection, Spiez, Switzerland,* ***2.*** *Institute for Infectious Diseases, University of Bern, Switzerland*

Background

Human adenoviruses (HAdV) are known to be emerging viral respiratory tract infections in humans along with Middle East respiratory syndrome coronavirus, H7N9 influenza A virus or swine-like influenza H3N2 variant virus. This DNA virus is associated with a wide range of illnesses ranging from benign colds to more serious conditions including gastroenteritis, acute respiratory infections, conjunctivitis, haemorrhagic cystitis, meningoencephalitis, and causes much severe disease in immunocompromised hosts, infections that sometimes result in death. We investigated the prevalence, epidemiology and clinical characteristics of HAdV infections observed in Bern since 1998.

Methods

Archive of clinical samples constituted since 1998 to 2018, including several hundreds of specimens confirmed positive for HAdV by virus culture sent by the Inselspital and other surrounding hospitals for HAdV diagnostic purposes was descriptively explored and analysed using statistical R environment.

Results

We analysed demographic data, clinical characteristics of HAdV cases, distribution and seasonal trends of HAdV infections. HAdV was most commonly detected in upper respiratory tract samples and in children aged 0-10 years. We noticed higher HAdV prevalence in the year 2009 and 2010 with a maximum of 114 and 123 positive cases, respectively. This corresponds to reports of increased incidences of HAdV seen in other parts of the world during the same time

Conclusions

We have provided epidemiological trends, demographic data and clinical characteristics of HAdV infections in Bern from 1998 to Jan 2018. This analysis provides foundation for molecular epidemiological work to be performed on the biobank. On-going genome sequencing results will be presented.

**Student paper*

Session 1 – Modern diagnostic microbiology / S-01

Optimization of carbapenemase detection in pathogenic bacteria using Matrix-Assisted Laser-Desorption/Ionization Time of Flight (MALDI-TOF)

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Background

Antibiotics, especially β-lactams, are the main defense against bacteria. However antimicrobial resistance is an increasing concern since last decades. In presence of multi-resistant strains, carbapenems are often the last resort due to their broad-spectrum activity. Though, we observed a dramatic emergence of carbapenem resistance due essentially to carbapenemases hydrolysing the drug. Classical methods cannot always properly detect carbapenemase especially those with week hydrolysis activity such as OXA48. A potent alternative for carbapenemase detection is MALDI-TOF MS (matrix-assisted laser desorption/ionization time of flight mass spectrometry).

Methods

- Optimization of meropenem spectra acquisition using pure meropenem, and 2 *klebsiella pneumoniae* strains as positive and negative controls.
- R-script development to calculate a ratio corresponding to hydrolyzed versus non-hydrolyzed meropenem peaks.
- Validation on a panel of 24 isolates taken from the CHUV Microbiology Laboratory collection, including carbapenemase producers and non-producers.

Results

We successfully obtained meropenem MALDI-TOF spectra and developed a bioinformatic pipeline of spectra analysis in R using the MALDIquant package. This pipeline consists in normalizing the spectra and calculating a logRQ ratio between hydrolyzed and non-hydrolyzed meropenem peaks. Applying this pipeline of analysis on 24 clinical strains, we obtained up to 100% specificity and sensitivity.

Conclusions

We show the feasibility of the method with success. Some parameters have to be optimized such as threshold to define significant hydrolysis or not. we propose to analyze more strains especially non-producing carbapenemases strains. For a routine diagnostic use, a web-based application is in development based on the R-script settled in this preliminary study to analyse spectra.

Session 1 – Modern diagnostic microbiology / S-02*

Evaluation of different Nuclear Magnetic Resonance (NMR) methods for metabolomic studies in Streptococcus pneumoniae

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Background

Growth environment has been shown to influence thickness of the capsular polysaccharide in *Streptococcus pneumoniae*, but the mechanisms of this influence are not precisely understood. Impaired production of specific metabolites under different growth conditions may be a limiting factor for CPS thickness. In this study we evaulate different Nuclear Magnetic Resonance (NMR) methods to determine the influence of the carbon source and capsular serotype on metabolite concentrations. With our results, we expect to discover implications for the regulation of different metabolic pathways.

Methods

Several pneumococcal strains were cultured under various growth conditions and whole cell extracts of the bacteria were then analysed using several different NMR methods.

Results

On the whole, our study shows that ^{1}H NMR spectroscopy is effective at detecting changes in the metabolome because of its high sensitivity. However, mixtures result in very complex spectra that are difficult to resolve, making 2D experiments and long measurement times necessary. Broader chemical shift ranges and no signal overlap make ^{31}P NMR measurements useful for investigating phosphorylated compounds, but low concentrations require large cultures and long measurement times to acquire good spectra. ^{13}C NMR is well-suited for investigating the metabolic pathways of specific compounds from the medium, as ^{13}C labelled substances are relatively easily available.

Conclusions

Our findings will allow us to establish a detailed protocol for the determination of differences in CPS metabolism in different serotypes and with different carbon sources. We also expect to make new findings on the regulation and efficiency of different pneumococcal metabolic pathways in the future.

** Student paper*

Session 1 – Modern diagnostic microbiology / S-03*

The intestinal microbiota predisposes to the occurrence of traveller's diarrhoea and to the carriage of multidrug-resistant Enterobacteriaceae after travelling to tropical regions

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Background

Travelling to tropical regions, characterized by suboptimal hygiene living conditions and uncontrolled antibiotic usage, has been associated with high risk of acquisition of multidrug-resistant Enterobacteriaceae (MRE) and of occurrence of traveller's diarrhoea (TD). The role of gut microbiota in MRE acquisition and TD is unknown. Here, we investigated the dynamics of changes of microbiota before and after a travel to tropics on a sub-cohort of subjects from the VOYAG-R study (trial nºNCT01526187).

Methods

We included 43 subjects who could provide faecal sample before and after a travel to tropical regions. When found positive by culturing for any MRE after travel, the subjects sent an additional sample one month later. We extracted the whole RNA from 104 faecal samples (43 before travel, 43 at return, 18 one month after travel), performed retrotranscription and sequenced the cDNA (MiSeq 2x300bp). Reads were mapped to the reference 16S Greengenes and 23S SILVA databases.

Results

Subjects experiencing diarrhoea during the visit to tropical regions had higher proportions of *Prevotella copri* after return, but also before departure. The presence of MRE at return was not associated with distinct microbiota profiles. Conversely, the clearance of MRE one month after return was linked to a specific pattern of OTUs that was also found before travel and at return.

Conclusions

Specific bacteria were associated to a higher risk of diarrhoea during a stay in tropical regions and to a faster clearance of MRE after their acquisition.

** Student paper*

Session 1 – Modern diagnostic microbiology / S-04*

Setting up of a functional metagenomics methodology to reveal the resistome in pig farms

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Background
In order to minimize zoonotic transmission and spread of antibiotic resistant (ABR) bacteria it is important to obtain a precise picture of the fate and persistence of resistance genes in the pig farm environment. Monitoring of resistance genes is classically done by isolation of indicator organisms with subsequent antibiogram characterization. Using such culture-dependent approaches, ABR genes in commensals and anaerobic bacteria, which might be accessible to pathogens via horizontal gene transfer, are neglected. These genes are detectable with culture-independent approaches (functional metagenomics), potentially providing a more accurate description of the collection of all resistance genes (resistome).
Methods
Within this project of the National Research Programme "Antimicrobial Resistance" (NRP 72) we so far aimed at setting up and validating the functional metagenomics approach. For this, DNA from faecal material of three pig farmers was extracted, purified and fragmented to approx. 5kb, before ligated into pCR-Blunt vector. Plasmids were electroporated into *E. coli.*Recovered transformed cells were then screened for resistance on agar containing antibiotics (10 µg/mL Tetracycline, 100 µg/mL Ampicillin, 23.75µg/mL Sulfamethoxazole + 1.25µg/mL Trimethoprim, 100 µg/mL Chloramphenicol, 32 µg/mL Amoxicillin + 16 µg/mL Clavulanic acid, 25 µg/mL Cefuroxime, 25 µg/mL Cefotaxime).
Results
Phenotypic resistances towards all tested antibiotics were detected. For the resistance genes, we will present some first preliminary sequencing results during the meeting.
Conclusions
As we managed to establish a culture-independent method to detect resistances in faecal material, it is planned to examine further stool samples from well-chosen pigs, farm workers and environmental probes to describe the Swiss pig farm resistome.

** Student paper*

Session 2 – Microbial pathogenesis / S-05

Full attenuation of a highly pathogenic Mycoplasma mycoides subsp. capri via synthetic genomics-based deletions

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Background

The knowledge regarding *Mycoplasma mycoides* subsp. *capri*(*Mmc*) virulence traits is limited and inconclusive. Here, we wanted to demonstrate that a highly virulent *Mycoplasma* species can be fully attenuated via targeted deletion of potential virulence traits. Genes encoding the l-alpha-glycerophosphate oxidase pathway, the immunoglobulin cleavage MIB-MIP system and certain lipoproteins were among the candidates to be deleted.

Methods

Deletions of 68 genes were engineered in a *Mmc*genome maintained in *Saccharomyces cerevisiae*. Five successive rounds of seamless deletions were performed using the tandem repeat coupled with endonuclease cleavage method. Afterwards, the genome was transplanted into *M. capricolum* subsp. *capricolum* and transplants were selected. To test attenuation, goats were inoculated transtracheally with 108 color changing units (CCU) of wtGM12 or its derivate GM12::YCP1.1D68. Animals were observed up to 31 days post infection.

Results

GM12::YCP1.1D68 had a similar doubling time as wtGM12 in vitro, but it did not convert glycerol to peroxide nor did it cleave immunoglobulin *in vitro* in contrast to its parental strain. The wtGM12 caused 100 % mortality, while the mutant strain did not cause any clinic and pathology. The WT strain induced a septicemia with titers of up to 109 CCU per ml.

Conclusions

This is the first time that a *Mycoplasma* strain edited via synthetic genomics tools has been tested *in vivo*for attenuation. The pathogenicity was completely abolished after deleting candidate virulence traits. Subsequent trials will dissect the role of individual candidate virulence traits towards pathogenicity and open the way for the development of an rationale vaccine.

Session 2 – Microbial pathogenesis / S-06

Identification and characterization of factors involved in cell wall homeostasis in pseudomonas aeruginosa

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1. *Harvard Medical School*

Background

Peptidoglycan (PG) is an essential cross-linked polymer that surrounds most bacterial cells and prevents them from osmotic rupture. The PG cell wall is a meshwork made of glycan strands cross-linked by short peptides. Its synthesis relies on penicillin-binding proteins (PBPs), the targets of a major class of antibiotics, the beta-lactams. Many Gram-negative bacteria, including the opportunistic pathogen *Pseudomonas aeruginosa*, are resistant to beta-lactam antibiotics due to a chromosomally encoded beta-lactamase called AmpC.

Methods

In *P. aeruginosa* expression of the *ampC*gene is tightly regulated and its induction is linked to cell wall stress. We reasoned that a *lacZ* fusion to the *ampC* promoter (P*ampC*::*lacZ*) would serve as a useful reporter for the identification of new factors involved in maintaining cell wall homeostasis. To this end, we mutagenized a strain encoding the P*ampC*::*lacZ* fusion with a transposon and plated the resulting mutant library on plates containing X-gal. Colonies displaying increased blue-color (*lacZ* induced) were selected for further analysis.

Results

As an indication that the screen was working as expected, mutants with transposons disrupting the *dacB* gene were isolated. This gene encode a cell wall remodeling factor that has previously been implicated in *ampC* induction and beta-lactams resistance. In addition, several novel mutants resulting in *ampC* induction were identified. Results characterizing the role(s) of these factors in PG homeostasis and beta-lactams resistance will be presented.

Conclusions

Our results add a new level of complexity to the regulation of the widespread beta-lactamase AmpC and offer new targets for the design of antimicrobial drugs.

Session 2 – Microbial pathogenesis / S-07

Short chain fatty acids control SPI-I phase variation post-transcriptionally

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1. *ETH Zurich/EAWAG*

Background

*Salmonella spp.*express *Salmonella* pathogenicity island I (SPI-I) genes to mediate the initial phase of interaction with host cells. A number of reports show only a subpopulation express these genes and that the proportion of SPI-I expressing cells is important for pathogenicity.

Methods

We used SPI-I transcriptional reporter strains in a combination of population and single cell assays. Strains were analyzed in multiple conditions aiming to replicate the environment *Salmonella* encounters within the human ileum.

Results

We show a combination short-chain fatty acids decrease the proportion of SPI-I expressing cells. This effect is mediated by HilE, a known regulator of SPI-I. Curiously, the addition of SCFAs do not affect *hilE* transcription, suggesting the response is mediated post-transcriptionally. An *in silico* analysis of HilE protein structure indicates a putative intrinsically disordered domain; these domains are dynamic, assuming a structure upon binding to its cognate molecule. Experiments are underway to assess the importance of this domain for SCFA mediated repression of SPI-I expressing subpopulation development.

Conclusions

Collectively, these results show physiologically relevant environmental signals affect *Salmonella* development, and by implication, infectivity.

Session 2 – Microbial pathogenesis / S-08*

Comparative genome analysis of Neisseria mucosa subspecies reveals a putative role for katA, sodC, pilE and fimT homologs in the pathogenesis of N. mucosa subsp. macacae endocarditis

Mr. Florian Tagini [1], Mr. Reto Lienhard [2], Mrs. Marie-Lise Tritten [2], Dr. Olivier Clerc [3], Dr. Antony Croxatto [1], Prof. Gilbert Greub [1]

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Background

Neisseria mucosa subsp. mucosa, macacae and *sicca* are commensal bacteria of the oral cavity and rare agents of endocarditis. We isolated a pathogenic *N. macacae* strain (CDF1) from a patient with endocarditis and present here a comparative genome analysis of *N. mucosa*.

Methods

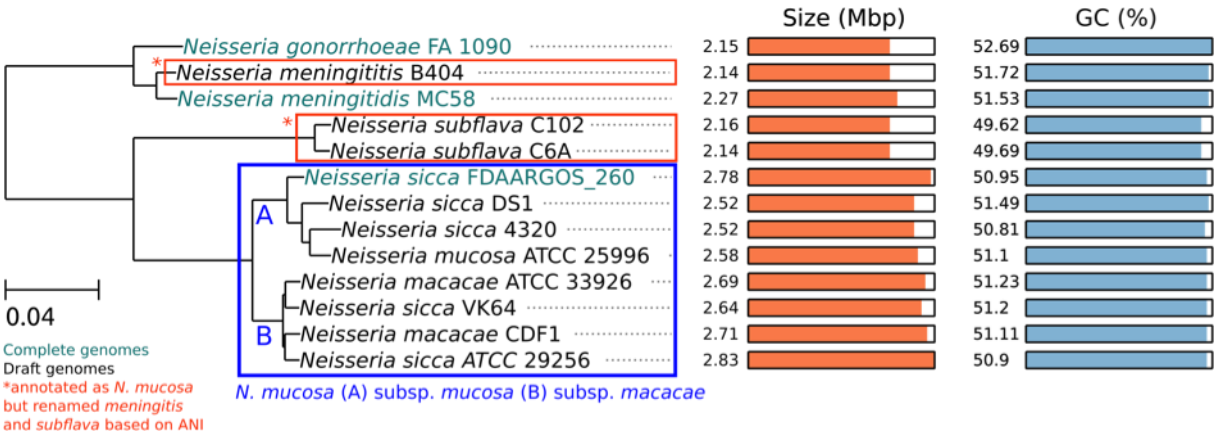
CDF1 was sequenced using a MiSeq (Illumina). All publically available *N. mucosa* subspecies genomes (n=10) as well as reference genomes of *N. meningitidis* and *N. gonorrhoeae*were used. Grouping of orthologs, core-genome phylogeny and comparisons were done using Orthofinder, FastTree and MySQL, respectively. We investigated presence/absence of orthologs of previously identified neisserial virulence factors.

Results

Core-genome phylogeny showed only two major clades (Fig 1) and subspecies *sicca* was not monophyletic. We thus propose to rename susbp. *sicca* as either subsp. *mucosa* or subsp. *macacae*. CDF1 encoded a catalase and was the only strain of its clade holding *sodC*, encoding for a copper/zinc superoxide dismutase. In addition, CDF1 encodes an ortholog of *pilE*, the major pilin subunit, sharing 55% amino acid identity with *pilE* of *N. meningititis,*while the other subsp. *macacae* strains exhibited <42% identity. The non-pathogenic *N. macacae* strain ATCC 33926 lacked *fimT*, essential for type four pili (TfP) biogenesis as well as *katA*, encoding for the catalase. Transmission electron microscopy and hydrogen peroxide assays showed respectively that CDF1 has both TfP and a catalase activity, contrarily to ATCC 33926.

Conclusions

We refined the taxogenomics of *N. mucosa* and our results suggest a role of catalase, superoxide dismutase and TfP in the pathogenesis of *N. mucosa* subsp. *macacae* endocarditis.



Attachment #120

**Student paper*

Session 3 – Antibiotic resistance / S-09*

Characterization of multidrug resistance plasmids harbored in enterovirulent Escherichia coli isolated from diarrheic pigs in Switzerland

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Background

Antibiotics represent the first therapeutic choice for porcine enteric colibacillosis caused by enterotoxigenic and Shiga toxin-producing *E. coli*(ETEC and STEC). An ETEC (14OD0056) and STEC strain (15OD0495) exhibited a multidrug-resistance (MDR) phenoptype and were further characterized by whole-genome sequencing (WGS).

Methods

Minimum inhibitory concentration of antibiotics were determined by broth microdilution and interpreted according to the EUCAST and CLSI criteria. WGS was performed by PacBio technology and the assembled scaffolds were corrected by read mapping paired-end MiSeq Illumina reads. Resistance gene identification and plasmid typing were obtained using on-line platforms (Center for Genomic Epidemiology). Conjugation was performed by filter mating at room temperature and 37°C.

Results

Both strains were resistant to ampicillin, gentamicin, sulfamethoxazole, tetracycline, trimethoprim and tobramycin. All resistance genes were located on a single plasmid (p14ODMR) in the ETEC and in the STEC they were located on 2 plasmids (p15ODMR, p15ODAR). Plasmid p14ODMR harbored the resistance genes *aac(3)-IIa*, *ant(3")-Ia*,*aph(6)-Id*,*aph(3")-Ib*,*aph(3')-Ia*, *bla*TEM-1,*dfrA1*,*sul1*,*sul2*,*tet*(B) and*tet*(C); plasmid p15ODMR carried *aph(6)-Id*, *bla*TEM-1,*dfrA14*,*sul2*and*tet*(A); and plasmid p15ODAR contained *aac(3)-IVa*,*aph(3")-Ib* and *aph(6)-Id*. All 3 plasmids were transferable by conjugation at both temperatures.

Conclusions

WGS of MDR ETEC and STEC revealed that almost all resistance genes were located on single conjugative plasmids, posing the risk of simultaneous selection of several resistance traits, even if only one antibiotic is used. Targeted therapy following antibiotic susceptibility testing are necessary for a prudent use of antibiotics in pigs. Supported by FSVO grant Nº1.15.07 (ERA-Net Project PRAHAD).

**Student paper*

Session 3 – Antibiotic resistance / S-10

Antimicrobial resistant bacteria in food at Swiss retail level: assessment of prevalence and consumer exposure

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Background
Antimicrobial resistance (AMR) in bacteria is a public health concern. However, the AMR bacteria (ARMB) exposure of humans via food is only partially assessed.
Methods
This study aimed to assess AMRB prevalence in retail food and subsequent exposure of Swiss consumers in a systematic literature review of data published between 1996 and 2016 covering the Swiss agriculture sector and relevant imported food.
Results
Out of 313 studies encompassing 122'438 food samples and 38'362 bacteria isolates, 30'092 samples and 8'799 isolates were AMR positive. Meat and seafood harboring *Campylobacter, Enterococcus, Salmonella, E. coli, Listeria*and *Vibrio* spp. featured a median AMRB prevalence of >50%. AMR against aminoglycosides, cephalosporins, fluoroquinolones, penicillins, sulfonamides and tetracyclines were observed at AMR exposures scores of levels 1 (medium) and 2 (high) for *Campylobacter, Salmonella, E. coli*in meat as well as *Vibrio*and*E. coli* in seafood. AMR against glycoproteins, lincosamides, macrolides and nitrofurans were observed in *Staphylococcus* and *Enterococcus* in meat sources, *Staphylococcus* in seafood as well as *Enterococcus* and technologically important bacteria (incl. starters) in fermented or processed dairy products. Knowledge gaps were identified for specific food categories indicator and starter culture bacteria and their mobile genetic elements.
Conclusions
The AMR exposure potential via foodborne pathogens and indicator bacteria was medium to high for raw meat, milk, seafood and certain fermented dairy products. Food at retail, additional food categories, starter culture bacteria and AMR genetics are recommended to be better integrated into systematic One Health AMR surveillance and mitigation schemes.

Session 3 – Antibiotic resistance / S-11

Functional characterization of co-adjuvants that prevent the spread of antibiotic resistance development in Streptococcus pneumoniae

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Background
Streptococcus pneumoniae (the pneumococcus) is part of the commensal microbiota of the nasopharynx. However, it is a major public health problem as it can cause severe life-threatening infections. The development of resistance during the course of an infection is very rare; in fact, it has been shown that HGT occurs mainly during colonization, due to the simultaneous carriage of multiple pneumococcal strains or by the presence of closely-related *Streptococci*(such as *S. mitis*). Many antibiotics can induce competence and subsequent HGT in *S. pneumoniae*, thus driving the spread of antibiotic resistance.
Methods
We used a competence-activated luciferase reporter, in combination with high-throughput screening, CRISPRi, microscopy, and a sort of depletion/induction genes.
Results
Importantly, we show that several of these small molecules act at sub-MIC concentrations and potently inhibit HGT even under antibiotic stress. We show that these compounds act by perturbing the proton motive force and ATP synthase, and thereby disrupt the critical signalling between the membrane-bound histidine kinase ComD and the response regulator ComE. Most of the here-described molecules are non-toxic for humans, providing a promising strategy for them to be used as adjuvants during antibiotic therapy.
Conclusions
Competence inhibition of pneumococci colonising the nasopharynx during antibiotic treatment, can mitigate the acquisition of new antibiotic resistance and virulence genes, decreasing the chance of future multi-drug resistant infections.

Session 3 – Antibiotic resistance / S-12*

Epigenetically inherited antibiotic resistance: another component of a multi-faceted problem

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Background

Streptococcus pneumoniae (the pneumococcus) is part of the commensal microbiota of the nasopharynx. However, it is a major public health problem as it can cause severe life-threatening infections. Antimicrobial resistance (AMR) in *S. pneumoniae* is particularly concerning. It reduces treatment success and increases healthcare costs. AMR mainly occurs via horizontal gene transfer or acquisition of point mutations; however, epigenetic mechanisms such as natural population heterogeneity or activation of antibiotic stress responses can also play an important role.

Methods

We have used population analysis profiles to observe the presence of heteroresisters in populations of pneumococcus and serial passaging to demonstrate the stability of this phenomenon. In the forthcoming weeks, we will sequence the genomes using short-read Illumina technology and PacBio to look for mutations, genomic inversions, and alternative DNA methylation patterns. We will also sequence the RNA to detect transcriptome differences. Using atomic-force and time-lapse fluorescence microscopy within microfluidic chips on several cell cycle markers for cell division, DNA replication, chromosome segregation, transcription and translation, we will provide more insights into the underlying mechanism of heteroresistance.

Results

Here we show that subinhibitory concentrations of the first-line betalactams, amoxicillin and cefotaxime, result in the occurrence of pneumococcal heteroresisters; bacteria with temporarily reduced antibiotic susceptibility. Strikingly, we have demonstrated that heteroresistance is heritable, and maintained after more than 40 generations of growth in the absence of the antibiotic.

Conclusions

Heteroresistance occurs in the pneumococcus in the presence of commonly used bacteriolytic antibiotics. Further experiments are necessary to fully describe and understand the mechanism of this phenomenon.

**Student paper*

Session 4 – Phages / S-13*

Mutations in the bcs operon of Erwinia amylovora abolish infection by bacteriophages S6 and M7

Ms. Leandra Knecht [1]***, Ms. Nadine Heinrich*** [2]***, Ms. Katja Felder*** [2]***, Dr. Yannick Born*** [3]***, Dr. Cosima Pelludat*** [4]***, Prof. Martin J. Loessner*** [5]***, Dr. Lars Fieseler*** [3]

1. *ZHAW/ ETHZ,* ***2.*** *ETHZ,* ***3.*** *ZHAW,* ***4.*** *Agroscope,* ***5.*** *ETH Zurich, Department of Health Science and Technology, Institute of Food Nutrition and Health, Laboratory of Food Biotechnology, Zurich*

Background

The pathogen *Erwinia amylovora*is the causative agent of fire blight, a devastating plant disease affecting members of the *Rosaceae* family. One of its virulence factors is the ability to form biofilms, which leads to clogging of the plant vessels and therefore provoke disease symptoms and eventually plant death. Although the application of streptomycin can effectively control infection, the antibiotic is banned in an increasing number of countries. Bacteriophages are a promising alternative to conventional antibiotics.

Methods

For effective phage cocktail formulation, the identification of phage receptors on the host surface is crucial. Hence, a high throughput screen of a Tn5 transposon mutant library was established, revealing genes of *E. amylovora* CFBP 1430 important for phage infection.

Results

Mutants with defective genes in the bacterial cellulose synthase operon (*bcs*) could no longer be infected by the Felix O1-like phage M7 and the N4–like phage S6. The constitutively expressed *bcs*-complex is responsible for synthesis and secretion of bacterial cellulose, a compound associated with biofilm formation. Deletion of the entire *bcs* operon or single *bcs*genes verified their importance for infection of *E. amylovora* with M7 and S6. Experiments with the cellulose binding dye Congo red and the fact that both phages harbour a set of putative cellulases and endoglucanases further indicate an interaction between M7 and S6 with cellulose.

Conclusions

These findings suggest that M7 and S6 rely on cellulose either for the adsorption to the host bacteria directly or to bring the phage into proximity with its receptor on the bacterial surface.

**Student paper*

Session 4 – Phages / S-14*

Transfer of pathogenic viruses and bacteriophages from liquid to skin

Ms. Ana Karina Pitol Garcia [1]***, Prof. Tamar Kohn*** [2]***, Prof. Healther Bischel*** [3]***, Prof. Alexandria Boehm*** [4]***, Dr. Tim Julian*** [1]

1. *Eawag, Swiss Federal Institute of Aquatic Science and Technology,* ***2.*** *École polytechnique fédérale de Lausanne (EPFL),* ***3.*** *UC Davis,* ***4.*** *Stanford*

Background
Understanding the transfer of viruses between liquid and skin is necessary to estimate indirect transmission of viruses during water-related activities.
Methods
In this work, we quantified the transfer of bacteriophages and pathogenic viruses between liquid-andskin. Firstly, we performed 210 transfer events in 27 sessions with seven volunteers and three bacteriophages (MS2, Φ6, QB). Secondly, we performed 196 transfers with one bacteriophage (MS2) and two pathogens (Coxsackievirus and Adenovirus). The transfer of MS2 was quantified using cadavers, volunteers, and synthetic skin. Finally, the transfer of viral pathogens was quantified using cadaver skin as surrogate.
Results
The concentration of bacteriophages in the liquid was the most influential predictor of virus transfer, explaining 79% of the variance observed in virus adsorption to skin. Liquid characteristics, contact time, and bacteriophage species had little-to-no influence on virus transfer. MS2 adsorbed more to cadaver and volunteer skin than to synthetic skin ($p<0.001$, $p<0.001$). Additionally, the adsorption of MS2 to cadaver skin was not significantly different than to volunteer skin ($p=0.12$). Using cadaver skin to study pathogenic virus transfer, we found that Adenovirus and Coxsackievirus adsorb less than MS2 ($p<0.001$, $p<0.001$). Specifically, MS2 adsorbed 0.56 log10more than Coxsackievirus and 0.71 log10 more than Adenovirus. We found no difference in the adsorption of Adenovirus and Coxsackievirus ($p=0.18$).
Conclusions
Our work demonstrates that pathogen transfer is significantly different than bacteriophage transfer, cadavers can be used as skin surrogate, and concentration of virus in the liquid is the most important predictor of virus transfer.

**Student paper*

Session 4 – Phages / S-15 + P-74

Optimization protocol for the extraction of bacteriophages from soil samples preceding metagenomics analysis

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1. *Department of Health Science and Technology, Institute of Food Nutrition and Health, Laboratory of Food Microbiology, Zurich*

Background

The present study is designed to clarify the role of bacteriophages as vehicles for antimicrobial resistance genes (AMRG) in soil by sequencing and functional metaviromics. To achieve this, an optimal soil viral DNA extraction protocol is crucial to retain viral richness and diversity coupled with lowest bacterial counts. Unfortunately, studies of the soil virome have been largely ignored due to major experimental and computational challenges. Here, an optimized soil viral DNA extraction method is pursuit, where the efficacy of several (i) bacteriophage elution, (ii) bacteriophage concentration and (iii) DNA extraction methods are tested.

Methods

Soil samples are spiked with an artificial viral community consisting of three phages (106PFU/g soil): ΦA511 – Myoviridae, Φ2639AΔLCR – Siphoviridae, and ΦT7 – Podoviridae. Bacteriophage count and bacterial reduction is assessed throughout the optimization steps using Epifluorescence Microscopy, Plaque Assays and 16S rRNA qPCR.

Results

Viruses resuspension in 1:2 Amended Potasium Citrate buffer+ 2% BSA, followed by O/N incubation, several rounds of centrifugation and filtration through 0.45 µm filter revealed the most optimal phage elution and separation approach. Tangential Flow Filtration versus Polyethylene glycol assays for viral concentration are under development to address most optimal concentration method. Essentiality of phage purification by CsCl ultracentrifugation will be then addressed followed by several DNA extraction approaches.

Conclusions

Establishing an optimized standardized workflow for extracting the soil virome is essential for downstream applications. This will provide novel genetic linkage from the environment to the clinic and will help us elucidate the role of the bacteriophages in the dissemination of AMRG.

Session 4 – Phages / S-16

Efficacy of phage therapy against lethal methicillin resistant Staphylococcus aureus (MRSA) ventilator associated pneumonia - an experimental study in rats

Dr. Josef Prazak [1]***, Dr. Manuela Iten*** [1]***, Prof. Yok-Ai Que*** [1]***, Dr. Grégory Resch*** [2]***, Dr. Denis Grandgirard*** [3]***, Prof. Stephen L. Leib*** [3]***, Prof. Stephan Jakob*** [1]***, Prof. Jukka Takala*** [1]***, Dr. Matthias Hänggi*** [1]

1. *Department of Intensive Care Medicine, Bern University Hospital, Bern,* ***2.*** *Department of Fundamental Microbiology, University of Lausanne,* ***3.*** *Institute for Infectious Diseases, University of Bern*

Background
Ventilator associated pneumonia (VAP) is common in critically ill patients and associated with high morbidity and mortality, especially when caused by antibiotic resistant bacteria. The goal of this study was to compare the efficacy of phage therapy versus antibiotics for the treatment of MRSA in a rat model of VAP.
Methods
Four hours after intubation and protective ventilation, rats were inoculated via the endotracheal tube with 6-9 x 10_9 CFU (LD_{100}) of the MRSA clinical isolate AW7. The animals were subsequently extubated. Two hours after bacterial challenge, rats were randomised to receive intravenously either teicoplanin (n=12), a cocktail of four lytic anti-S. aureus bacteriophages (n=12) or combination of both (n=11). 10 additional animals served as control (no treatment). Primary outcome was survival at 96h. Secondary outcomes were bacterial counts in lungs, spleen and blood.
Results
Treatment with either phages, antibiotics or combination of both significantly increased survival (58%, 50%, 45% respectively, compared to 0% survival for controls, ($p<0.01$;Fig.1). Phages were as good as antibiotic in controlling infection, combination of both did not further improve survival. There was no significant difference in bacterial count in lungs, spleen and blood between treatment and control groups. However, surviving animals had significantly lower CFU/g in the lungs and a trend towards less CFUs in blood and spleen. While no phages could be isolated from uninfected lungs, we observed phage multiplication upon pneumonia.
Conclusions
Phage therapy was equivalent to antibiotic in controlling MRSA VAP. Further studies are needed to assess whether phage delivered as aerosols further improve outcome.

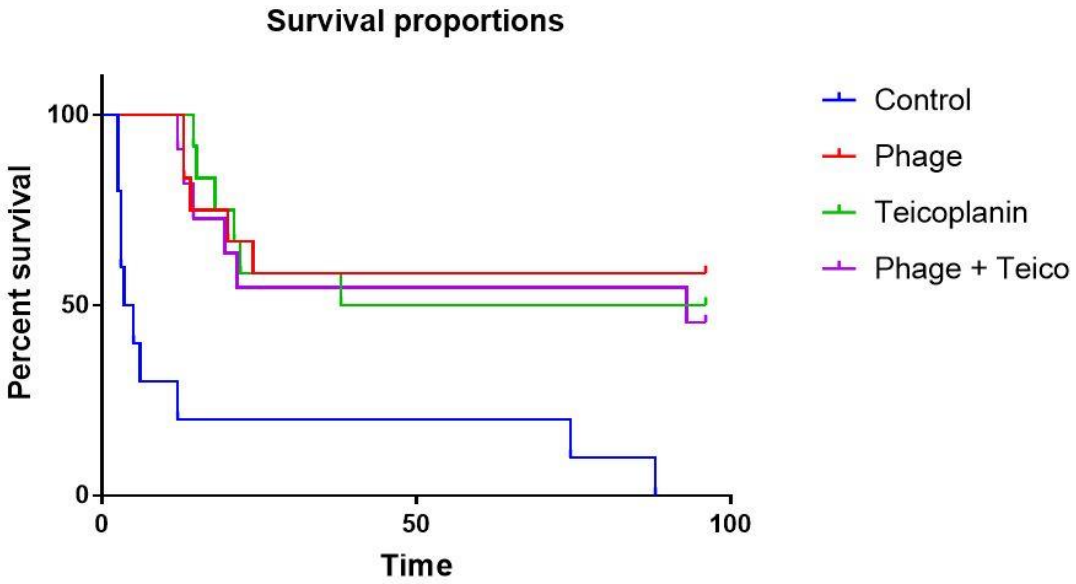

Fig 1. Survival of treated groups compared to untreated control group

Attachment #312

Session 5 – Vector-borne diseases / S-17* + P-55*

Current and future spatial distribution of the tick Ixodes ricinus–host of the Rhadbochlamydiae bacterial pathogens–in Switzerland

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Background

The bacteria *Rhabdochlamydiae*are candidate pathogens causing possible complications such as pneumonia. To estimate the risk of contracting *Rhabdochlamydiae* in Switzerland, we modelled the spatial distribution of one of their known host, the tick species *Ixodes ricinus*. This is also useful to precise the potential transmission risk of other *Ixodes*-associated pathogens such as *Borrelia*spp., *Anaplasma phagocytophilum* and tick-borne encephalitis virus.

Methods

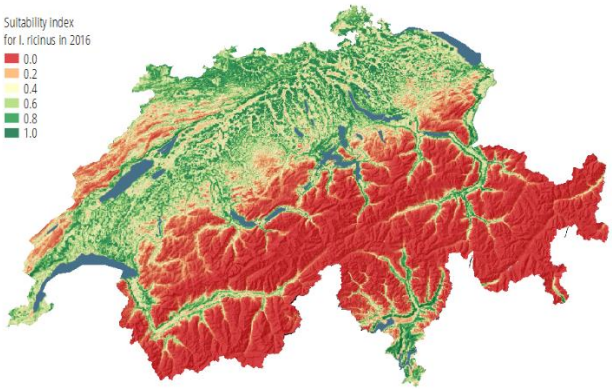
We used 175 location sites of ticks collected by a Swiss Army field campaign and 787 tick positions reported by users of a smartphone application (A&K Strategy, https://zecke-tique-tick.ch). For each location, we retrieved from Swiss federal datasets the environmental factors reflecting the topography, climate and land cover. We then used two presence-only modelling techniques (MaxLike and MaxEnt) to estimate the current and future spatial distribution of *I. ricinus*. Future distribution are estimated up to year 2090 and according to various scenarios for climate and land-use changes.

Results

The most influencing factors explaining the current suitability for *I. ricinus'*presence are PCA-components correlated with monthly temperatures, vegetation indexes (NDVI) and precipitation (Figure 1). From 2016 to 2090, the suitability is predicted to increase in many areas above 800 meters in altitude and to decrease in areas converted from natural to artificial land-use or where the climate will be significantly dryer.

Conclusions

The distribution maps offer an important support for the delimitation of health risk areas, where *Rhabdochlamydiae's* presence should be monitored in priority and where specific prevention or control campaigns may be useful to prevent transmission of all *Ixodes*-associated pathogens.



Attachment #159

**Student paper*

Session 5 – Vector-borne diseases / S-18

Organotypic culture slices of rat cerebellum to study antivirals against tick-borne encephalitis virus

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Background
The neurotropic tick borne encephalitis virus (TBEV), causing potentially life-threatening disease after breaching the blood brain barrier, accounts for most cases of tick-transmitted infections in Central and Eastern Europe as well as in Russia. No specific treatment of TBEV infections exists so far and vaccination is recommended for people at risk of exposure. Nevertheless, among potential antiviral substances, various nucleoside analogues (RNA chain terminators) have been identified as promising candidates for treatment of TBEV infections in previous studies in *in vitro* cell culture testing. So far, however, *in vitro*primary cell culture systems such as organotypic culture slices, much more precisely modeling the complex cell-to-cell interactions of the target tissue than cell lines do, are lacking.
Methods
In this study, we investigated the suitability of organotypic culture slices of rat cerebellum (OCS) to study the effectiveness of selected nucleoside analogues to inhibit replication of neurotropic viruses.
Results
50µMof 2'-C-methyladenosine (2'-CMA) and especially 7-deaza-2'-C-methyladenosine(7-deaza-2'-CMA) exhibited strong inhibitory effects on TBEV growth in OCS reducing titers on average 103-fold. On the other hand, the influence of2'-C-methylcytidine (2'-CMC) on TBEV propagation was weak reducing titers just 10-fold. There was no noticeable difference in TBEV titers between OCS treated with 50µMof Ribavirin and the control. All tested nucleoside analogues exhibited excellent cytotoxicity profiles at 50µM.
Conclusions
Our findings in OCS were comparable to cell line culture systems and thus compose a ideal approach to study antivirals against TBEV in vitro in target tissue.2'-CMA and 7-deaza-2'-CMA are promising candidates for future studies on therapeutic interventions of TBEV infections.

Session 5 – Vector-borne diseases / S-19

Pathogen prevalence in ticks parasitizing humans; a citizens science application

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Background
The tick species *Ixodes ricinus* serves as a vector for numerous human pathogens of bacterial, viral, or protozoic origin. To date, only few studies on pathogen prevalence in ticks parasitizing humans have been performed.
Methods
Using the smartphone application "Zecke – Tick Prevention" anonymized data on tick bites were collected; some users took the opportunity of sending removed ticks to the Swiss National Reference Centre for Tick-Transmitted Diseases (NRZK / CNRT) for pathogen screening. Using quantitative (RT-)PCR as well as PCR with melting curve analysis or sanger sequencing ticks were screened for the presence of various pathogens.
Results
In 2017, a total of 84 app users provided ticks for pathogen screening. Overall carrier rates of 0 % for tick-borne encephalitis virus, 7.14 % for *Borrelia burgdorferi* sensu lato, 0 % for *Borrelia miyamotoi*, 16.67 % for *Rickettsia* spp., 3.49 % for *Anaplasma phagocytophilum*, 3.57 % for *Babesia* spp., 1.19 % for *Candidatus* Neoehrlichia mikurensis, and 2.4 % for *Parachlamydia* spp. were found. Carriage of multiple pathogens was found in 2.4 % of all analysed ticks.
Conclusions
Our data documents the presence of pathogens in ticks parasitizing humans, with carrier rates comparable to those found in questing ticks. Carriage of multiple pathogens was observed, demonstrating the potential risk of acquiring multiple infections as a consequence of a tick bite.

Session 5 – Vector-borne diseases / S-20

Collecting ticks and tick-bite data by users of the smartphone app “Zecke – Tick Prevention”

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Background

The interactive application ‘Zecke–Tick prevention’ shows the correct procedure to follow after tick bites. It explains how to remove ticks and supports the users’ health by means of a tick bite diary. The app collects data of reported tick bites.

Methods

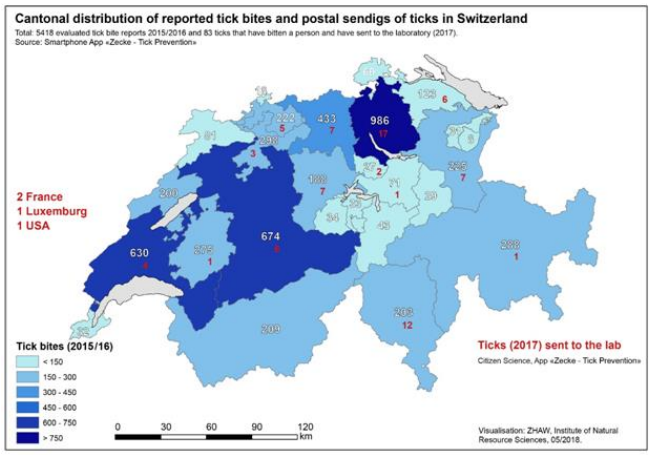
App-users voluntarily and anonymously transmitted information relating to the recreational activity at the time of the tick bite, the body part bitten, as well as their age and gender. The app reminds the user after 5, 10 and 28 days to check the site of the tick bite for symptoms being possible signs of an infection. Some users sent removed ticks by post to the National Reference Centre for Tick-borne Diseases (NRZK/CNRT) for pathogen screening. These users did not receive results.

Results

The tick bite map of Switzerland (Figure1) shows their distribution in 2015 and 2016 (n=5418 of total 6489 datasets). The majority of tick bites were registered in the urbanized ‘Central Plateau’. A total of 84 ticks were sent to the NRZK/CNRT for pathogen screening. Most of them originated from canton of Zurich. A few individuals have reported many tick bites by sending ticks. Considering pathogens’ prevalence and distribution a sample size of at least 1’000 ticks would be needed to obtain significant statistics. Profile of persons bitten in 2017 will be presented.

Conclusions

Citizens are highly interested in recording tick bites in the application “Zecke–Tick Prevention”. Motivation to support science by sending engorged ticks for pathogen screening to the NRZK/CNRT will be promoted in 2018.



Attachment #308

Session 6 – Combating antibiotic resistance (NRP72) / S-21

Evolution of Tolerance shapes evolution of antimicrobial Resistance

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Background
The widespread use of antibiotics promotes the evolution and dissemination of resistance mechanisms. Whereas resistant microorganisms are able to grow in the presence of the antibiotic, drug tolerance enables bacteria to survive during antibiotic treatment windows.
Methods
To uncover the different strategies used by bacteria to survive antibiotic treatment and their implications for drug resistance development, we use *in vitro* evolution experiments and *in vivo* clinical microevolution of P*seudomonas aeruginosa*, an important human pathogen causing both acute and chronic infections.
Results
We show that development of tolerance precedes and promotes acquisition of resistance traits *in vitro* and we present evidence that similar processes shape drug response in the human patient. We observe that in patients, microevolution leads to two distinct drug survival strategies that rely on high levels of multi-drug tolerance or on multiple resistances.
Conclusions
These studies propose that while early development of antibiotic tolerance predisposes bacteria for the acquisition of resistance, both mechanisms eventually represent viable options to survive chemotherapy in human patients.

Session 6 – Combating antibiotic resistance (NRP72) / S-22

Engineering peptidoglycan hydrolases as specific antimicrobials for treatment of Staphylococcus aureus infections

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Background

Staphylococcus aureus shows a high degree of antibiotic resistance and has been shown to invade and persist within different types of mammalian cells. These cells are often metabolically inactive, which enables them to evade the host immune response and antibiotic therapy.

Methods

To address the need for novel antimicrobial agents effective against these pathogens. we employed peptidoglycan hydrolases (PGHs), including phage endolysins, as novel antimicrobials featuring strong bactericidal activity, high specificity, and lack of resistance development. Moreover, the modular enzymes can be engineered to feature specifically desired properties. However, systemic administration of PGHs is hampered by several factors, including possibly reduced activity at the site of infection, lack of cell-penetrating properties, and insufficient serum circulation half-life.

Results

We created a large collection of staphylococcal PGHs, and developed methods to rapidly screen this library for enzymes with high activity in human serum and intracellular environments. We fused them to cell-penetrating peptides (CPPs) to enable transduction into eukaryotic cells. In *S. aureus*-infected eukaryotic cell lines, PGH-CPP fusion proteins reduced intracellular *S. aureus* by up to 5 logs compared to PGHs without CPPs. The circulation half-life of PGHs in mice can be extended by fusion to an albumin binding domain (ABD). Upon intravenous injection, the ABD mediates high-affinity binding to serum albumin, reducing renal filtration and lysosomal degradation. Finally, we demonstrated anti-biofilm activity of PGHs in dynamic models and in synergy with an exopolysaccharide depolymerase.

Conclusions

Overall, our results show the high potential of engineered PGHs as therapeutics for treatment of staphylococcal infections.

Session 6 – Combating antibiotic resistance (NRP72) / S-23

Do Long-term Land-use practices Affect Soil Bacterial and Viral Profiling, Antimicrobial Resistance Evolution and Spread?

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Background

This study analyzes whether long-term soil fertilization practices influence microbial composition and antimicrobial resistance (AMR) content in one of the oldest experimental agricultural field soils in Europe (ZOFE).

Methods

Treatments investigated were organically fertilized (manure, sludge, compost) (OF), chemically fertilized (PK, NPK) (CF), mixed fertilized and non-fertilized (control). Bacterial and viral DNA were extracted, shot-gun sequenced and analyzed for microbial composition and AMR profiles.

Results

16S rRNA analysis of the bacterial fraction revealed remarkable differential patterns (treatments with increased counts) for: Acidobacteria (OF), Actinobacteria (control, CF), Bacteroidetes (OF), Candidatus saccharibacteria (control, NPK), and Chloroflexi (control). Low percentage of ID was observed between our samples and those from NCBI, with new bacterial genomes being reconstructed and quantified. High viral diversity was observed, with only 4% of reads classified (ViralRefSeq NCBI). Siphoviridae were predominant followed by Myoviridae, Podoviridae and Phycodnaviridae. Except for Sludge treatment, general synteny was observed. Using MEGAres (AMR DB) (≥90% ID) 0.07% of reads retrieved hits, with highest counts in Sludge+PK and PK, and lowest in Control and NPK. Multidrug-resistant efflux pumps were predominant. Reads- and proteins-based, resistance to bacitracin and β-lactams was increased in all treatments but control. 2.4% of contigs carried ≥3

AMR determinants. Selected contigs are being thoroughly investigated. Similar procedures are being followed with the viral fraction.

Conclusions

Our system shows high bacterial and viral diversity with low ID rates to former published data. Fertilization practices slightly modify microbial composition and, at least, bacterial AMR pattern. Additional variables are being analyzed to facilitate biological interpretation of treatment-associated outlines.

Session 6 – Combating antibiotic resistance (NRP72) / S-24 + P-75

Procalcitonin and lung ultrasonography point-of-care testing to decide on antibiotic prescription in patients with lower respiratory tract infection at primary care level: a pilot study

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Background

Only 5-12% of outpatients with lower respiratory tract infection (LRTI) have community acquired pneumonia (CAP) requiring antibiotics. The tools used for diagnosis (chest X-ray, C-reactive protein) have limited diagnostic accuracy.

Procalcitonin (PCT), a host biomarker, can be safely used to decide on antibiotic prescription in patients with LRTIs(5). However, it has a low specificity for CAP. Lung ultrasound (LUS) is effective in detecting lung consolidation in pneumonia and might compensate for the lack of specificity of PCT. We hypothesize that the combination of both LUS and PCT might safely lead to more accurate identification of LRTI patients requiring antibiotics in the primary care setting.

Methods

Observational pilot study to test the feasibility of a point of care intervention combining PCT measurement and LUS (figure 1.) for the management of lower respiratory tract infections (LRTI) in primary care. Table 1 summarizes inclusion and exclusion criteria. The primary outcome of the pilot study is feasibility of the intervention.

Results

8 GPs were trained for participation in the study. They recruited 19 patients (*67%*female, median age 42 years old). PCT measurement was successful in 17/19 patients (mean value 0.1 µg/L (95% CI 0.09-0.11)). LUS was performed in all patients (21 minutes median duration, 88% of exams with good or above quality). 89% agreement of antibiotic prescription with the algorithm's recommendation

Conclusions

We conclude that a cluster randomized controlled trial, testing the impact of the intervention on antibiotic prescription rates for LRTIs in primary care in Switzerland is feasible.

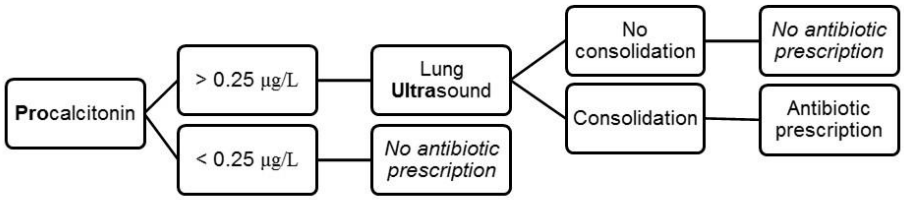

Figure 1: The algorithm for the decision of antibiotic prescription in patients included in the UltraPro study

Patients Screening Criteria	
All consecutive patients presenting with a LRTI	
Inclusion criteria	Exclusion criteria
Signed informed consent and aged ≥ 18 years	Working diagnosis of acute sinusitis or non-infective disorder
No antibiotics prescribed for the current episode	Cystic fibrosis
Acute cough (< 21 days) and ≥1 symptom or sign: • History of fever for more than 4 days • Dyspnoea • Tachypnoea (≥22 cycles per minutes) • Abnormal focal finding during lung auscultation	Antibiotics for COPD exacerbation in the last 6 months
	Known pregnancy
	Severe immunodeficiency
	Admission of the patient
	Patient not available for follow-up

Table 1: Screening, inclusion and exclusion criteria for the study.

Attachments #144

Session 7 – Microfluidics and innovative diagnostic tests / S-25*

Evaluation of EDTA- and DPA-based tests for detecting Mcr producers

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Background

We evaluated the ability of dipicolinic acid (DPA)- and EDTA-based broth microdilution (BMD) and combineddisk (CDT) tests to detect mcr-1*/-2/-3/-4/-*5 producers.

Methods

Ninety-two *Enterobacteriaceae* (85 colistin-resistant) of which 44 *mcr*-positives (39 *E. coli*, three *K. pneumoniae*, two *Salmonella* spp.) were tested. For the BMD tests, DPA (900 µg/ml) was used in cation-adjusted Mueller- Hinton broth (CAMHB), while EDTA (100 µg/ml) was tested in MHB. Results were categorized as positive if in presence of chelator strains exhibited ≥3 two-fold MIC decrease compared to the colistin MIC alone. EDTA-based DCTs were performed on both MH and CAMH agar using colistin disks plus chelator (292 or 584 µg), while the DPA-based CDT was performed only on CAMH agar with colistin disks plus 1000 µg of chelator. Results were considered positive if in presence of chelator strains exhibited ≥3 mm inhibition zone increase compared to colistin alone.

Results

The DPA-based BMD assay detected 37 *mcr*-positives, with seven false-negative (two *E. coli*, three *K. pneumoniae*, two *Salmonella* spp.) strains (sensitivity, 84.1%; specificity, 100%). The EDTA-based BMD assay detected 41 *mcr*positives, but 22 false-positives (including 12 *E. coli*) were recorded (sensitivity, 93.2%; specificity, 54.2%). Both EDTA- and DPA-based CDTs did not reached sufficient performance to be routinely implemented.

Conclusions

The EDTA-based BMD assay is not accurate to detect mcr producers, whereas the DPA-based BMD test demonstrated a good accuracy, especially when implemented for *E. coli*(sensitivity, 94.9%; specificity, 100%). This work was supported by NRP-72 No. 177378 to AE.

**Student paper*

Session 7 – Microfluidics and innovative diagnostic tests / S-26

Bacterial Microcolonies in Gel Beads for High-Throughput Screening

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Background
High-throughput screening of a DNA library expressed in a bacterial population for identifying potentially rare members displaying a property of interest is a crucial step for success in many experiments such as directed evolution of proteins and synthetic circuits and deep mutational scanning to identify gain- or loss-of-function
mutants.
Methods
I will describe a protocol for a high-throughput screening of bacterial (*E. coli*) microcolonies in gel beads. Single cells are encapsulated into monodisperse water-in-oil emulsion droplets produced with a microfluidic device. The aqueous solution also contains agarose that gelates upon cooling on ice, so that solid gel beads form inside the droplets. During incubation of the emulsion, the cells grow into monoclonal microcolonies inside the beads. The latter are recovered from the emulsion and sorted by fluorescence activated cell sorting. The bacteria are recovered from the gel beads and are then ready for a further round of sorting, mutagenesis or analysis.
Results
We determined enrichment rates and demonstrated that we can measure the average fluorescent signals of microcolonies containing phenotypically heterogeneous cells, obviating the problem of cell-to-cell variability. Finally, we applied this method to sort a pBAD promoter library at ON and OFF states.
Conclusions
This method could also be easily amended to screen for other readouts than the expression of a fluorescent protein. For example, it is possible to assay cell growth by staining microcolonies with a biomass indicator dye. When encapsulating multiple cells per droplet, cell-cell interactions could also be screened for. Thus, the described method is broadly applicable in biology.

Session 7 – Microfluidics and innovative diagnostic tests / S-27

Performance evaluation of Accelerate Pheno system in antimicrobial susceptibility testing of highly resistant bacteria strains in bloodstream infection, compared to standard laboratory testing

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Background

The Accelerate Pheno system (AXDX) is a fully automated test system capable of performing identification (ID) and antimicrobial susceptibility testing (AST) directly from positive blood cultures within an average of 7 hours. The aim of this study was to evaluate the performance of the instrument, testing highly resistant bacteria strains, in comparison to the conventional colturebased identification methods and AST.

Methods

17 Gram positive and negative MDR strains and 4 ATCCs were selected, identified with MALDI-TOF (Bruker) and analyzed with 2 different AST panels in Phoenix (BD). For each isolate, 10 to 100 CFU/ml were spiked into blood culture bottles along with 10 mL healthy donor blood, incubated in Bactec (BD) until signaled positive, then loaded on AXDX. As reference method for AST, Broth microdilution (BMD) was performed using SensititreTREK panel (ThermoSCIENTIFIC).

Results

The correct identification was achieved in 18 of 21 samples (85.7%), as showed in table 1. Regarding AST evaluation, 247 antimicrobial-microorganism combinations were analyzed. The overall category agreement (CA) between AXDX and BMI was

97.2%. Discrepancies were expressed as very major errors VME (3), major errors ME (3) and minor errors mE (2).

Conclusions

AST results of AXDX were accurate for the most of clinical strains selected for the study. These data clearly highlight the potentiality of this system to provide realiable results in a timely manner. Further studies are needed to optimize the identification phase and the AST performance for ATCCs, in order to guarantee the opportunity to routinely use standardized internal quality controls, according to the Eucast guidelines.

TABLE 1 ID results of the Accelerate Pheno system compared to MALDI-TOF organisms identification					
Organisms Identification (colture-based ID)				Accelerate Pheno	
Organism	Resistance Mechanism		Inoculum	ID	AST
Klebsiella pneumoniae	CRE	CarbA: KPC	21 UFC/ml	Klebsiella spp	yes
Klebsiella pneumoniae	CRE	CarbD: OXA-48	32 UFC/ml	Klebsiella spp	yes
Escherichia coli	CRE	CarbB: NDM-1	28 UFC/ml	**No (x2)**	**No (x2)**
Escherichia coli	CRE	CarbD: OXA-48	37 UFC/ml	Escherichia coli	yes
Escherichia coli	CRE	CarbB:NDM-1	21 UFC/ml	Escherichia coli	yes
Escherichia coli	CRE	ESBL+AMC	17 UFC/ml	Escherichia coli	yes
Klebsiella pneumoniae	CRE	ESBL+AMC	89 UFC/ml	Klebsiella spp	yes
Klebsiella pneumoniae	ESBL	CTX-M	32 UFC/ml	Klebsiella spp	yes
Escherichia coli	wild-type	none	30 UFC/ml	Escherichia coli	yes
Pseudomonas aeruginosa	CR	CarbB: VIM1	87 UFC/ml	Pseudomonas aeruginosa	yes
Acinetobacter pittii	wild-type	none	53 UFC/ml	Acinetobacter baumannii	yes
Escherichia coli	ESBL	R colistina	12 UCF/ml	Escherichia coli	yes
Pseudomonas aeruginosa	CRE	CarbB: VIM1	57 UFC/ml	Pseudomonas aeruginosa	yes
Staphylococcus aureus	MRSA	MecA	81 UFC/ml	**P. aeruginosa + S.aureus**	**No (x2)**
Enterococcus faecium	VRE	VanB	13 UFC/ml	Enterococcus faecium	yes
Enterococcus faecalis	wild-type	none	74 UFC/ml	Enterococcus faecalis	yes
Staphylococcus aureus	wild-type	none	16 UFC/ml	Staphylococcus aures	yes
ATCC 25922 E.coli			27 UFC/ml	Escherichia coli	yes
ATCC 27853 P.aeruginosa			91 UFC/ml	Pseudomonas aeruginosa	yes
ATCC 29213 S.aureus			30 UFC/ml	Staphylococcus aures	yes
ATCC 29212 E.faecalis			86 UFC/ml	Enterococcus faecalis	yes

Attachment #116

Session 7 – Microfluidics and innovative diagnostic tests / S-28

Incorporation of liquid-based-microbiology enhances diagnostic outcome of neisseria gonorrhoeae in asymptomatic female carriers

Ms. Anna Roditscheff [1], Ms. Nadia Wohlwend [2], Dr. Martin Risch [3], Prof. Lorenz Risch [3], Dr. Thomas Bodmer [3]

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Background

To identify asymptomatic female carriers of *Neisseria gonorrhoeae* (NG), Swiss diagnostic laboratories often screen urogenital specimens by adding selective culture media. After Implementation of liquid based microbiology (LBM) in May 2013, we reviewed the diagnostic work flow to determine the effects of a combination of LBM, nucleic acid amplification testing (NAAT) and culture on laboratory automation and diagnostic outcome. Furthermore we investigated the performance of liquid transport media on NAAT.

Methods

Overall, 9'575 liquid-based urogenital swabs were collected and simultaneously cultured on selective chocolate agar PolyViteX VCAT3 plates and tested by NAAT (RealTime CT/NG assay m2000 Abbott, Anyplex™II, Seegene). If culture growth was detectable, antimicrobial susceptibility testing (AST) was performed. Furthermore, the performance of SwabAX (Axonlab) to cobas® PCR Media (Roche) for NAAT was tested on 360 urogenital specimens, using m2000 Abbott and cobas® 6800.

Results

NG was detected in 0.40 % (38/9'575) of all specimens. 100 % (38/38) off all NG positive specimens were detected by NAAT and 57.90 % (22/38) were identified by culture, revealing a better NG detection rate for NAAT than for culture. Importantly, testing the performance of SwabAX for CT/NG detection on m2000 Abbott and cobas® 6800, did not differ significantly from Cobas PCR Media.

Conclusions

Our findings demonstrate that an altered diagnostic work flow for NG, combining LBM, NAAT and culture, improves sensitivity of NG detection. A transport media that allows for specific as well as syndromic orientated diagnostics and application of molecular and culture techniques is a crucial point for successful laboratory Automation.

Session 8 – Innate Immunity / S-29

Cell-to-cell phenotypic heterogeneity in macrophage-Mycobacterium tuberculosis interaction

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Background

Mycobacterium tuberculosis (*Mtb*), the etiological agent of tuberculosis, is a major cause of global morbidity and mortality. Previous studies have shown that marked cell-to-cell differences often exist within clonal *Mtb* populations and this intrinsic phenotypic heterogeneity provides a chance for subpopulations to survive extended periods of stress. Phenotypic heterogeneity is amplified when *Mtb* is exposed to stresses as well as *in vivo*. Therefore, to understand the dynamics of *Mtb* persistence it is essential to study the physiology and behaviour of *Mtb* within the context of the host cell, taking into account the intrinsic heterogeneity of the two populations of cells.

Methods

We used micro-fabrication techniques to develop a system to trap hundreds of individual host cells (macrophages), infect them with *Mtb* and monitor their behaviour by time-lapse microscopy for several days.

Results

This approach allowed us to observe how *Mtb*grows and behaves when it is internalized in macrophages, the primary residence of this pathogen. We observed a marked heterogeneity in the inhibition of growth of *Mtb* within different macrophages; in the survival time of infected macrophages; in the dissemination of intracellular bacteria to other macrophages. Moreover, we are currently investigating how the cell-to-cell heterogeneity observed affects *Mtb* persistence and antibiotic tolerance.

Conclusions

Thanks to our approach we can investigate phenomena that cannot be measured in conventional batch culture experiments and we can better understand the dynamics of the interactions between the macrophages and *Mycobacterium tuberculosis*.

Session 9 – Host pathogen interaction & invasion / S-30

Identification and characterization of a W. chondrophila-specific inclusion membrane protein (Inc) secreted by the Type III Secretion System

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Background

Waddlia chondrophila is a strict intracellular bacterium implicated in adverse pregnancy outcomes in humans and abortion in ruminants. Similarly to *Chlamydiae,* it displays a biphasic replication cycle and secrete multiple virulence factors to modulate its environment and facilitate survival and replication. In particular, it possesses a Type Three Secretion System (T3SS) devoted to the injection of effectors into the host cell cytosol or in the membrane of the bacteria-containing vacuole. While several T3SS effectors are described in classical *Chlamydiae*, nothing is known about *W. chondrophila* effectors.

Methods

To identify virulence factors of *W. chondrophila*, we developed a plaque assay with amoebae (that normally feed on bacteria) and *E.coli* containing a *W. chondrophila* genomic library packed in cosmids. Depending on the virulence factors expressed, amoebae are more or less able to produce lysis plaques. A first screening of about 200 cosmids allowed the identification of 3 putative virulence factors, also predicted *in silico* to be secreted by the T3SS. We first focused on Wcw_1131 and characterized its temporal expression by RT-qPCR and immunoblots. We also assessed its subcellular localization in infected cells by immunofluorescence and its T3SS-dependent secretion in a heterologous system.

Results

Our results demonstrate that Wcw_1131 is a T3SS effector produced during the early steps of an infection cycle and that is localized at the inclusion membrane. It is the first Inc protein described in *Chlamydia*-related bacteria.

Conclusions

Further experiments are needed to precisely determine its function, which will potentially open novel perspectives for the development of drugs active against *Chlamydiae*.

Session 9 – Host pathogen interaction & invasion / S-31

Type VI secretion in plant-beneficial Pseudomonas protegens contributes to gut microbiome invasion and pathogenicity towards a plant pest insect

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Background

Pseudomonas protegens strains are known for their plant-beneficial activities, which include pathogen suppression, plant growth promotion and plant defense induction. These rhizobacteria also exhibit insecticidal activities towards Lepidopteran and Dipteran pest insects. Following ingestion by herbivorous larvae, the insecticidal pseudomonads establish in the gut, breach the intestinal barrier, invade the hemocoel and kill the insects. Various antimicrobial, lytic and toxic compounds contribute to the pathogen and pest control abilities of *P. protegens*. The molecular basis of their niche competitiveness on plant and insect hosts that are densely populated by competing bacteria, however, remains largely unexplored.

Methods

Here, we used infection assays in combination with mutational and metagenomic analyses to investigate the role of the type VI secretion system (T6SS) of *P. protegens* in interbacterial competition, insect gut colonization and pathogenicity. The T6SS is a phage tail-like contractile transmembrane apparatus of Gram negative bacteria that functions like a deadly syringe to inject toxic and lytic effector proteins into neighboring cells.

Results

The genome of model *P. protegens* strain CHA0 harbors a gene cluster encoding the T6SS core apparatus and two distinct gene clusters encoding VgrG spike, effector and cognate immunity proteins. We demonstrate that these clusters are required for pathogenicity towards larvae of the cabbage pest *Pieris brassicae* following oral infection. The core apparatus and one of the VgrG clusters contribute to colonization of the insect gut, gut microbiome invasion and competition with specific microbiome members.

Conclusions

Our results highlight the importance of T6SS in niche competitiveness and insect pathogenicity of biocontrol pseudomonads.

Session 9 – Host pathogen interaction & invasion / S-32*

Elucidation of the molecular microenvironment of coronavirus replicase complexes reveals close association with the host cell translation machinery

Mr. Philip Vkovski [1], Mr. Markus Gerber [1], Dr. Jenna Kelly [1], Dr. Stephanie Pfaender [1], Dr. Nadine Ebert [1], Dr. Sophie Lagache [2], Dr. Cedric Simillion [3], Ms. Jasmine Portmann [1], Mr. Hanspeter Stalder [1], Prof. Remy Bruggmann [4], Prof. Manfred Heller [2], Prof. Ronald Dijkman [5], Prof. Volker Thiel [5]

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Background
Viruses rely on the host cell's functions to fulfill their requirements during virtually every step of the viral life cycle. The replication of positive-sense RNA viruses depends on intracellular membranes to accommodate viral RNA replication on virus-induced organelles that provide an optimal microenvironment within the cytosol of the infected cell. However, a global view of host factors associated with the viral replication and transcription complex (RTC) and host pathways assisting viral RNA replication is lacking.
Methods
We constructed a recombinant coronavirus mouse hepatitis virus (MHV) harboring a promiscuous biotin ligase embedded within the replicase complex, allowing proximity-dependent labelling and mass spectrometric identification of replicase-proximal factors during the entire course of the infection.
Results
We thereby identified a comprehensive library of >500 viral and host proteins residing in the vicinity of the MHV RTC. Specific siRNA-mediated silencing of each of these replicase-proximal host factors revealed, amongst others, the importance of vesicular trafficking pathways between the endoplasmic reticulum and the Golgi apparatus for coronavirus replication. In addition, numerous eukaryotic translation initiation factors were detected near the RTC. They were required for efficient viral replication, and moreover, active translation was visualized near the RTC upon puromycin labeling.
Conclusions
Collectively, our data establish a spatial link of translation and coronaviral mRNA synthesis and may serve as a paradigm for other positive-strand RNA viruses. Our data also provide a starting point for a comprehensive analysis of critical virus-host interactions at the site of viral RNA synthesis.

**Student paper*

Session 10 – Intracellular bacteria / S-33

Waddlia chondrophila plasmid: a genetic tool to understand the biology of Chlamydia-related bacteria

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Background

Waddlia chondrophila is a *Chlamydia*-related intracellular Gram-negative bacterium able to grow in a broad range of host cells, including free-living amoebae as well as mammalian, fish and insect cell lines. *W. chondrophila* seropositivity has been associated with miscarriages and respiratory infections, and this species is therefore recognized as an emerging pathogen in humans. However, despite the interest for human health the study of chlamydial biology has strongly been limited by genetic intractability of *Chlamydiales*. Recent advances on the genetic manipulation of *Chlamydia trachomatis* now allow functional studies of chlamydial genes, but this remains limited to *Chlamydiaceae*.

Methods

We aim to design a strategy to transform *W. chondrophila* based on the recent techniques developed for *C. trachomatis*, which take advantage of the fact that in addition to the circular chromosome most chlamydial genomes feature a plasmid, which can be modified to create a shuttle vector that can be amplified and modified in *Escherichia coli*.

Results

We are constructing a shuttle vector based on the 15.6-kb plasmid of *W. chondrophila* in order to express genes of interest under control of an inducible promoter, an essential step to decipher gene functions. Moreover, chlamydial plasmids are species-specific, as most of the proteins encoded on *W. chondrophila* plasmid have no homologs in other *Chlamydiae*.

Conclusions

Therefore we are investigating the functions of the *W. chondrophila* plasmid-encoded proteins, first by expressing these genes in heterologous hosts (*E. coli* and *C. trachomatis*), then by over-expressing or deleting them in *W. chondrophila*, once the transformation method is established.

Session 10 – Intracellular bacteria / S-34*

Characterization of cell division mechanisms of Waddlia chondrophila, a Chlamydia-related bacterium

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Background

Waddlia chondrophila is an obligate intracellular bacterium, member of the *Chlamydiales* order and suspected to cause abortion in ruminants and miscarriage in humans. The *Chlamydiales* division differs from the classical bacterial division machinery, by lacking the division organizer FtsZ, certain division proteins homologues and by exhibiting an atypical peptidoglycan. We recently described septal proteins, MreB, RodZ and NlpD, which play important roles in *W. chondrophila* cell division.

We aim here to describethe exact role of the division septum proteins and their interactors.

Methods

To better describe the role of septal protein RodZ in chlamydial division we identified by Yeast-two-hybrid assay its potential interactors. Among them, SecA, FtsH and SufD proteins were selected for further studies, given data on these proteins in other bacteria. We investigated expression and localization of the interactor candidates by immunofluorescence and quantitative RT-PCR.

Results

Expression pattern of these genes was consistent with a potential role in chlamydial division. Heterologous overexpression of each protein in*E. coli*caused morphology and growth defects, indicating a potential role in bacterial division. To further investigate the conservation of the function of the waddlial gene,we performed complementation studies in *E. coli*. Interestingly, another Potential RodZ interactor was YbbP/CdaA, which is known to have a diadenylate cyclase function (DAC)*and to be responsible for* cyclic di-AMP*synthesis. Thus, we investigated*YbbP/CdaA function in *W. chondrophila*, especially regarding its ability to produce c-di-AMP.

Conclusions

More work will now be required to better characterize the role of these proteins in chlamydial division and c-di-AMP synthesis.

**Student paper*

Session 10 – Intracellular bacteria / S-35*

New approaches for in vitro assays to evaluate the antibiotic susceptibility of Chlamydia

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Background
The aim of this study was to develop new approaches to determine the *in vitro* susceptibility of *Chlamydia* to different antibiotics in view of existing protocols as there is no clear consensus among the research community for antibiotic resistance determination in obligate intracellular bacteria.
Methods
To determine the minimal inhibitory concentration (MIC), infected cells were exposed to cultivation media containing two-fold dilutions of the antibiotic in question, or drug-free media as an unexposed control. MIC determination was based on a consensus of both inclusion number reduction and alteration of inclusion size and morphology upon antibiotic exposure. In addition to the MIC, we employed a recovery assay, allowing observation of the chlamydial response to drug removal and subsequent recovery, as compared to both continued exposure and to the unexposed control. For proof of principle, we evaluated the susceptibility of three *Chlamydia suis*field strains and the reference strain S45/6 to tetracycline, sulfamethoxazole and penicillin, antibiotics commonly used to prevent respiratory and gastrointestinal diseases on fattening pig farms.
Results
Tetracycline sensitive strains can easily be distinguished from resistant strains using the evaluation parameters proposed in this study. Moreover, we report that S45/6 is sensitive to sulfamethoxazole while all evaluated *C. suis*field strains showed some degree of sulfamethoxazole resistance. Finally, Penicillin G induces the chlamydial stress response in all evaluated *C. suis*strains.
Conclusions
We propose a two-step protocol evaluating two major parameters, the MIC and the recovery, allowing initial assessment after two workdays, and a detailed report after eight workdays.

**Student paper*

Session 10 – Intracellular bacteria / S-36

Lessons learned from a false-negative result with a laboratory-developed Legionella pneumophila PCR in an external quality control challenge

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Background
In 2015, we adapted a previously published PCR for the detection of *Legionella pneumophila,*targeting the *mip*gene.Based on an alignment of the 235 sequences available in the NCBI database at the time, degenerated positions were included in the oligonucleotides to take into account frequent polymorphisms. The assay was validated by testing an array of positive and negative samples, including external quality control (EQC) samples of previous years. The performance of the optimized assay was very satisfactory and the assay performed well in the EQC challenges of 2015 and 2016. In 2017 however, the results of the QCMD EQC panel revealed that a strain of type Sg2-14 was not detected by our assay, even at high concentration.
Methods
To investigate the causes of this unsatisfactory EQC result, we analyzed the sequence of the strain Sg2-14 and identified several mismatches in the primers binding sites. Adapted degenerated oligonucleotides were tested in various combinations and compared to a primer set fully matching the strain Sg2-14.
Results
A modified reverse primer taking into account the polymorphism of the strain Sg2-14 at nucleotide 19 of 24 was sufficient to restore full sensitivity of our PCR for this particular strain, without loss of specificity.
Conclusions
Our data illustrates that a single mismatch in a primer binding site, even 5 nucleotides from the 3'-end, can lead to complete absence of detection and stresses the importance of EQC program and of continuous improvement to ensure quality of laboratory-developed PCR assays.

Session 11 – Combating antibiotic resistance (NRP72) / S-37

Optimised use of antibiotics in acute care hospitals : a multicenter cluster-randomized trial

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Background
One third of hospitalized patients receive antibiotics, leading to a high selective pressure. However, data on appropriateness of antibiotic therapies in Swiss hospitals are lacking and the extent of room for improvement is unknown. We initiated a multicenter study consisting in the evaluation of the impact of weekly clinical audits and multifaceted feedback strategies on reducing the use of anti-Gram-negative antibiotics that deserve a restrictive prescription: quinolones, 3rd- and 4th-generations' cephalosporins, piperacillin/tazobactam, carbapenems (= protected antibiotics).
Methods
Internal medicine, general surgery and intensive care units of 8 hospitals located in the French-speaking part of Switzerland were allocated to either intervention or control group. The intervention consists in one-day weekly review of protected antibiotic prescriptions by a tandem of an infectious diseases specialist and a senior physician in charge of the patients, using a standardized checklist, followed by immediate feedback to prescribers and monthly reports to the medical team with key messages. Evaluation of appropriateness includes indication, duration, route of administration, spectrum and dosage.
Results
The intervention started in March in 3 hospitals (2 medical units, 2 surgical units and 1 ICU). Results of the first month of intervention are presented. Among 748 patients reviewed, 149 (20%) received a protected antibiotic. Optimisation of 41/149 (27.5%) ongoing prescriptions have been proposed by the auditing tandem: 21 stops, 12 de-escalations, 9 switchs to the oral route, 4 adaptations of dosage.
Conclusions
Preliminary results confirm that there is room for improvement.

Session 11 – Combating antibiotic resistance (NRP72) / S-38*

Contribution of natural competence to antimicrobial resistance in Acinetobacter baumannii

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1. *GHI, École polytechnique fédérale de Lausanne (EPFL), Lausanne*

Background

*Acinetobacter baumannii*is a Gram-negative bacterium and an opportunistic human pathogen. It can carry numerous antibiotic resistance genes, which are frequently localized on the chromosome. Natural competence for transformation, a physiological state that enables the bacterium to internalize and integrate exogenous DNA, could explain how these antibiotic resistance genes spread among bacteria.

Methods

We developed and used a new protocol to test *A. baumannii* transformability and established a suicide plasmidbased deletion method, which enabled us to delete competence genes of interest.

Results

When transforming *A. baumannii* strain A118 under different pH conditions (pH 6 - 8), we found that its transformability was significantly increased at a pH of 6. Secondly, we compared the transformability of strains A118, ATCC17978 and ATCC19606 and observed that solely A118 was transformable under the tested conditions when using genomic DNA or a non-replicating plasmid with homologous regions to the A118 chromosome. Other types of DNA (replicating plasmid, PCR-derived DNA fragments) were not stably maintained in the bacterium. Consistent with its function in other organisms, the deletion of *comEA* resulted in abolished transformability.

Conclusions

A. baumannii strain A118 is naturally transformable under laboratory conditions and dependent on the competence gene *comEA*. It is able to take up genomic and plasmid DNA, when the latter contains sequences that are homolog to the bacterium's genome. Moreover, acidic medium enhanced the natural transformability of *A. baumannii*. Future work will identify the underlying regulatory circuits and the exact composition of the DNAuptake complex in *A. baumannii*.

** Student paper*

Session 11 – Combating antibiotic resistance (NRP72) / S-39

Assessing phylogenetic relatedness and plasmid transmission in ESBL-producing Enterobacteriaceae

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Background

Transmission of extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae challenges healthcare facilities regarding the implementation of effective infection control measures. The complexity of the process relies on the ESBL genes, that are generally encoded on self-transmissible plasmids, which can be exchanged among the same and different bacterial species. The aim of this study was to identify possible transmission events in a group of 50 ESBL-producing Enterobacteriaceae strains from different origin based on whole genome sequencing (WGS) analysis.

Methods

Bacterial strains were sequenced by Illumina and MinION and *de novo* whole genome assembly was performed by SPAdes, Canu and Unicycler. The obtained genomes were annotated with Prokka and plasmids and resistance genes were identified using Plasmidfinder and Resfinder databases. Phylogenetic relationships of the chromosomes and plasmids were established based on SNPs and gene composition (core and accessory genes).

Results

Closed bacterial chromosomes and plasmids were obtained when long read sequencing data was available. ESBL genes were identified in both chromosomes and plasmids. Based on the phylogenetic analysis, similar plasmids carrying ESBL and other resistance genes were detected in different strains, suggesting plasmid transmission events.

Conclusions

The combination of high quality short Illumina reads and long read sequencing data is crucial to get complete and closed chromosomes and plasmids. SNPs- and gene composition-based phylogeny approaches have enough resolution to decipher chromosome and plasmid transmission events, as confirmed in known transmission pairs included in our study.

Session 11 – Combating antibiotic resistance (NRP72) / S-40

Rapid nanomotion-based antibiotic susceptibility test

Dr. Sandor Kasas [1], Dr. Petar Stupar [1], Dr. Onya Opota [2], Dr. Leonardo Venturelli [1], Mr. Wojciech Chomicki [1], Dr. Kamila Guerin [1], Mr. Anton Malovichko [1], Dr. Anne-Celine Kohler [1], Prof. Gilbert Greub [3], Prof. Giovanni Dietler [1]

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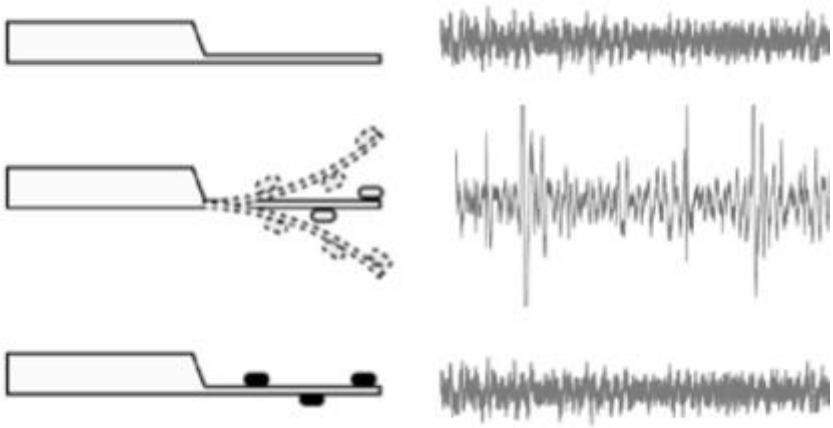
Background
Finding the right antibiotic and dose at the right time is of utmost importance in clinical microbiology. A rapid antibiotic susceptibility tool would improve the patient care significantly. Within our research, we have demonstrated that depositing living bacteria onto an atomic force microscope (AFM) cantilever is a simple and rapid method to assess their metabolic level or live /death state.
Methods
The technique consists in attaching the living organism of interest onto a traditional AFM cantilever and in monitoring its nanometric scale oscillations with a commercial or dedicated AFM-like device. We recently demonstrated that living organisms induce cantilever oscillations that stops once the organism is dead.
Results
In our laboratory, we use this technique to detect sensitivity of bacteria to antibiotics in a timeframe of minutes. Nanomotion antibiotic susceptibility test delivers rapid and reliable results, independently on the bacterial type or its replication rate. Furthermore, our laboratory currently develops different types of nanomotion-based prototypes that are planned to be implemented in microbiology laboratories and hospitals.
Conclusions
Such a rapid antibiotic susceptibility test could reduce treatment costs and more importantly diminish health risks. The new strategy for detecting antimicrobial resistance would be particularly useful in cases of infectious disease where the treatment lasts several weeks or months, thus helping to avoid the illness recurrence. We will present the working principle of the method, the different prototypes we already developed in our laboratory as well as the applications of the technique in various basic science and medical domains.



Attachment #314

Session 12 – Lay communication / S-41

Board and card games involving microbes: anxiogenic or educational?

Prof. Gilbert Greub *1*

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Background
In recent years, several board and card games involving microbes have been commercialized such as “Pandemics”, “Panic Lab”, “Viral” and “Virulence”. These games generally have appealing titles to increase their attractiveness. Often their content is based on scientific aspects, but an evaluation of their educational value and scientific content is missing.
Methods
We compared five games and assessed their scientific pertinence by looking at various characteristics, including (i) ease of understanding for laypersons, (ii) level of scientific reliability, (iii) iconography quality, (iv) use of anxiety-generating words.
Results
Some games were easily accessible to laypersons, including children, but others (such as “viral”) were mainly designed to game addicts, due to complex rules. Scientific reliability varied from game to game. “Viral” and “Virulence” for instance provide extended educational information. Nevertheless, scientific inaccuracies were present in most games supporting the importance of biomedical advices during game conception. Thus, in one game, viruses were represented dividing by binary fission alike bacteria. Quality of the iconography was generally good with two different game categories, those using cartoons representing microbes in a fun way without any scientific background and those representing microbes in an also fun but more realistic way, that makes the microbes easy recognizable. Thus, viruses from “Viral” are fanciful whereas the *Dictyostelium*morphotypes from “Panic Lab” are easy to recognize.
Conclusions
Some of these games may be used to increase awareness of the population about the importance of microbes and might even be used for teaching if supplemented with additional scientific information.

Session 12 – Lay communication / S-42

KROBS: an innovative game to develop people's awareness of pathogenic microorganisms

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Background
People are usually only aware of a few viruses and bacteria (such as those causing AIDS or tuberculosis) and have no knowledge of the multiple microbes to which they are exposed in their daily life. Furthermore, important changes recently occurred in the general population lifestyle (air conditioning systems, outdoor leisure activities, frequent journeys abroad) which provided opportunities for microorganisms to emerge as pathogens and enhanced the risks to get in contact with them. It is thus important to inform the public on this topic.
Methods
In collaboration with a professional game designer, we developed an innovative and playful card game that supports our communication project.
Results
While having fun, players encounter diverse situations of everyday life and learn what are the safe or risky behaviors regarding exposure to pathogens. Using four colors of cards, we inform about four major modes of transmission of microbes: by arthropods, water aerosols, contact with animals and food. For each of these transmission modes, we chose four microbes (mainly bacteria, but also viruses and parasites) that people could be infected with, if they adopt a risky behavior. In addition, a fifth color includes microorganisms that are transmitted by insects or rodents in endemic countries. A QR code tags each card and gives access, on a dedicated website, to more detailed scientific information such as reservoirs for these microorganisms, infection risks, prevention opportunities, symptoms of related diseases and existing treatments.
Conclusions
KROBS is an exciting and fun-filled game that will undoubtedly increase the general population knowledge on pathogenic microbes.

Session 12 – Lay communication / S-43

«break'd!»: supporting educators with up-to-date microbiological research

Mr. Massimo Caine [1]***, Dr. Pierre Brawand*** [2]***, Dr. Perron Karl*** [1]***, Prof. Patrick Linder*** [3]

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Background

The world of academic research stands on facts made of data and results. Altogether these pieces of information are diffused within the scientific community through the drafting of scientific publications. Even though the redaction of a single article may take years of hypothesis, discussions and experiments, the latest technological and societal developments led us to a situation where every 20 seconds a new scientific publication is produced. Outside the academic environment, these publications are also very important not only for the technological advancements that they represent, but also for the debates that they foster on different levels, from policy-making to pedagogical teaching. However, the jargon of the scientific writing makes it difficult to fully comprehend what it has been achieved by the described research. To address this situation and to support the teaching of modern biology in classrooms, we created an innovative and easy-to-read mini-magazine called "*break'd!*". The objective of *break'd!* are (i) to provide teachers with a continuing professional development related to their curricula, (ii) foster classroom debates and discussion between teachers and their students (iii) be the ice-breaker for topic-focused webinars with active researchers.The mini-magazine will be distributed in six different publications, each of them addressing a specific topic - two of them will be focusing on Microbiology (microbiota and antimicrobial resistance) providing an example of how modern microbiological research can be brought into classrooms with an engaging and exciting format.

Methods

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Results

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Conclusions

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Session 12 – Lay communication / S-44 + P-68

Getting to know microbes: outreach activities in the Cadagno region

Dr. Aurelia Weber [1], Mr. Massimo Caine [1], Dr. Cristina Fragoso Corti [2], Prof. Mauro Tonolla [2], Prof. Raffaele Peduzzi [3], Prof. Reinhard Bachofen [4], Dr. Perron Karl [1], Prof. Patrick Linder [1]

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Background

The largest part of the planet's living material consists of bacteria and microorganisms that play a vital role in many biological processes. In spite of their huge importance, microbes are largely unknown and mostly negatively perceived. In this context, outreach activities that address the public are crucial, to give a better understanding of this hidden world.

The aim of this project is to raise awareness about the importance of microorganisms in preserving biological and ecological systems, by offering to the public and schoolchildren the opportunity to see microbes and learn about their metabolism, ecology and utility.

Methods

To this purpose, a "microbiological path" was developed in the Val Piora in Ticino. To guide the visitors along the didactic path, informative material is available on panels and in a descriptive leaflet. Visits and activities with local experts can also be organized for schools, to provide complementary education.

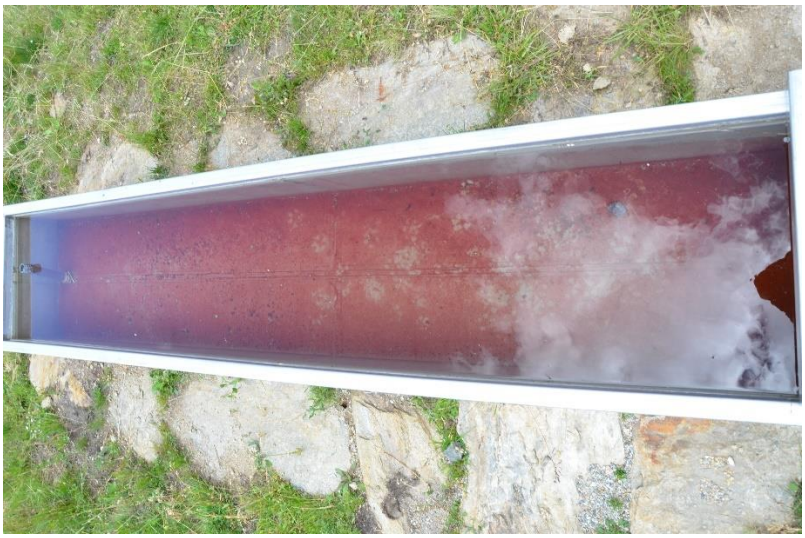
Results

Several colonies and specimens can be observed directly in nature without any special equipment. For example, the microscopic algae *Haematococcus pluvialis* and the bacteria *Chromatium okenii* form very distinct red coloration in the water. Lichens, microscopic fungi and other bacteria (like lactic acid bacteria for the production of cheese) are also highlighted.

Conclusions

Brochure and complementary information available onhttps://www.bioutils.ch/parcours-didactique

This project was supported by the FNS with an Agora grant for 3 years (2014-2017)



Attachments #309

Session 13 – Bacterial toxins & Cell microbiology / S-45

Pilus locus organization is a key virulence determinant in Streptococcus gallolyticus causing infective endocarditis

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2. *Radboudumc, Radboud Institute for Molecular Life Sciences, Department of Pathology, Nijmegen*

Background
Streptococcus gallolyticus subsp. *gallolyticus* (*SGG*) is an emerging cause of infective endocarditis. Pili are important contributors to pathogenicity in *SGG*and other *Streptococcus bovis/Streptococcus equinus* complex (SBSEC) members. They are organized in three loci *pil1*, *pil2* and *pil3*, each encoding for a sortase, a fimbrial protein and an adhesin. Their genetic organization in correlation with virulence, habitat and SBSEC species is only partially established.
Methods
Comparative genomics was performed on 60 SBSEC genomes using amino acid sequences encoded by the nine genes in *pil1*, *pil2* and *pil3* of *SGG* UCN34 as reference. Search hits were manually curated and analyzed for partial matches, truncated genes and mutations.
Results
pil3 was the most commonly shared loci among SBSEC, present in *S. equinus (*27/31), 15/18 *SGG*(15/18), other SBSEC species (4/4) but not *S. lutetiensis* (0/2). *pil1* and *pil2* were found in complete form among six bloodderived *SGG* and *pil2* also among three commensal *SGG*. Commensal *SGG* featured truncated or mutated genes for *pil1* (12/12) and *pil2* (9/12). These loci were also absent or featured truncated adhesin genes in commensal *S. equinus* (31/31), *S. lutetiensis*(0/2), *S. infantarius* subsp. *infantarius* (0/3), *S. gallolyticus* subsp. *macedonicus* (0/2) and pathogenic *S. gallolyticus* subsp. *pasteurianus* (0/4).
Conclusions
Complete *pil1*, *pil2* and *pil3* loci seem to be key virulence determinants of infective-endocarditis-causing *SGG*. Truncation of loci in other strains and SBSEC species suggests niche adaptation by loss of function events from a shared ancestral strain. Virulence among other SBSEC pathogens might thus be related to pili-independent and yet unknown factors.

Session 13 – Bacterial toxins & Cell microbiology / S-46

One step back, two steps forward: Toxin-antitoxin modules and persister formation of Escherichia coli

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Background
Bacterial persisters are antibiotic-tolerant cells notorious for their contribution to the recalcitrance of chronic and relapsing infections. Our group previously proposed that persister formation in the *Escherichia coli*model would be primarily driven by stochastic peaks of the second messenger (p)ppGpp through a signaling cascade involving polyphosphate, protease Lon, and the activation of toxin-antitoxin (TA) modules (Maisonneuve et al., *Cell*(2013)). Here, we critically revisited this previous work as a model to identify and overcome sources of the prevalent reproducibility problems in the field.
Methods
Classical microbiological techniques were used to construct series of new bacterial mutants and to analyze the effect of different biological or technical parameters on antibiotic killing.
Results
Most importantly, we discovered that several bacterial mutants used to support our previous view on persister formation had been inadvertently infected with bacteriophage ϕ80, a notorious laboratory contaminant. New experiments demonstrated that lysogenization with ϕ80 and not the inactivation of any specific genetic pathway was the cause of previously observed defects in antibiotic tolerance of, e.g., mutants deficient in polyphosphate metabolism or TA modules. Moreover, our work revealed and solved a number of biological and technical artifacts that are inherent to commonly used procedures of antibiotic killing assays like, e.g., a high variation of persister levels due to insufficient control of bacterial growth dynamics.
Conclusions
Our work caused a major paradigm shift in the field by demonstrating that the previous view on *E. coli* persister formation based on TA module activation is wrong (Harms et al., *mBio*2017).

Session 13 – Bacterial toxins & Cell microbiology / S-47*

A reservoir for Salmonella conjugative plasmids in the intestinal mucosa

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Background

Antibiotic resistance is of grave concern. Its emergence and spread are not well understood. Persisters are bacterial cells that phenotypically adapt to survive antibiotic exposure. They can form reservoirs in host tissues and promote the emergence of resistance mutations. We hypothesized that persisters may also promote the spread of resistance plasmids. *Salmonella enterica*Typhimurium (*S*. Tm) is an invasive pathogen that forms persisters in the host's gut tissue. We asked if such persisters may form a tissue reservoir for subsequent transfer plasmids to bacteria occupying the gut luminal niche.

Methods

First, we colonized mice with *S.*Tm bearing an antibiotic resistance-encoding conjugative plasmid. Then, we cleared the gut luminal niche with oral ciprofloxacin and ampicillin, and allowed a plasmid-deficient recipient strain to occupy the freed niche post antibiotic treatment (Fig. 1A-B). Plating quantified pathogen loads and transconjugant populations.

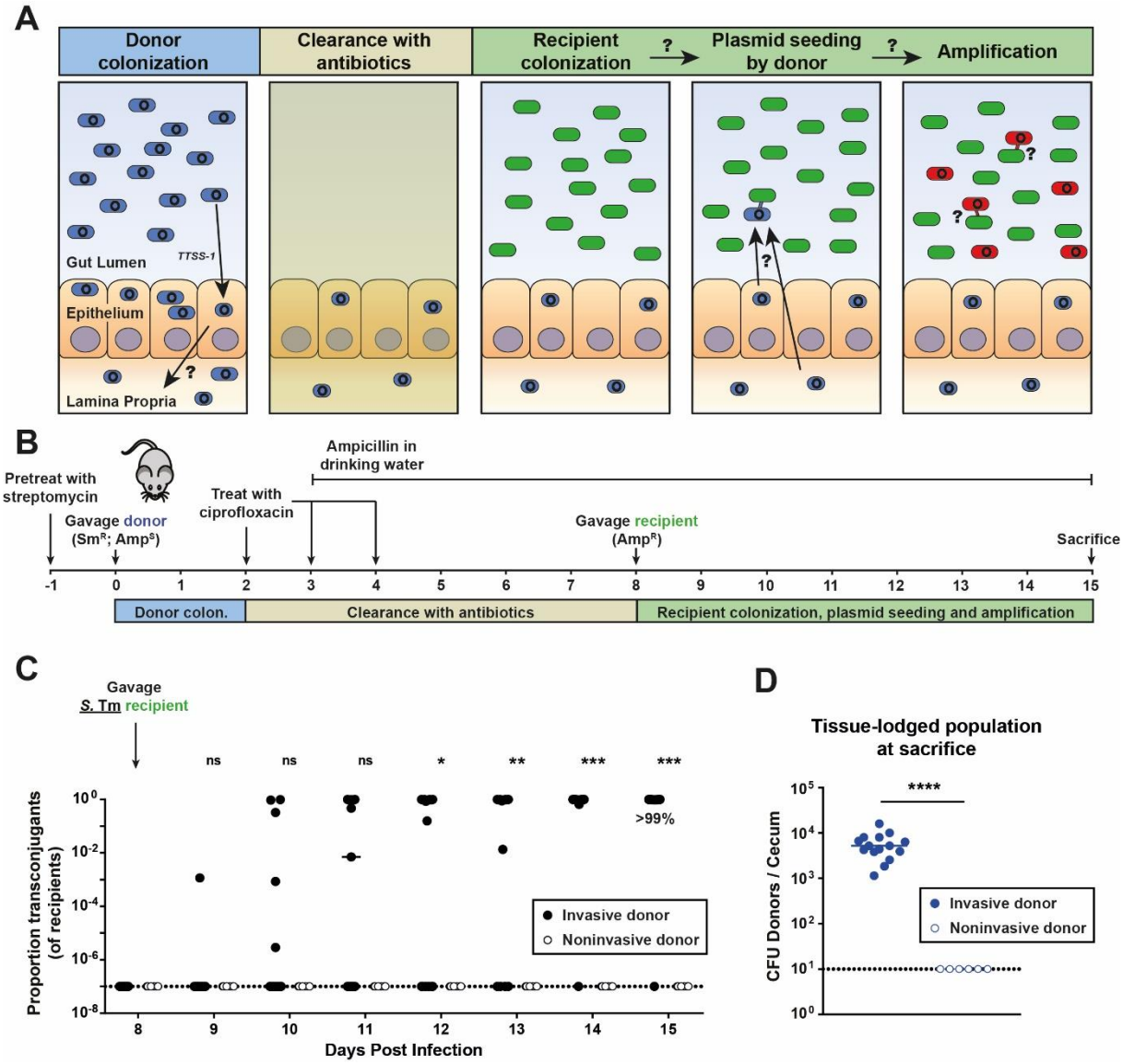
Results

>99% of the recipient population acquired the resistance plasmid within 6 days (Fig. 1C). Vaccination (which limits tissue invasion) and non-invasive donors (which cannot form tissue-lodged persisters) verified that tissuelodged persisters are an important reservoir for subsequent plasmid transfer. Genetic barcoding and population dynamics analyses established that the transconjugant population arises from rare conjugation events from the tissue-lodged persister population.

Conclusions

The intestinal mucosa could bean important reservoir for mobile genetic elements including conjugative plasmids harboring clinically relevant resistance genes.

**Student paper*



Attachment #108

Session 13 – Bacterial toxins & Cell microbiology / S-48* + P-60*

In situ architecture, function and evolution of a contractile injection system

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Background
To interact with other cells, bacteria use different contractile machines that function similarly to membranepuncturing bacteriophages. In contrast to extracellular systems, the so-called type 6 secretion system (T6SS) is defined by intracellular localization and attachment to the cytoplasmic membrane.
Methods
We used cryo-focused ion beam milling, electron cryotomography, and functional assays to study a T6SS in *Amoebophilus asiaticus* in its cellular context.
Results
The in situ architecture revealed three modules, including a contractile sheath-tube, a baseplate, and an anchor. All modules showed large-scale conformational changes upon firing. T6SSs are organized in multi barrel gun-like hexagonal arrays by lateral baseplate interactions. The system mediated interactions with host membranes and may participate in phagosome escape. Evolutionary sequence analyses predicted that T6SSs are more widespread than previously thought.
Conclusions
Our insights form the basis for understanding T6SS key concepts and exploring T6SS diversity.

**Student paper*

Session 14 – Microbial evolution / S-49

Adaptation to new environment: the ph challenge

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Background
Perturbations of environmental parameters such as pH or carbon sources induce microbes to develop various strategies to maintain high fitness in novel conditions. In this study, we focus on an *Agrobacterium tumefaciens* strain isolated from a four-species community growing in a metal-working fluid (MWF), a coolant and lubricating agent.
Methods
To untangle the metabolic pathways of *A. tumefaciens* in this environment, we designed a minimal medium in which different concentrations of carbon sources commonly present in the MWF were added separately. Among these tested compounds, citric acid got our attention: after several days of incubation, *A. tumefaciens* switched from no growth to a sudden 20-fold increase compared to the starting inoculum. Growth occurred only at the highest-tested citric acid concentration simultaneously among all the replicates. Moreover, this specific concentration has a very acidic pH, and we verified that cells collected from the stationary phase could grow instantly in the same fresh medium but not in a neutralized citric acid medium.
Results
Our hypothesis is that the exposure to the acidic condition triggered an adaptive response. We are evaluating what has changed both at the genomic and transcriptomic level. Our aim is to understand the impact of pH on metabolic functions of *A. tumefaciens* and its ability to adapt to extreme environments.
Conclusions
The result of this experiment will give us more insight on how bacteria react to external variations, highlighting which are the pathways responsible for fitness preservation in a new environment.

Session 14 – Microbial evolution / S-50*

Endofungal bacteria – New insights into bacterial-fungal coexistence

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Background

Bacteria and fungi coexist in various microhabitats and establish interactions ranging from mutualism to antagonism. Such interactions can impact higher trophic levels, as well as nutrient cycling. The interaction between fungal-bacterial interacting partners is dynamic and can rapidly change in response to changes in environmental factors. The same is true for interactions occurring at the cellular level. Bacteria and fungi in close physical contact show relationships ranging from random coexistence to nearly total physiological interdependency. The most intimate relationship yet consists in bacteria colonizing inner hyphae (endobacteria).

Methods

We have investigated the diversity of both endobacteria and bacteria firmly attached to hyphae in 130 fungal strains. Amplicon sequencing of the 16S rRNA gene was used to identify bacterial species in DNA extracted from individual fungal cultures.

Results

We have discovered that endobacteria are much more frequent than assumed. Moreover, they seem to appear equally distributed in the phyla Basidiomycota, Ascomycota and Zygomycota, and also occur in the distinct phylogenetic lineage of the eukaryotic fungus-like Oomycota. We have started to investigate the rules underpinning this close association. Under environmental conditions affecting negatively the fitness of the fungal host, we have observed for several fungal models that this tight association turns to a loose coexistence.

Conclusions

Defining the conditions triggering changes in the type of interaction between both partners are key to understand the dynamics of bacterial-fungal interactions. Such a discovery is essential for a better definition of the general mechanisms behind these interactions and their role in microbial ecosystem functioning.

**Student paper*

Session 15 – Viral hepatitis / S-51

Recombinant hepatitis E viruses harboring tags in the ORF1 protein

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Background
Hepatitis E virus (HEV) is believed to be the most common cause of acute hepatitis and jaundice in the world. Current understanding of the molecular virology and pathogenesis of hepatitis E is scarce, especially due to the lack of appropriate functional tools. Here, we report the development of tagged HEV genomes as a novel tool to investigate the viral life cycle.
Methods
A selectable subgenomic HEV replicon was subjected to random 15-bp sequence insertion using transposonbased technology. Viable insertions in the open reading frame 1 (ORF1) protein were selected in a hepatoblastoma cell line. The identified insertion sites were used to tag HEV with the green fluorescent protein (GFP) and other reporters.
Results
Functional insertion sites were identified downstream of the methyltransferase domain, in the hypervariable region (HVR) and downstream of the helicase domain of the ORF1 protein. HEV replicons harboring an HA tag, GFP or the NanoLuc luciferase in the ORF1 protein were found to be functional. Full-length HEV genomes harboring HA or NanoLuc tags in the ORF1 allowed for the production of infectious viral particles. The HA tag allowed to localize HEV replication complexes in infected cells and the NanoLuc allowed to quantitatively monitor HEV infection and replication by luciferase assay. Recombinant HEV harboring these and other tags are currently being further characterized.
Conclusions
The development of tagged functional HEV genomes should allow to track viral replication complexes in live cells and should facilitate the identification of host factors, yielding new insights into the HEV life cycle.

Session 15 – Viral hepatitis / S-52

Palmitoylation determines the membrane association and subcellular localization of hepatitis E virus ORF3 protein

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Background
Hepatitis E virus (HEV) is a positive-strand RNA virus encoding 3 open reading frames (ORF), namely ORF1, ORF2 and ORF3. HEV ORF3 protein is a small, hitherto poorly characterized protein involved in viral particle secretion and possibly other functions.
Methods
A panel of ORF3 constructs and the full-length protein expressed by infectious HEV were investigated by confocal laser scanning microscopy and immunoblot using GFP fusion proteins and newly established recombinant antibodies. Oligomerization was studied by coimmunoprecipitation and fluorescence resonance energy transfer. Posttranslational modifications were probed by site-directed mutagenesis and different biochemical assays.
Results
HEV ORF3 protein forms membrane-associated oligomers. HEV ORF3 proteins produced in cell-free and mammalian cell expression systems displayed different apparent molecular weight. Sequence analyses revealed the presence of 8 conserved cysteine residues within the first 21 amino acids which were found to be palmitoylated, as corroborated by ^{3}H-palmitate labeling, the investigation of cysteine-to-alanine substitution mutants and treatment with the palmitoylation inhibitor 2-bromopalmitate (2-BP). Abrogation of palmitoylation by sitedirected mutagenesis or 2-BP treatment relocalized ORF3 protein from the plasma membrane to the cytoplasm and decreased stability of the protein. Moreover, we found, using selective permeabilization conditions coupled to immunofluorescence, that HEV ORF3 protein is entirely exposed to the cytosolic side of the membrane.
Conclusions
HEV ORF3 protein forms membrane-associated oligomers and is palmitoylated at conserved N-terminal cysteine residues. Palmitoylation determines the subcellular localization, stability and likely the function of HEV ORF3 protein. These findings provide new insights into the life cycle of HEV and may yield new angles for therapeutic intervention.

Session 15 – Viral hepatitis / S-53

Hepatitis E in a one-year old child led to the identification of transfusion-associated Hepatitis E virus infections

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Background
Hepatitis E virus (HEV) can be transmitted via blood transfusions but general screening is currently not recommended in most European countries.
Methods
Serum samples were analyzed for HEV IgM and IgG as well as HEV-RNA. HEV-RNA positive samples were sequenced.
Results
The detection of HEV-RNA in a one-your-old child after allogenic stem cell transplantation led to the retrospective identification of transfusion-associated transmissions.
HEV-RNA was detected in three samples of an asymptomatic donor over a 4-week period. Donation 1 was HEV seronegative (700 HEV-RNA IU/ml), donation 2 (27,000 HEV-RNA IU/ml) was positive for HEV IgM only and donation 3 (10 HEV-RNA IU/ml) for HEV IgM and IgG. Apheresis platelets from all three donations were transfused to a total of 5 immunocompromised recipients.
Besides the child (recipient donation 2) another recipient tested HEV RNA positive (63-year old women, recipient donations 2, 3). The total dose of transfused HEV-RNA was higher in infected patients compared to not infected patients (mean 6.3 log10 HEV-RNA IU versus 4.6 log10 HEV-RNA IU, $p<0.001$). The child developed chronic HEV infection whereas the women cleared the infection. Interestingly, the time between receipt of the HEV-contaminated apheresis platelets and the first detection of HEV-RNA in the child was 83 days (exceeding the known incubation time for HEV). Before transplantation he received intravenous immunoglobulins (IVIG) on multiple occasions. The nucleotide sequences were 100% identical proving transfusion-associated transmission.
Conclusions
(i) Increased awareness to detect HEV infection in immunosuppressed patients seems mandatory (ii) Residual HEV antibodies through IVIG products/donor might mitigate the clinical course.

Session 15 – Viral hepatitis / S-54

Detection of Hepatitis E virus (HEV) RNA in Raw Sausages and Raw Sausages Containing Pig Liver from Retail Level in the Southern Part of Switzerland

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Background

Hepatitis E virus (HEV) is the causative agent of an acute and self-limiting hepatitis and is increasingly detected in food products containing pork. Recently, the first confirmed foodborne HEV infection in Switzerland linked to the consumption of a raw sausage containing pig liver was described. This sausage type called “Mortadella di fegato” is very popular in the canton of Ticino, Switzerland. The aim of this study was to screen raw sausages containing pig liver and raw pork sausages collected at retail level in the Ticino region, to assess the occurrence and the viral load of HEV in these types of products

Methods

In this study, 102 raw sausages containing pig liver (mortadella di fegato cruda) and 18 raw pork sausages (salami) collected between January 2016 and June 2017 at retail level throughout the canton Ticino. HEV was detected by quantitative real-time reverse transcription PCRs using commercial kits (ceeramTools and mengovirus extraction control kit, bioMerieux).

Results

HEV was detected in 12 of 102 (11.8%) mortadella di fegato products, but not in any of the salami sausages. Viral loads in the mortadella di fegato sausages ranged from logHEV 2.3 to 5.7 genome copies per gram (gc/g) food product.

Conclusions

Although the presence of HEV RNA alone does not confirm viral infectivity, mortadella di fegato type sausages made with raw pig liver should be considered a potential source of HEV infection in humans. Consumers at risk, such as the immunocompromised, persons with underlying liver conditions, and pregnant women should avoid eating products containing raw pig liver.

Session 16 – Metagenomics / S-55 + P-61

Stunted childhood growth is associated with decompartmentalization of the gastrointestinal tract and overgrowth of oropharyngeal taxa

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Background

Linear growth delay (stunting) affects 155 Million under five children globally. Treatment has been limited by a lack of understanding of the underlying pathophysiological mechanisms. Stunting is most likely associated with changes in the microbial community of the small intestine, a compartment vital for digestion and nutrient absorption. Efforts to better understand the pathophysiology have been hampered by the difficulty to get access to small intestinal fluids. Here, we describe for the first time the microbial community found in the upper gastrointestinal tract of stunted children.

Methods

We studied 46 duodenal, 57 gastric and 404 fecal samples from stunted and non-stunted children aged 2-5 years living in Bangui, Central African Republic and in Antananarivo, Madagascar, using 16S Illumina Amplicon sequencing and classical, semi-quantitative culture methods. Bacterial identification was performed using metabolic growth tests (API galleries) and Mass spectrometry-based identification.

Results

A majority of stunted children showed small bacterial overgrowth (SIBO) largely dominated by bacteria that normally reside in the oropharyngeal cavity. The overrepresentation of oral bacteria was reflected in fecal samples of stunted children, but not non-stunted controls, offering a non-invasive diagnostic marker. In addition, two bacterial enteropathogenic groups were more prevalent in stunted children, while *Clostridia*, well-known butyrate producers, were reduced.

Conclusions

Our data suggests that undernutrition is associated with a “microbiome decompartmentalization” of the gastrointestinal tract characterized by increased presence of oropharyngeal bacteria. Undernutrition having traditionally been seen as a consequence of overstimulation through enteropathogens, this study proposes a true paradigm change in the pathophysiology of stunting.

Session 16 – Metagenomics / S-56* + P-39*

"Fondue Chinoise" as a potential source of campylobacteriosis: whole genome sequencing (WGS) surveillance over 2 years in Basel, Switzerland

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Background
Campylobacter jejuni and *Campylobacter coli* are common causes of foodborne disease, causing diarrhea, fever and complications. Over 7000 confirmed cases are reported annually in Switzerland, with peaks over summer and winter. Infection is generally associated with poultry: the summer peak through barbecuing, and the winter peak possibly linked to festive consumption of "Fondue Chinoise".
Methods
Campylobacter isolates were collected from symptomatic patients and chicken meat in Basel, Switzerland, from April 2015 - March 2018 (n=700). Whole Genome Sequencing (WGS) was performed using Illumina technology, supplemented with MinION and PacBio long read technologies providing high quality reference assemblies within putative transmission clusters. An established core genome MLST (cgMLST) scheme was used to cluster the isolates, with whole genome single nucleotide polymorphism (SNP) analysis for high-resolution phylogenetic analysis of transmission events. Detailed genomic analysis also identified virulence factors and compared antimicrobial resistance determinants to phenotypic findings.
Results
This comprehensive longitudinal study shows a high diversity of *Campylobacter* in Basel. Patient and chicken isolates were both found in multiple clonal complexes, in both *C. jejuni* and *C. coli*. Interim analysis of 119 human and 81 chicken meat isolates shows clusters containing only patient isolates (n=11), only butcher isolates (n=12), and those containing both butcher and patient isolates, suggestive of transmission (n=7).
Conclusions
Our data confirms that chicken is a source of campylobacteriosis, presumably through undercooking or crosscontamination at kitchen level. This is the largest *Campylobacter* WGS surveillance study to date, investigating links between clinical isolates and isolates from contaminated chicken meat.

**Student paper*

Session 16 – Metagenomics / S-57

Community-acquired blood culture-negative infective endocarditis case elucidated by clinical metagenomics

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Background

Despite significant progress in diagnostics and therapies, Infective endocarditis (IE) remains a deadly disease. The most common causes of IE are *Staphylococcus*, *Streptococcus*, *Enterococcus* and HACEK-related organisms. We aimed at identifying the *causative agent* of a blood-culture-negative IE by whole metagenome shotgun sequencing (WMGS).

Methods

We extracted and sequenced DNA from the cardiac valve tissue of a patient who presented a community acquired native valve endocarditis and whose blood cultures were repeatedly negative. Sequence reads were mapped against bacterial genomic sequences, 16S rRNA genes and clade-specific taxonomic markers.

Results

Most of the 103,136 reads classified as bacterial in the sample data set were assigned to *Neisseria meningitidis*, followed by other *Neisseria* species. In line with these data, mapping of reads against clade-specific and 16S rRNA gene markers revealed *N*. *meningitidis* as most represented species. The negative control was dominated by organisms other than *N*. *meningitidis*. Assembled metagenomic fragments covered 81% of the genomic sequence of *N*. *meningitidis* MC58 (serotype B) and suggested that the strain in question belongs to the ST-41/44 complex/Lineage 3, one of the major causes of meningococcal infections in European countries.

Conclusions

*N. meningitidis*is commonly associated with meningitis and/or septicaemia but should not be neglected as a causative agent of IE, which became exceedingly rare with the introduction of antibiotics. Our data show that WMGS may be used as a diagnostic procedure to strengthen the diagnosis of IE and to obtain draft genomic sequence of the pathogen and typing information, bypassing the need for bacterial culture.

Session 16 – Metagenomics / S-58

Ecology of human lung microbiota and its impact on the host

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Background
Human lung microbiota research is in its infancy, with compelling evidence for a link between microbial composition and lung health. But little is known about its ecology and causal relationships. We focus on the microbiota of transplanted lung because different bacterial communities have been associated with different immune states influencing allograft health. The central hypothesis of this study assumes that there are key species within lung microbiota, which are metabolically active and differentially interact with immune cells.
Methods
We correlate 16S rRNA community data, untargeted metabolomics and recipient metadata to understand lung bacterial ecology and generate microbiota-association networks. We culture resident lung bacteria *ex vivo*under different conditions. We use human macrophages to study the influence of resident lung bacteria on the host innate
immunity.
Results
We have deciphered the most abundant and prevalent lung bacteria, and also provide initial insights on lung bacterial metabolism. We have established the Lung Microbiota culture Collection (LuMiCol) that contains diverse representatives genotypes found in 16S rRNA community analysis. We have employed novel assays to provide early insights on the stimulatory effects of lung bacteria on the innate immune cells.
Conclusions
There is a core set of lung bacteria that contribute significantly to lung microbial ecology. LuMiCol is by far the largest collection of resident lung bacteria from transplanted lung exhibiting genotypic and phenotypic diversity. Here, we reveal bacterial consortia that can be categorised into “low” and “high stimulatory”. This brings us one step closer towards understanding microbial influence on lung homeostasis.

Session 17 – Cell surface structures in prokaryotes / S-59

Investigating the antibacterial mode of action of an anticancer peptide

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Background

Antibiotic resistance is a rising concern, since many bacteria become resistant to a whole range of antibiotics, complicating their treatment. There is thus an urgent need of innovative strategies for the development of novel antibacterial molecules. In this perspective, antimicrobial peptides are promising candidates. These peptides are found in many different organisms, from bacteria to human and are often part of the innate immune system. Interestingly, we discovered fortuitously the antimicrobial effect of the anticancer peptide TAT-RasGAP$_{317-326}$. We could show that this peptide has a broad antibacterial activity against both Gram-positive and Gram-negative bacteria *in vitro*. Moreover, the peptide showed an *in vivo* activity when injected simultaneously with the bacteria in a mouse model of peritoneal infection (Heulot et al., 2017).

Methods

In this study, we took advantage of a fluorescent version of TAT-RasGAP$_{317-326}$ to characterize the dynamic of its entry and accumulation in *Escherichia coli*. Using confocal microscopy, we could observe accumulation of the peptide in the cytosol and DNA condensation. This was confirmed by electron microscopy.

Results

Then, in order to better understand the mechanism of action of the peptide, we challenged *E. coli* with increasing concentrations of the peptide and could thus select partially resistant mutants. Sequencing of these mutants highlighted potential resistance mechanisms that involve a regulator of outer membrane proteins.

Conclusions

We now need to go further in the characterization of the effect of TAT-RasGAP$_{317-326}$ on different bacteria in order to better understand the mode of action of this peptide and to estimate the probability of resistance appearance.

Session 17 – Cell surface structures in prokaryotes / S-60* + P-76*

Antimicrobial Peptide Dendrimers from Chemical Space and their Mechanism of Action

Ms. Bee Ha Gan [1]***, Mr. Thissa Siriwardena*** [1]***, Ms. Alice Capecchi*** [1]***, Prof. Jean-Louis Reymond*** [1]

1. *University of Bern*

Background

By exploring synthetic peptides with unusual branching topologies of the peptide chain not found in natural peptides, we have identified regularly branched peptide dendrimers composed of lysine and leucine such a **G3KL**exerting strong antimicrobial effects against multidrug resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*(Stach et al., *Angew. Chem. Int. Ed.***2014**, 53, 12827).We have recently optimized this dendrimer for in vivo use by combinatorial library screening (Siriwardena et al., *J. Am. Chem. Soc.***2018**, 140, 423).

Methods

We used virtual library enumeration, pharmacophore-shape analysis, synthesis and antimicrobial testing to identify improved variants of dendrimers G3KL. We synthesized fluorescence labeled G3KL analogs, and studied their interaction with bacterial cells by confocal microscopy and TEM. We characterized all dendrimers by CD and NMR and investigated their predicted structure by MD simulation.

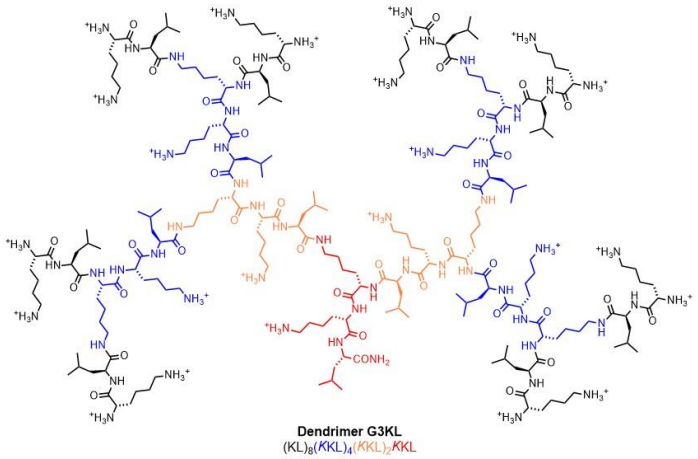
Results

Here I will present our ongoing efforts to optimize G3KL to address *Klebsiellae pneumoniae*, and to understand its mechanism of action. Most strikingly, we identified a new dendrimer with an expanded activity range including *Klebsiellae pneumoniae*, increased serum stability and promising activity in an in vivo infection model against a multidrug resistant strain of *Acinetobacter baumannii*. Imaging, spectroscopic studies and a structural model from MD simulations suggest that our dendrimer acts by membrane disruption.

Conclusions

These experiments provide the first example of using virtual screening in the field of dendrimers and show that dendrimer size does not limit the activity of AMPDs, and show that the dendrimer interacts with the bacterial membrane and ultimately enters the cell, leading to rapid killing.

**Student paper*



Attachment #198

Session 17 – Cell surface structures in prokaryotes / S-61* + P-40*

Listeria monocytogenes L-form cells, rebuilding the wall for cell virulence

Ms. Virginie Grosboillot [1]***, Ms. Isabelle Keller*** [1]***, Dr. Markus Schuppler*** [1]***, Prof. Martin J. Loessner*** [1]

1. *Department of Health Sciences and Technology, Institute of Food, Nutrition and Health, Zurich*

Background

*Listeria monocytogenes*is a Gram-positive intracellular foodborne pathogen capable of infecting humans and animals. Although the incidence of infection through contaminated food is low, *L. monocytogenes* is considered as the leading cause of death among all foodborne bacterial pathogens, due to the high listeriosis mortality rate (~30%). Under specific conditions like treatment with cell wall active antibiotics, phage predation, or immune defence activities, *L. monocytogenes* is assumed to convert to cell-wall deficient forms, called L-forms. While it is known that *L. monocytogenes*L-forms are able to grow and revert to a walled state *in vitro*, a lack of evidence remains concerning the presence and persistence of L-forms during infection of eukaryotic host cells, and their potential ability to revert intracellularly, thus causing a new infection cycle.

Methods

Fluorescence *in situ* hybridization (FISH) in combination with confocal laser scanning microscopy was used to detect intracellular L-forms after infection of HeLa cells with parental *L. monocytogenes* and Ampicillin treatment.

Results

Shape and size of the observed FISH signals suggested the presence of intracellular L-forms. Furthermore, viability of intracellularly visible L-forms was assessed by the growth of L-forms that were previously retrieved from HeLa cells by the use of a micromanipulator into osmotically protective liquid medium lacking antibiotics.

Conclusions

In conclusion, our results suggest that Ampicillin treatment of host cells infected by parental *Listeria monocytogenes* may trigger intracellular conversion to viable L-forms that possess the capability to persist and proliferate in the host cells.

**Student paper*

Session 17 – Cell surface structures in prokaryotes / S-62

DNA-uptake pilus of Vibrio cholerae capable of kin-discriminated auto-aggregation

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Background
Natural competence for transformation is a widely used and key mode of horizontal gene transfer that can foster rapid bacterial evolution. Competent bacteria take-up DNA from their environment using Type IV pili, a widespread and multi-purpose class of cell surface polymers. The Gram-negative bacterium *Vibrio cholerae* is an important human pathogen that causes the pandemic disease cholera. It becomes competent and produces a DNA-uptake pilus during growth on chitinous surfaces, which are abundant in its natural aquatic environment. However, how these pili facilitate DNA-uptake and whether or not they have additional functions has remained unclear.
Methods
Here, we have used a combination of bacterial genetics and bacterial cell biology to visualise Type IV pili in live cells and dissect the mechanisms underlying their activity.
Results
Using direct labelling, we show that DNA-uptake pili are abundant, highly dynamic and that they retract prior to DNA-uptake. Unexpectedly, these pili can self-interact to mediate auto-aggregation of cells into macroscopic structures, reminiscent of those formed in the intestine during infection. Furthermore, extensive strain-tostrain variability in the major pilin subunit PilA controls the ability to aggregate without affecting transformation, and enables cells producing pili composed of different PilA to discriminate between one another.
Conclusions
Our results suggest a model whereby DNA-uptake pili function to promote inter-bacterial interactions during surface colonisation and moreover, reveal a simple and potentially widespread mechanism for bacterial kin recognition.

Session 18 – Zoonotic pathogens / S-63

The hidden risk in snake breeding: an anecdote of human leptospirosis

Prof. Simone Schuller [1], Dr. Mathieu Picardeau [2], Mrs. Isabelle Brodard [3], Dr. Marga Goris [4], Dr. Cécile Bassi [5], Dr. James Tataw Ashu [5], Mr. Reto Lienhard [6], Dr. Sabrina Rodriguez-Campos [3]

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Background

An epidemiological investigation was performed after acute leptospirosis was diagnosed in a middle-aged male Swiss patient. The patient bred mice and rats, which are known reservoir hosts for *Leptospira* spp., as live food for snake breeding.

Methods

To investigate the source of infection, litter samples from rodent cages (n=2) were collected, mice (n=42) and rats (n=14) were euthanized and renal tissues examined by *lipL32* real-time PCR. The MLST 7L scheme was performed on real-time PCR positive samples with a Ct<30. Kidney tissues from a subsample of 10 mice and 10 rats were cultured in semi-solid EMJH medium supplemented with 6% rabbit serum and 2% 5-fluorouracil. Microscopic agglutination testing (MAT) was carried out against 13 leptospiral serovars depending on availability of serum. Serological typing of strains was performed with 43 antisera and monoclonal antibodies.

Results

Figure 1 represents results from real-time PCR and MLST. Positive MAT results of rodent sera are summarized in Table 1.To date, 4 *Leptospira* spp. strains were isolated (3 strains ST155, 1 strain ST149). One of each was serotyped: ST155 resembles serovar Polonica (serogroup Sejroe), ST149 resembles serovars Castellonis and Guangdong (both serogroup Ballum).

Conclusions

Rodent breeding may pose a risk to humans through shedding of pathogenic *Leptospira* spp. in urine as indicated by positive kidney and litter samples matching the ST from the patient. The data moreover suggest that multiple strains may circulate in a population of cage-bred rodents.

Figure 1. Molecular detection of *lipl32* specific of pathogenic *Leptospira* spp. from the total of 60 samples (mouse tissue, n=42; rat tissue, n=15; mouse litter, n=1; rat litter, n=1; human blood, n=1) by real-time PCR and results of Multilocus Sequence Typing (MLST).

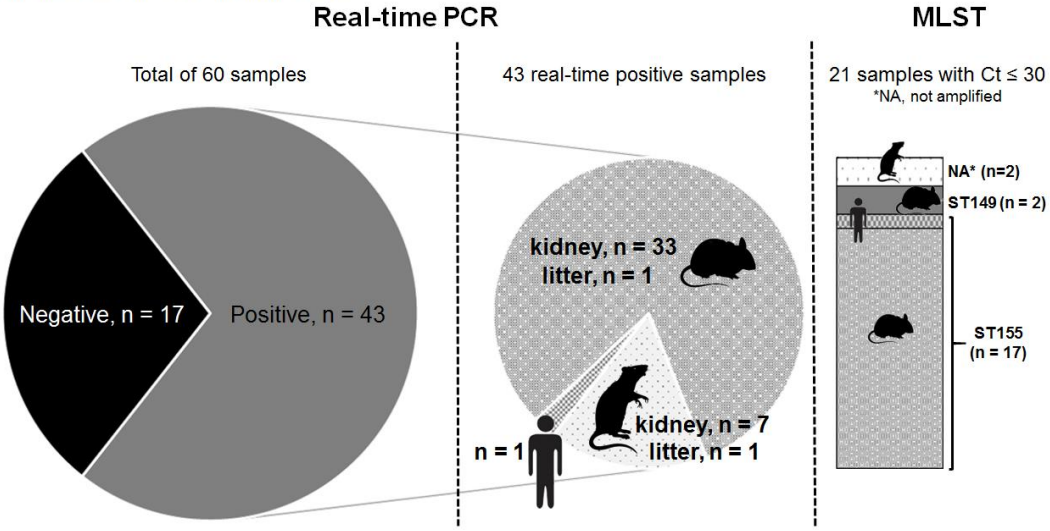

Table 1. Summary of positive results from a total of 11 mouse and 12 rat sera examined by microscopic agglutination testing (MAT).

Leptospiral serovar (strain used as antigen in MAT)*	Number of positive serum samples (titer range)	
	Mouse	Rat
Hardjo (strain Hardjoprajitno)	7 (1:40 to 1:400)	6 (1:20 to 1:100)
Sejroe (strain M84)	4 (1:40 to 1:400)	- -
Australis (strain Ballico)	7 (1:20 to 1:200)	7 (1:100 to 1:200)
Copenhageni (strain M20)	4 (1:20 to 1:40)	2 (1:400 to 1:800)

*strains provided by OIE and National Collaborating Centre for Reference and Research on Leptospirosis, Amsterdam, The Netherlands

Attachments #155

Session 18 – Zoonotic pathogens / S-64*

Analysis of the zoonotic potential of the Influenza D virus for humans

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1. *University of Bern, Vetsuisse Faculty, Institute of Virology and Immunology, Bern and Mittelhäusern*

Background
Influenza D virus (IDV) is a new genus in the Orthomyxoviridae family and was first detected in pigs with influenza-likesymptoms in 2011. IDV-directed antibodies were detected in a broad range of livestock like camelids, cattle and small ruminants, suggesting that the virus has a wide host tropism. Interestingly, IDVdirected antibodies were also found in humans, albeit only in those with occupationally exposure to livestock.
Methods
To elucidate the zoonotic potential of IDV for humans, we infected well-differentiated human airway epithelial cells (hAECs) from several donors with IDV and analyzed the viral replication kinetics and cell tropism at 33°C and 37°C. In addition, we assessed the induction of the innate immune response during the course of infection by qRT-PCR. Upon infection, apical washes were collected every 24 hours to monitor the production and secretion of viral progeny using qRT-PCR and virus titration.
Results
It turned out that IDV is able to efficiently replicate in hAECs at both 33°C and 37°C. Moreover, immunofluorescence analysis showed that IDV has a preferential cell tropism for ciliated cells, which is similar to what we have observed for the related Influenza C virus. Finally, analysis of the innate immune response revealed a pronounced upregulation of traditional interferon-stimulated genes such as IFIT1, MxA and OAS1, as well as interferon lambda (IFN-λ) 1, 2 and 3 at 48 and 72 hours post infection.
Conclusions
These results demonstrate that IDV replicates efficiently on hAECs with a preference for ciliated cells and induces an innate immune response during the late stage of infection.

** Student paper*

Session 18 – Zoonotic pathogens / S-65

Isolation, characterization and rapid human adaptation of tick-borne tamiami virus

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Background
The New World Arenaviruses are diverse family of emerging negative strand RNA viruses comprised of Clades A, B, C, and D. North American Clade D viruses, including Whitewater Arroyo (WWAV), Tamiami (TAMV), Bear Canyon, Skinner Tank, and Catarina virus, are carried in nature by Cricetidae rodents. TAMV was isolated in 1970 from *Sigmodon hispidus* in Florida and clusters phylogenetically closely with WWAV, which has been associated with human disease. Recent field studies resulted in the first isolation of the New World Arenavirus Tacaribe (TCRV), from host-seeking *Amblyomma americanum* ticks in Florida.
Methods
Using next generation sequencing (NGS), we detected genetic traces of TAMV in these tick-derived isolates. The ability of TAMV to prevent super-infection of TCRV but not *viceversa,*allowed enrichment of TAMV with concomitant extinction of TCRV by passaging in human cells, evidenced by NGS and immunofluorescence.
Results
The new tick-borne viral TAMV-FL isolate shares only 85% of homology with available TAMV reference sequence, suggesting strong selection and adaptation, and excluding the possibility of a laboratory contamination. During serial passaging, we further observed rapid selection of two mutations (N149K and D154N) located in the putative interaction region between the TAMV envelope glycoprotein (GP) and its cellular receptor, transferrin receptor 1.
Conclusions
The rapid acquisition of point mutations, in particular in the GP suggests high plasticity and the potential for alter receptor binding, providing first evidence for a more dynamic host range of TAMV that may include ticks as transmission hosts and reveals the capacity for rapid human adaptation.

Session 18 – Zoonotic pathogens / S-66*

Phosphatidylserine receptors TIM-1 and Axl promote hantavirus infection

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Background

Hantaviruses are emerging human pathogens responsible for severe human diseases and represent a challenge for public health. Currently, there are no effective antivirals or licensed vaccines against hantaviruses.

Viral attachment and entry represent the first steps in virus transmission and are promising targets for antiviral therapeutic intervention. We investigated the largely unknown receptor use of hantaviruses in human respiratory epithelial cells of the Old World Hantaan virus (HTNV) and New World Andes virus (ANDV), the most important pathogens of the major hantavirus Clades. A range of emerging enveloped viruses can use phosphatidylserine (PS) receptors of the human T cell immunoglobulin and mucin domain containing protein (TIM) and the Tyro3/Axl/Mer (TAM) families to enter human cells via "apoptotic mimicry".

Methods

We established and validated a hantavirus pseudotype system based on replication competent and propagation deficient recombinant vesicular stomatitis virus as a suitable BSL2 surrogate model to study hantavirus entry into the host cell.

Results

Flow cytometry and Western blot revealed expression of TIM-1 and Axl on A549 cells, consistent with their known expression pattern in human lung epithelia *in vivo*. Overexpressed TIM-1 and Axl in cells naturally lacking both receptors markedly increased entry of HTNV and ANDV. In a complementary approach, we treated A549 cells with blocking antibodies to TIM-1 and Axl and found a major contribution of TIM-1 and to a lesser extend Axl in entry of HTNV and ANDV.

Conclusions

We provide first evidence that HTNV and ANDV may hijack the phosphatidylserine receptors to enter human epithelial cells during airborne transmission.

**Student paper*

Session 19 – Microbial pathology & Immunity / S-67*

Th17 cell immunity to the commensal fungus Candida albicans

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Background

*Candida albicans*is a commensal fungus colonizing mucosal epithelia in humans. If host defenses are breached,*C. albicans* can turn into a pathogen and cause disease. This may occur in individuals with inherited or acquired defects of the IL-17 pathway. Available mouse models of superficial candidiasis are not well suited to study the host response to fungal colonization as *C. albicans* is usually cleared rapidly from mice. Here we introduce a novel mouse model of persistent oral candidiasis. This study aims at dissecting the regulation of protective T cell-mediated antifungal immunity in the oral mucosa.

Methods

Immunocompetent wildtype mice were infected sublingually with an avirulent natural isolate of *Candida albicans* (strain 101). The *C. albicans*-specific T cell response was analyzed by flow cytometry following antigenspecific T cell re-stimulation.

Results

Persistent colonization of mice with *C. albicans* resulted in a IL-23-dependent induction of fungus-specific T helper 17 cells. Even after a prolonged period, we did not observe plastic T cells producing IFN-γ and there was no compensation by Tregs for the continuous activation of Th17 cells. The relevance of T cells in controlling *C. albicans* in persistently colonized mice was confirmed by increased susceptibility of T cell-deficient mice and the rescue of fungal control in these mice by adoptive transfer of *C. albicans*-specific T helper cells.

Conclusions

Together, our results confirm a key role of Th17 immunity in the control of *C. albicans* colonization in mucosal barrier tissues and set the stage for future mechanistic studies of the antifungal T cell response.

** Student paper*

Session 19 – Microbial pathology & Immunity / S-68

Is Antifungal Treatment Needed in Non-Candidemic Patients with a Positive Catheter Tip Culture for Candida ? A Multi-Center Cohort Study of the Fungal Infection Network of Switzerland (FUNGINOS)

Dr. Yvonne Schmiedel [1], Dr. Veronique Erard [2], Dr. Peter Schreiber [3], Dr. R Zbinden [4], Dr. Nina Khanna [5], Dr. Reno Frei [6], Dr. Anna Conen [7], Dr. Dionysios Neofytos [8], Prof. Christian Van Delden [9], Dr. Konrad Mühlethaler [10], Dr. Pierre-Yves Bochud [2], Dr. Jacques Bille [2], Dr. Frederic Lamoth [2], Dr. Katia Boggian [11], Dr. Christian Ruef [12], Dr. Ursula Flueckiger [13], Prof. Oscar Marchetti [2], Prof. Stefan Zimmerli [1]

1. *Inselspital Bern,* ***2.*** *CHUV,* ***3.*** *USZ,* ***4.*** *Institute of Microbiology, Lausanne University Hospital and Lausanne University, Switzerland, 3Servicio de Microbiología, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain,* ***5.*** *University of Basel, University Hospital Basel,* ***6.*** *USB,* ***7.*** *Institute of microbiology, laksa,* ***8.*** *hcuge,* ***9.*** *Unit for Transplant Infectious Diseases, Service of Infectious Diseases, University Hospital Geneva and Department of Microbiology and Molecular Medicine, University of Geneva,* ***10.*** *IFIK,* ***11.*** *ST. Gallen,* ***12.*** *Zürich Infektiologie,* ***13.*** *Basel*

Background

Candidemia belongs to the top ten bloodstream infections clearly indicating treatment. Endovascular catheters are a frequent source of infection. The impact of their removal is uncertain. The benefit of antifungal therapy in non-candidemic patients with a catheter tip culture positive for *Candida*is a matter of debate. Aim of this study was to analyze clinical outcome according to the presence/absence of candidemia and antifungal treatment.

Methods

In this multi-center cohort study over a 3-year period in Swiss university hospitals, characteristics and outcome of patients with a positive catheter tip culture for *Candida*were investigated and compared in two settings: Absence vs. presence of concomitant candidemia.

Antifungal treatment vs. no treatment in absence of concomitant candidemia.

Results

Among 212 adult patients with a positive catheter tip culture for *Candida*, 84 (40%) had candidemia. Amongst non-candidemic patients, 75 (58%) received antifungal treatment.

Patients with concomitant candidemia compared to non-candidemic patients had more frequent signs of infection at the catheter insertion site (89% vs. 25%, p<0.001) and received more antifungal therapy (96% vs. 57%, p<0.001).

Non-candidemic patients who received antifungal therapy compared with those who did not had more frequent signs of infection at the insertion site (31% vs. 17%, p=0.08), endovascular devices (23% vs. 6%, p=0.04) and sepsis (15% vs. 6%, p=0.08).

No difference was found in 100-day mortality and 12-month rehospitalisation.

Conclusions

In non-candidemic patients, outcome is similar in those who did or did not receive antifungals: signs of infection, severity criteria and risk for endovascular complications probably guide the decision to start therapy.

Session 20 – Mycobacteria / S-69*

Genomics of Mycobacterium kansasii, does horizontal gene transfer mediate pathogenicity?

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

Background

Mycobacterium kansasii is an environmental bacterium and a human opportunistic pathogen. Among 7 subtypes described, subtype 1 is the most pathogenic subtype. Two major mechanisms of horizontal gene transfer occur in *M. kansasii*: conjugation of plasmids and distributive conjugal transfer (DCT), allowing transfer and recombination of genomic fragments ranging from 50bp to >200Kb. Using comparative genomics, we evaluated the frequency of such events and their association with pathogenicity.

Methods

Whole-genome sequencing was performed using MiSeq on 51 *M. kansasii* strains isolated at the Lausanne University Hospital and 25 publically available genomes were added to the dataset. Each strain was classified as pathogenic or non-pathogenic. Plasmids were identified (1) using PacBio sequencing for one strain, (2) by homology with plasmid sequences, (3) by looking for homologs of genes involved in conjugation and (4) using GC-coverage plots. DCT were identified using BratNextGen.

Results

Twenty-three plasmids were identified. Mean size was 120,430bp and GC-contents were lower than chromosomal DNA ($p<0.00001$). Confirming their conjugative properties, type-VII secretion loci and relaxase homologs were found in 23/23 and 19/23 plasmids, respectively. Three DCT events, leading to the loss of a chromosomal gene involved in the resistance to oxido-nitrosative stress (selectively present in subtype 1), were detected in 3/5 non-pathogenic subtype 1 strains. No DCT occurred at this location in 28/28 pathogenic subtype 1 strains.

Conclusions

Large conjugative plasmids sharing the same backbone structure were found in 30% of *M. kansasii* strains. While no specific plasmidic gene was associated with disease, DCT events may play a role in modulating pathogenicity.

**Student paper*

Session 20 – Mycobacteria / S-70

Molecular epidemiology integrating whole genome sequencing, a powerful approach for the investigation of Mycobacterium tuberculosis outbreaks

Dr. Fathiah Zakham [1], Dr. Sacha Laurent [2], Dr. Claire Bertelli [3], Prof. Laurent Nicod [4], Prof. Gilbert Greub [5], Dr. Katia Jaton [6], Dr. Jesica Mazza-stalder [4], Dr. Onya Opota [3]

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Background

Whole genome sequencing (WGS) stands at present as the most discriminant method for *Mycobacterium tuberculosis* genotyping. Recently, clinical and epidemiological investigations suggested a local outbreak of tuberculosis (TB) in the canton de Vaud with two potential TB clusters. The aim of this study was to investigate this outbreak suspicion using a molecular epidemiology approach integrating epidemiological and clinical information as well as WGS-based genotyping.

Methods

M. tuberculosis DNA extracted from liquid cultures using a rapid method applicable in the BSL 3 conditions that we integrated to our daily workflow, was prepared with the Nextera XT kit for shotgun sequencing using a MiSeq Illumina platform. Genotyping was achieved using an in-house bioinformatics pipeline. Clinical and epidemiological data collected for all cases were integrated with genotyping data.

Results

The analysis of single nucleotide polymorphisms (SNPs) identified 2 potential clusters consisting of 3 strains (SNPs < 1) and 2 strains (SNPs = 0), supporting epidemiological and clinical findings. In addition, the SNP analysis revealed that the 2 clusters were more distantly related (>>100 SNPs). Finally, a last strain did not group with either cluster 1 or 2.

Conclusions

The molecular epidemiology approach integrating WGS-based genotyping supported the existence of 2 clusters but could successfully delineate and discriminate them preventing overestimation of the outbreaks. While, further standardizations is ongoing to define reproducibility and reliability of the method this multidisciplinary approach represents a powerful tool for contact investigation and TB surveillance.

Session 20 – Mycobacteria / S-71

Lung-on-a-chip microtechnologies for studies of host-pathogen interactions in M. tuberculosis infections

Dr. Vivek Thacker [1]***, Dr. Riccardo Barrile*** [2]***, Dr. Katia Karalis*** [2]***, Prof. John Mckinney*** [1]

1. *Global Health Institute, Ecole Polytechnique Fédérale de Lausanne, Lausanne,* ***2.*** *Emulate Inc*

Background
A majority of host-*M.tuberculosis* encounters lead to latent infections in which the bacteria exist in a poorly characterized host-pathogen equilibrium, usually within granulomatous lesions in the lung. Although the structure and components of these lesions are well characterised, the dynamic nature of this equilibrium, and the contribution of phenotypic heterogeneity within the bacterial population towards this process is less well understood.
Methods
We reconstitute, entirely *in vitro*and using primary cells, the murine alveolar interface using a lung-on-a-chip microfluidic device. This builds upon the previously reported human lung-on-a-chip system by incorporating aspects of the innate immune system such as alveolar and bone-marrow derived macrophages. This system is then exposed to *M. tuberculosis*, and the dynamic progression of the infection is monitored through a combination of long-term live-cell time-lapse microscopy (7-10 days) and confocal microscopy. This "bottom-up" approach allows for single-cell imaging and temporal tracking of host-pathogen interactions and enables us to closely follow the chain of events from initial infection to the development of lesions.
Results
We are currently investigating unresolved aspects about the innate immune response to the initial infection focusing on the interplay between resident macrophages and alveolar epithelial cells. Here, we report on the insights gained from initial infection experiments. As the system develops further, we will extend it to study how granulomatous lesions develop, and the roles played by bacterial phenotypic variants in this process.
Conclusions
This work address challenging questions of immense significance for our understanding of latent TB and persistence during antibiotic therapy.

Session 20 – Mycobacteria / S-72

Whole genome sequencing characterization of an isoniazid resistant Mycobacterium tuberculosis strain undetected by conventional molecular methods

Dr. Onya Opota [1], Dr. Fathiah Zakham [1], Dr. Sacha Laurent [2], Dr. Claire Bertelli [1], Dr. Laurent Merz [3], Prof. Laurent Nicod [4], Dr. Jesica Mazza-stalder [4], Prof. Gilbert Greub [5], Dr. Katia Jaton [6]

1. *Institute of Microbiology, Lausanne University Hospital and Lausanne University, Switzerland,* ***2.*** *Institute of Microbiology, Lausanne University Hospital and Lausanne University, Switzerland.,* ***3.*** *Établissements Hospitaliers du Nord Vaudois Site de St Loup, St Loup,* ***4.*** *Pneumology Service, Lausanne University Hospital and Lausanne University, Switzerland,* ***5.*** *Lausanne University Hospital, Lausanne,* ***6.*** *Institute of Microbiology, Lausanne University Hospital and Lausanne University*

Background

Resistance to isoniazid (INH) in *Mycobacterium tuberculosis* is a very complex mechanism involving, among others, mutations in *katG* gene encoding a catalase-peroxidase activating the pro-drug and mutation in *inhA* causing drug target modification. Here we report a delay in the introduction of a complete efficient anti-TB regimen due to a strain displaying a high-level of resistance to INH (Minimum Inhibitory Concentration, MIC>10mg/L), undetected by conventional molecular methods.

Methods

The molecular mechanism of INH resistance was investigated by Sanger sequencing of selected resistance genes as well as by whole genome sequencing (WGS) using an Illumina MiSeq platform and an in-house bioinformatics pipeline.

Results

Partial sequencing of *rpoB*, *katG* and *inhA* genes, directly on the clinical sample and on the cultured *M. tuberculosis* strain did not identify any known mutation associated with rifampicin or isoniazid resistance despite the high level of resistance to INH. Sequencing of the promotor of *ahpC* gene encoding for alkyl hydroperoxide reductase revealed a mutation, C(-57)T, previously associated with INH resistant. WGS confirmed this mutation and the absence of mutation in *inhA*and*katG*. Comparison with the genome of the drug-sensitive reference strain *M. tuberculosis*H37rv revealed, in the INH-R strain, an insertion sequence in *katG* gene, leading to gene disruption.

Conclusions

These results suggest a *katG* disruption mechanism leading to high-level resistance to INH in *M. tuberculosis* associated with compensatory *ahpC* mutation to maintain bacterial fitness (oxidative stress resistance). Benefits of *ahpC* screening and WGS will be discussed as such resistance mechanism may escape conventional molecular methods.

Session 21 – Diagnostic microgiology / S-73

Phylogeographical analysis reveals the historic origin, emergence and evolutionary dynamics of methicillin-resistant Staphylococcus aureus ST228 in Switzerland

Dr. Mohamed Abdelbary [1], Prof. Ed Feil [2], Dr. Laurence Senn [1], Dr. Guy Prod'hom [1], Prof. Jacques Schrenzel [3], Prof. Hajo Grundmann [4], Dr. Dominique Blanc [1]

1. *Lausanne University Hospital, Lausanne,* ***2.*** *Univerity of Bath, Bath,* ***3.*** *Geneva University Hospitals,* ***4.*** *University Klinik, Freiburg*

Background
MRSA remains a major public health concern. Sequence type 228 (ST228) was first described in Germany and represented a successful MRSA clone in several European countries. Since 2000, ST228 had emerged in Lausanne causing several large outbreaks. Here, we aimed to elucidate the evolutionary history of this clone and to determine the genetic content correlating with its expansion and successful spread in Switzerland.
Methods
Whole genome sequencing was performed on 531 ST228 isolates collected from 14 countries between 1997 and 2012. Maximum-likelihood phylogenetic reconstruction was performed and the resulting phylogenetic tree was rooted using N315 reference genome.
Results
The phylogenetic analysis revealed that clades of ST228 isolates were associated with their geographical origin. This clone appears to have originated from a distinct German lineage. The highly successful Swiss ST228 lineage was associated with the loss of *ant(6)-Ia* and *aph(3')-III* genes and mupirocin resistance, and was introduced first in Geneva university hospital and, subsequently, emerged in Lausanne on several occasions suggesting that patients transfer between these two hospitals have played a significant role in its spread.
Conclusions
Our results suggest that ST228 has most likely originated from its progenitor in Germany and diverged into different European countries. In Switzerland, ST228 was introduced first in Geneva and, subsequently, spread in Lausanne. The clades that spread in Switzerland showed the acquisition of multiple mobile genetic elements.

Session 21 – Diagnostic microgiology / S-74* + P-41*

Comparison of classical microscopy and culture with the Seegene AllplexTM BV Assay for laboratory diagnosis of Bacterial vaginosis

Mrs. Shawmiya Ratnasabapathy [1], Dr. Marianne Affolter [2], Mrs. Arunthasha Pavananthan [2], Dr. Dobrila Dimitrijevic [2], Dr. Angelika Stroehle [2]

1. *MCL Medical Laboratories/ ;medi Bern,* ***2.*** *MCL Medical Laboratories*

Background
Bacterial vaginosis (BV) is characterized by an overgrowth of anaerobic bacteria leading to a replacement of beneficial lactobacilli by anaerobes. Microscopy using the modified Nugent-Ison score and culture are currently used methods for diagnosis of BV in our laboratory. The Seegene Allplex™ BV Assay as a multiplex PCR for *Lactobacillus sp (Lacto)*, *Gardnerella vaginalis (GV), Atopobium vaginae (AV), Megasphera 1*, BVAB2, *Bacteroides fragilis*and *Mobiluncus sp*. is a promising new tool for BV diagnosis.
Methods
A total of 119 E-swabs™ (Copan), 61 positive, and 58 negative were included and stored at -20°C prior to DNA extraction and PCR. DNA was extracted on MagnaPure 96 (Roche), Allplex™ BV assay done on CFX96 Realtime PCR instrument. Calculation was done by two quantitative thresholds a Qt1 of 3.25 for Lacto, *GV* and *AV* (Seegene Viewer RUO) and Qt2 with individual thresholds: GV 5.5, AV 4.11, Lacto 3.53 (Seegene Viewer submitted for CE-IVD).
Results
Quantitative threshold (Qt) calculation of Allplex™ BV Assay :
Qt1: 49 of 61 positive samples were positive, 11 intermediate, 1 negative.
Qt2: 48 of 61 positive samples were positive, 10 intermediate, 3 negative.
Qt1: 52 of 58 negative samples were negative , 5 intermediate, 1 positive.
Qt2: 56 of 58 negative samples were negative, 2 intermediate.
Conclusions
The Seegene Allplex™ BV Assay is a new tool for BV diagnosis. Calculated with a Qt1 of 3.25 for Lacto, *GV* and *AV*showed a specificity 89.7% and a sensitivity 80.3%, calculation with individual thresholds of Qt2 a specificity of 96.6% and a sensitivity of 78.7%.

**Student paper*

Session 21 – Diagnostic microgiology / S-75 + P-42

To be or not to be molecular? Application to Streptococcus agalactiae screening in late pregnancy

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1. *ADmed Microbiology,* ***2.*** *ENIL-Mamirolle*

Background
Group B streptococci (GBS) remain a leading cause of serious neonatal infections. Actual SSGO guidelines recommend the screening of all pregnant women at 35-37 weeks gestation for vaginal and rectal colonisation. Currently we use a simple method based on a direct B agglutination of the LIM enrichment broth after incubation. Our objective was to control this procedure with a molecular based method (LAMP) performed on incubated LIMs and to test a rapid cassette PCR directly on the sample upon arrival.
Methods
Vaginal/rectal swab samples in LIM broth (N=87) were prospectively tested with three methods to detect GBS. Upon arrival in the laboratory a GeneXpert GBS PCR cassette (Cepheid) was immediately launched before overnight incubation. On the next day an Illumigene GBS (Meridian) LAMP amplification test and a direct agglutination of the broth with the OXOID Streptococcal grouping Kit were performed. Discrepant results were resolved by culture.
Results
Overall 10 % (9/87) samples tested positive but only five showed positive with all three methods. All positive samples (9/9) were detected by Illumigene, 7/9 by direct GeneXpert and only 5/9 by agglutination. Eleven direct GeneXpert tests (12%) gave an error message probably due to the presence of mucus. These cassettes are not intended originally for swabs in LIM broth.
Conclusions
We observed a lack of sensitivity of our actual procedure. A simple molecular method like the Illumigene LAMP performed on incubated LIM offers excellent results. To our knowledge the GeneXpert is the only commercial rapid direct PCR test currently available.

Session 21 – Diagnostic microgiology / S-76

Evaluation of Xpert MTB/RIF Ultra, the new highly sensitive molecular point-of-care Test for tuberculosis diagnosis

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Background
Xpert MTB/RIF (Xpert) the molecular POCT that detects *M. tuberculosis* and resistance to rifampicin has dramatically improved the diagnosis of tuberculosis (TB) by reducing the turnaround time, shortening patient's isolation and time to initiate anti-TB drugs. The new version, Xpert MTB/RIF Ultra (Ultra), displays increased analytic sensitivity by targeting the multi-copy genes IS6110 and IS1810. The aim of this study was to evaluate the performance of this new assay.
Methods
Respiratory specimens (n=72) from patients with suspected pulmonary TB collected in our hospital previously tested with Xpert were tested with Ultra in a retrospective study. Performance and accuracy of both tests were compared using culture as gold standard and clinical data to investigate discrepant results. Relation with smear microscopy for the detection of acid-fast bacilli (AFB), was also addressed.
Results
The overall sensitivity was 81.1% (30/37) for Xpert and 94.6% (35/37) for Ultra. Both assays displayed an overall sensitivity of 100% (17/17) on smear positive specimens. The sensitivity on smear negative specimens was 65.0% (31/20) for Xpert and 90.0% (18/20) for Ultra. Specificity of Xpert and Ultra were respectively 94.3 % (33/35) and 91.4% (32/35). The semi-quantitative result of both test positively correlated with the semi-quantitative result of the AFB detection.
Conclusions
Our data support an increased sensitivity of Ultra compared to Xpert; culture remaining the most sensitive method. A particular attention is paramount when introducing Ultra, due to its extremely high sensitivity that can detect low amount of DNA, which can correspond to patient with a past history of TB.

Session 22 – Environmental microgiology / S-77* + P-56*

Low molecular weight organic acids as key molecules in bacterial-fungal interactions

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Background

Low molecular weight organic acid (LMWOA) production is widespread in fungi and can have important roles in processes like pathogenesis, competition, mineral weathering or lignocellulose degradation. More recently, LMWOA production has been involved in the interaction between fungi and bacteria. This is particularly true in the case of oxalic acid (OA). For example, in the oxalate-carbonate pathway, oxalogenic fungi are known to interact with oxalotrophic bacteria in soils. Moreover, OA can also serve as a signaling molecule for mycophagous bacteria to localize their fungal target. Due to this multitude of functions, LMWOA appear crucial in bacterial-fungal interactions and might be part of a general mechanism mediating reciprocal bacterial-fungal behavior. Therefore, the aim of this study is to assess the role of LMWOA, and in particular OA, as key molecules in bacterial-fungal interactions.

Methods

Co-cultures confronting LMWOA-producing fungi to three soil bacteria (two oxalotrophic and one nonoxalotrophic) were performed in media with differing nutrient composition. LMWOA consumption was assessed in the co-cultures.

Results

Over 8 fungal strains screened, 5 produced large amounts of various LMWOA. For the latter, oxalotrophic bacteria appear to control LMWOA production and fungal growth, but this depends on the media composition.

Conclusions

With this study, new insights into the functional role of LMWOA in bacterial-fungal interactions are highlighted. This offers a new venue for the biological control of fungal growth using bacterial consumption of LMWOA.

**Student paper*

Session 22 – Environmental microgiology / S-78*

Targeted mycophagous lifestyle of Lysinibacillus sphaericus against the phytopathogenic fungus Rhizoctonia solani

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Background
Bacteria are well known for their diversified nutritional capabilities, which may lead to positive or negative interactions with other organisms. Mycophagy is a behavior allowing bacteria to obtain nutrients from living fungi, with a negative impact on the fungal partner. Bacilli are an important component of the soil microbial community and are widely used in biofertilization and biocontrol.
Methods
In order to understand the mechanisms underlying this mycophagous lifestyle, a confrontation assay was performed, where *R. solani* biomass and its exudates were the sole carbon source for the bacterium. In order to assess the specificity of the interaction, we confronted *L. sphaericus* to a saprophytic fungus, *Trichoderma rossicum*.
Results
We observed a mycophagous lifestyle in *Lysinibacillus sphaericus* against the soil-borne phytopathogenic fungus *Rizoctonia solani*. *L. sphaericus* inhibited the growth of *R. solani* but not of *T. rossicum*. *R. solani* hyphae that were in direct contact with bacteria were adversely damaged and unable to re-grow and this was not observed for *T. rossicum*. Microscopic observations revealed the deformation of *R. solani*'s cell wall and bacteria penetrating fungal hyphae. Bacterial growth occurred at the expense of *R. solani* fungal mycelium only, but no growth was measured on either *T. rossicum* hyphal biomass or in fungal exudates of both species.
Conclusions
The results confirms that the living mycelium of *R. solani* is required for bacterial growth and demonstrates the ability of the bacterium to select its target. This yet unexplored mycophagous lifestyle of *L. sphaericus* could be exploited for the control of phytopathogens in sustainable agriculture.

**Student paper*

Session 22 – Environmental microgiology / S-79

High time-resolution simulation of E. coli on Vietnamese farmers' hands based on videography and environmental microbial sampling

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Background

Infectious disease transmission is frequently mediated by the environment, where people's interactions with the environment dictate infection risks. Capturing and quantifying these offers important insights into effective interventions.

Methods

We capture high time-resolution activity data using videography for twenty-five Vietnamese farmers during collection and land application of human excreta for agriculture. The activity data are integrated with environmental microbial sampling data for *E. coli* concentrations on hands and surfaces into a stochastic-mechanistic simulation. The simulation models *E. coli* contamination on hands and *E. coli* ingested.

Results

Videography identifies frequent and variable contact rates for farmers' hands (from 34 to 1,344 objects contacted per hour per hand) and highly variable hand-to-mouth contact rates (from 0 to 9 contacts per hour per hand). Environmental microbial contamination data highlighted ubiquitous *E. coli*contamination in the environment, including excreta, hands, toilet pit, handheld tools, soils, and water. Results from the simulation show dynamic changes in *E. coli* contamination on hands, and predicted contamination levels of mean (standard deviation) 1.4 (1.3) log10 CFU *E. coli*/ cm2. There was also substantial inter-farmer variation in simulated *E. coli* ingested, with most (80%) ingesting no *E. coli.* Amongst farmers exposed, estimated ingestion rates were 0.7-10.4 CFU *E. coli*/ hr. Sensitivity analysis suggests that ingested *E. coli* is influenced by contamination of handheld tools, excreta, and the toilet pit, and frequency of hand-to-mouth contacts.

Conclusions

Integrating high time-resolution activity data into exposure assessments highlights variation in exposures amongst farmers, and offers greater insight into effective interventions and their potential impacts.

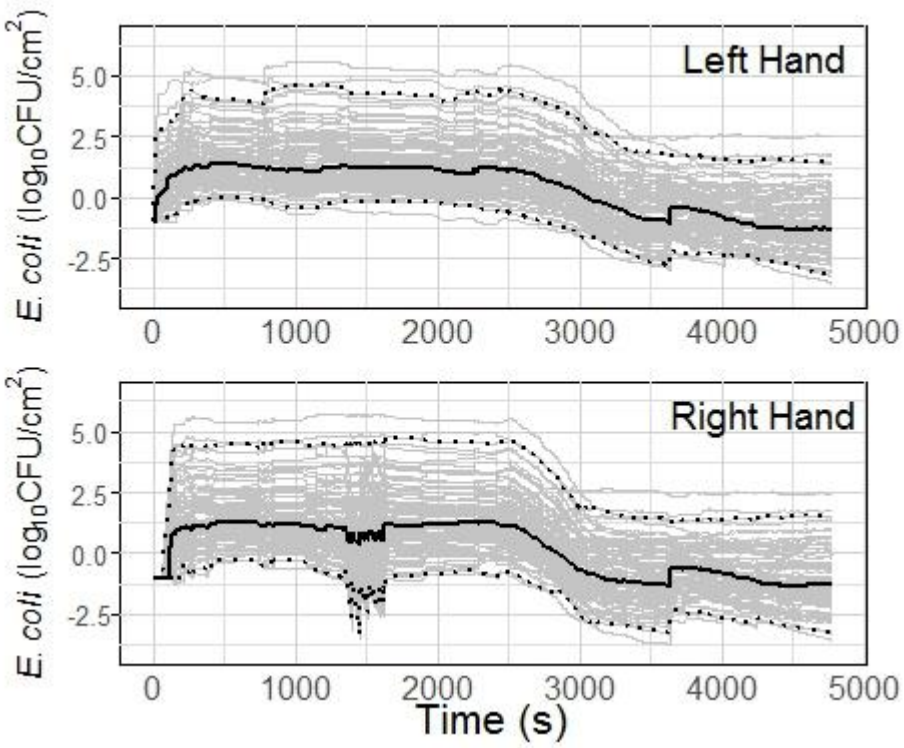

Figure5

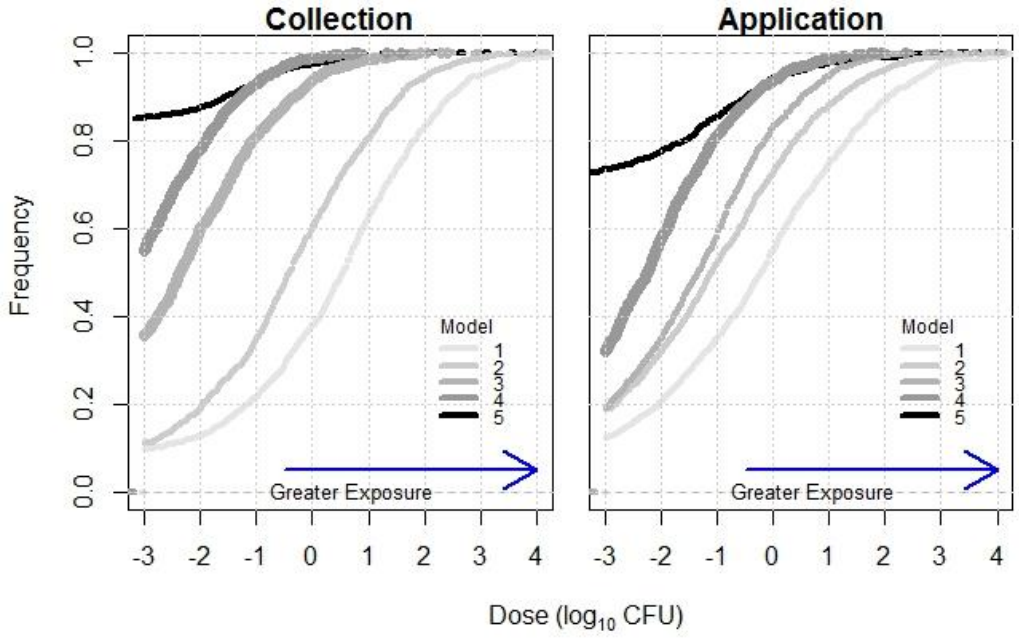

Figure6

Attachments #192

Session 22 – Environmental microgiology / S-80*

Iron reduction by the fermentative Gram-positive bacterium Clostridium acetobutylicum

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Background
Iron is an essential element for almost all living organisms. In addition, its biogeochemical cycling influences processes in the geosphere, like the mobilization or immobilization of trace elements and contaminants. Redox transformations of Fe(III) to Fe(II) can be catalyzed microbially, particularly by metal-respiring bacteria, that utilize Fe(III) as a terminal electron acceptor. Furthermore, Gram-positive fermentative iron reducers can also reduce Fe(III) by utilizing it as a sink for excess reducing equivalents, as a form of enhanced fermentation. While these microorganisms are likely to contribute to iron reduction significantly, particularly in environments where iron respirers fare poorly (e.g., contaminated sites), knowledge of their iron reduction mechanism is limited.
Methods
Here, we use the Gram-positive fermentative bacterium *Clostridium acetobutylicum* as a model system due to its ability to reduce heavy metals and its genetic tractability. We investigated the reduction of soluble and solid phase Fe(III) by *C. acetobutylicum* during fermentation, characterized the associated transcriptome, and used metabolic modeling to decipher the underlying pathways.
Results
We found that iron reduction buffers the pH, and elicits a shift in the carbon and electron flow. During standard fermentation, electrons are directed to the production of solvents and hydrogen, but if iron is provided, the electrons shift towards iron and the production of solvents and hydrogen decreases. Genes upregulated during iron reduction were targeted for transposon mutagenesis.
Conclusions
This study underscores the role fermentative bacteria can play in iron cycling and provides insight into the molecular mechanism of coupled fermentation and iron reduction.

**Student paper*

Detection and genomic diversity of Escherichia coli from soils and fecal sources in households in rural Bangladesh

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Background

Soils in households in low- and middle-income countries may be important in the transmission of *Escherichia coli*, including pathogenic and antibiotic-resistant variants.

Methods

E. coli was recovered from soil and human, chicken and cattle fecal samples from 52 households in rural Bangladesh. Associations between *E. coli* in soil and household-level factors and soil characteristics were investigated. Among 175 isolates, susceptibility to 16 antibiotics and the presence of intestinal pathotypes were evaluated. Whole-genome comparative analyses were performed in 60 isolates.

Results

E. coli was detected in 44.2% of the soils with an average of 1.95 log10CFU/g-dry-soil. Soil moisture (ρ=0.48, p=0.0003) and clay content (ρ=-0.47, p=0.0095) were associated with soil *E. coli* concentration but no household factor was correlated. Resistance patterns in *E. coli* isolated from soil were similar to *E. coli* from chicken and human feces (44% resistant to ≥1 antibiotic and 13% multidrug resistant). Intestinal pathotypes were identified in 10% of the *E. coli,*but prevalence varied among sources. The phylogenetic tree indicated high diversity, however the majority of isolates clustered with representatives from phylogenetic groups B1 (58%) and A (30%). A total of 36 sequence types (STs) were identified, but no ST was significantly prevalent in a particular reservoir. Resistance gene profiles among the isolates were also diverse with 35 genes/gene variants identified.

Conclusions

Overall these findings indicate that household soils in rural Bangladesh are reservoirs of antibiotic-resistant and pathogenic *E. coli,* and also showed that *E. coli*circulating in domestic settings are remarkably diverse at the core and accessory genome.

Session 22 – Environmental microgiology / S-82*

Changing the ligand-binding specificity of Escherichia coli periplasmic binding protein RbsB by rational design and screening

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Background
Bioreporter bacteria have proven to be a powerful and cheap tool for analytics of specific target molecules or conditions. The design of bioreporter bacteria was mainly exploited for natural compounds, but it has been proposed that computational prediction of substrate binding interactions in a well-known receptor protein could lead to design of mutant proteins with novel target recognition specificities for non-natural compounds.
Methods
Here a mutant library of the ribose binding protein of *Escherichia coli*(RbsB) was produced based on Rosetta binding pocket simulations, with the goal to find mutants that could bind ribose-analogues such as 1,3–cyclohexanediol and cyclohexanol.
Results
Screening of the library by a microcolony-in-bead fluorescence-assisted cell sorting procedure, resulted in six mutants with 1.2–1.5 times induction in presence of 1 mM 1,3–cyclohexanediol. These mutants completely lost the capacity to react to ribose. Purified RbsB mutants bound 1,3–cyclohexanediol, but not ribose, in isothermal microcalorimetry confirming the observed reporter induction. Purification and quantification of periplasmicspace abundance suggested most mutants to be more prone to protein misfolding compared to wild-type.
Conclusions
Although bioinformatics can thus help to predict mutations potentially yielding new ligand-binding pockets, secondary effects such as protein folding and stability are poorly predicted and may hamper successful mutant recovery. Future work will focus on improving mutant protein stability, which may eventually enable molecular binding mechanism predictions for non-natural compounds, as well as avoid secondary effects.

**Student paper*

Session 23 – Microbial pathology and Cell microbiology / S-83 + P-77

Visualization of sterols with a bacterial toxin bio-sensor reveals a novel sterol internalization pathway

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Background
Sterols are crucial components of biological membranes that help maintain membrane integrity and regulate various cellular processes. Although extremely important for the cell, the sterol molecules are difficult to visualize. Therefore, despite extensive studies focusing on membranes and its lipid components, the exact distribution of sterols have remained unclear.
Methods
Here we apply a genetically encoded sterol biosensor (D4H) based on the *Clostridium perfringens* theta toxin, to visualize sterols in the fission yeast *Schizosaccharomyces pombe*.
Results
Purified mCherry labelled D4H binds to giant liposomes (GUVs) containing cholesterol, confirming *in vitro* its functionality.*In vivo* D4H is detected at the plasma membrane (PM). External staining of the plasma membrane with the sterol-specific antibiotic filipin suggests a distinct organization of sterol-containing domains between the PM inner and outer leaflet. Unexpectedly, actin depolymerization leads to reversible relocalization of the probe towards the cell interior, underlying a possible role of actin in proper distribution of plasma membrane sterols. The intracellular D4H mCherry signal corresponds to a new kind of vesicles (**SRV** ***S****terol* ***R****ich* ***V****esicles*) whose formation is distinct from canonical endocytosis and instead depends on Stm2, a protein that localizes at ER-PM contact sites. Generation of D4H vesicles is also observed during the sporulation process, suggesting a physiological role of SRV in the redistribution of sterols to the newly forming spore membrane.
Conclusions
In summary, live-observation of intracellular sterols with the D4H sensor reveals a novel trafficking process of sterol-rich membrane.

Session 23 – Microbial pathology and Cell microbiology / S-84*

Hsp90 Controls Caspofungin Paradoxical Effect of Aspergillus fumigatus via the Mitochondrial Respiratory Chain

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Background

Aspergillus fumigatus is an opportunistic pathogen mold responsible of invasive aspergillosis in immunosuppressed patients. While voriconazole is the first-line treatment, echinocandins (e.g. caspofungin) could be used as second line treatment but has limited action, and a paradoxical effect (PE) (i.e. loss of efficacy at higher concentrations) is observed.

The heat shock protein 90 (Hsp90) is involved in caspofungin PE, and we tried to elucidate Hsp90-dependant mechanisms of the caspofungin PE.

Methods

To identify genes under the control of Hsp90 in response to caspofungin, we performed RNAseq in the wildtype strain (Ku80) and in the Hsp90 mutant repression strain (pthiA-Hsp90, with PE abolished) exposed or not to caspofungin.

We assessed the effect of mitochondrial respiratory chain (MRC) on the caspofungin PE using MRC inhibitors. The activity of mitochondria was assessed by fluorescent microscopy (Mitotracker Red CM-H2XRos, Thermofisher) and ATP production was measured in mycelial lysates.

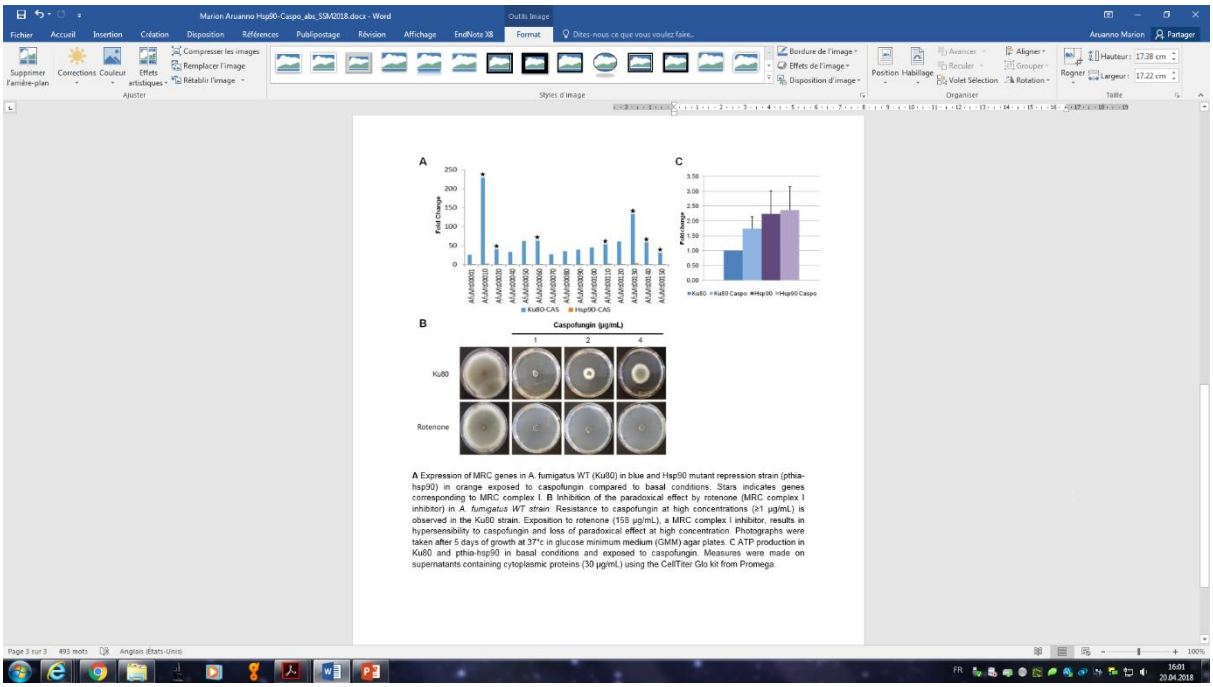
Results

Most genes involved in caspofungin stress response were able to increase in pthiA-Hsp90 similar to Ku80 except for the genes of the mitochondrial respiratory chain (MRC), in particular complex I. Caspofungin PE could be abolished with an MRC complex I inhibitor (rotenone), but not with others MRC inhibitors, supporting the role of MRC complex I. Caspofungin induced a significant increase of fluorescence and ATP production in Ku80 but no significant increase in pthiA-Hsp90 was observed under caspofungin exposure.

Conclusions

These results suggest a role of the mitochondrial respiratory chain and ATP generation in the caspofungin PE , which is under the control of Hsp90 at the transcription level.

**Student paper*



A Expression of MRC genes in A. fumigatus WT (Ku80) in blue and Hsp90 mutant repression strain (pthia-hsp90) in orange exposed to caspofungin compared to basal conditions. Stars indicates genes corresponding to MRC complex I. B Inhibition of the paradoxical effect by rotenone (MRC complex I inhibitor) in *A. fumigatus WT strain*. Resistance to caspofungin at high concentrations (≥1 µg/mL) is observed in the Ku80 strain. Exposition to rotenone (150 µg/mL), a MRC complex I inhibitor, results in hypersensibility to caspofungin and loss of paradoxical effect at high concentration. Photographs were taken after 5 days of growth at 37°c in glucose minimum medium (GMM) agar plates. C ATP production in Ku80 and pthia-hsp90 in basal conditions and exposed to caspofungin. Measures were made on supernatants containing cytoplasmic proteins (30 µg/mL) using the CellTiter Glo kit from Promega.

Attachment #225

Session 24 – Bacterial genomics / S-85

A comparative genomics database of the phylum Chlamydiae

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Background

All known *Chlamydiae* are obligate intracellular bacteria exhibiting a biphasic life cycle. They are difficult to cultivate and genetic manipulations are impossible for most of them, which drastically slows down the understanding of their fascinating biology. While an increasing number of genomes of new chlamydial species, genus, and families are described, no central resource allows to easily investigate their similarities and differences.

The aim of this project was to build a database enabling comparisons of chlamydial genomes and annotation transfers from well-studied genomes that could assist researchers in identifying interesting genes and investigating their evolution.

Methods

The annotation was updated consistently for all publically available genomes using KEGG, interproscan, Uniprot, COG, the DOOR operon database and the Transporter Classification Database (TCDB). Proteins were clustered into orthologous groups with OrthoFinder. Candidate type III secretion system effectors were identified using published algorithms and based on the identification of eukaryotic domains.

Results

A web interface was set up, allowing users to browse the annotation and to identify orthologs in *Chlamydiae* genomes. Genome-scale comparative analyses allow the identification of genome-specific or clade-specific orthologous groups, as well as highly conserved genetic features. Precomputed multiple sequence alignments, phylogenetic reconstructions and BLAST against RefSeq and UniProt databases help users to infer function of uncharacterised proteins. All entries are cross-referenced with major public databases.

Conclusions

The *Chlamydiae* database provides a high-quality resource for the chlamydia research community, facilitating their access to up-to-date and comprehensive genomic and proteomic data.

Session 24 – Bacterial genomics / S-86

Building an open source bioinformatic pipeline for routine high throughput sequencing in a microbiology diagnostic lab

Dr. Sacha Laurent [1], Dr. Trestan Pillonel [2], Mr. Florian Tagini [2], Mr. Valentin Scherz [3], Ms. Helena Gil Campesino [4], Dr. Fathiah Zakham [3], Dr. Onya Opota [3], Dr. Katia Jaton [3], Prof. Gilbert Greub [3], Dr. Claire Bertelli [3]

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Background

High throughput sequencing techniques are widely available in most laboratories and enabled countless breakthroughs in fundamental and applied microbial research. However, the implementation of routine genomics and metagenomics analyses to complement classical and molecular microbiology diagnostics is still in its infancy. Procedures for bioinformatics analyses need to be specifically developed to address the needs for laboratory accreditation.

Methods

We have chosen to develop our bioinformatics procedures using fully open-source principles to favour scrutiny and collaboration across labs, and using community standard tools to ensure reproducibility, scalability, deployability, modularity and portability. Complete code is publicly available at https://github.com/metagenlab/diag_pipelines/ with links to documentation and Docker images provided.

Results

We will show how the development of standardized bioinformatic procedures enabled to prepare our genomic services for accreditation and the added values for the everyday analyses alongside microbiological and molecular facilities. We will present examples of routine analyses regarding epidemiological investigations for hospital outbreaks, antimicrobial resistance in *Klebsiella pneumoniae* isolates, and virulence factor identification for *Staphylococcus aureus*. Moreover, we will show how automatic screening of public genomic databases using our tool helped us gain insights into mechanisms conferring antimicrobial resistance and devise new diagnostic approaches.

Conclusions

We have developed a reproducible bioinformatic pipeline to fit the needs of our microbial diagnostic lab. Due to its openness and modularity, any bioinformatician can build upon our work to develop its own set of analyses. Extension of the pipeline will be also carried in our lab, to accommodate for future applications, such as pathogen detection, or new sequencing techniques.

Session 24 – Bacterial genomics / S-87*

Challenges in using whole genome sequencing for Pseudomonas aeruginosa investigation

Ms. Bárbara Magalhães [1], Dr. Benoit Valot [2], Dr. Mohamed Abdelbary [1], Dr. Guy Prod'hom [3], Prof. Gilbert Greub [3], Dr. Laurence Senn [1], Dr. Dominique Blanc [1]

1. *Service of hospital preventive medicine, Lausanne University Hospital, Lausanne,* ***2.*** *Chrono-environment, Franche-Comté University, Besançon, France,* ***3.*** *Institue of Microbiology, Lausanne University Hospital, Lausanne*

Background

An increased *P. aeruginosa* incidence was observed in the CHUV. Molecular typing revealed three major clusters belonging to sequence types (ST) 1076, 17, and 253. ST1076 isolates were part of an outbreak in the burn unit, whereas the remaining STs showed sporadic occurrence throughout the remaining ICUs. Whole genome sequencing (WGS) was used to further investigate these three clusters.

Methods

Clinical and environmental isolates from all STs were sequenced using Illumina HiSeq. Two analyses approaches were applied: 1) a core genome SNPs alignment was obtained with Snippy, followed by Gubbins, and subsequently used for maximum likelihood tree construction; 2) a homemade filtering pipeline was used to acquire a SNPs alignment more stringent in quality. Isolates phylogeny was constructed through Bayesian inference.

Results

Default quality parameters lead to a high number of SNPs between ST1076 isolates. The resulting tree topology did not corroborate with the assumption that this ST was responsible for an outbreak. A subclade of burn unit ST17 isolates for which epidemiological links were previously suspected similarly demonstrated a high number of SNPs. When applying more stringent quality thresholds, the number of SNPs decreased for both STs and tree topologies were closer to epidemiological data. ST253 isolates SNPs number and tree topology was coherent in both approaches.

Conclusions

Additional quality filtering of the obtained SNPs alignment helped to eliminate possible false SNPs and consequently confirm previously suspected epidemiological links. However, future adaptation of the chosen thresholds to the different *P. aeruginosa* datasets would culminate in more reliable results.

**Student paper*

Session 24 – Bacterial genomics / S-88

Air-conditioner cooling towers as complex reservoirs and continuous source of Legionella pneumophila infection evidenced by genomic analysis

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Background

Water supply and air-conditioner cooling towers (ACCT) are known potential sources of *Legionella pneumophila*. Traditional typing methods have low resolution and may not allow reliable identification of transmissions. The advent of whole genome sequencing (WGS) allows high-resolution analysis, and the study of complexity within environmental compartments.

Methods

In summer 2017, the health administration of the City of Basel detected an increase of *Legionella pneumophila* infections compared to previous months. An epidemiological and WGS-based microbiological investigation was performed, involving isolates from the local water supply and two ACCTs (n=60), clinical outbreak and non-outbreak related isolates from 2017 (n=8) and those collected between 2003-2016 (n=26). Finally, we also compared the sequenced strains to already published bacterial genomes from 17 countries (n=539).

Results

Phylogenetic analysis of the ACCT isolates showed clustering into two groups separated by a few hundred allelic differences. Several strains were found in both ACCTs. Furthermore, we found that isolates from the two ACCTs were highly related to three clinical isolates from 2017. Five clinical isolates from the last decade were also found to be closely related to the recent isolates from ACCTs. Finally, we found several clinical isolates to be related to published genomes.

Conclusions

Current outbreak-related and historic isolates were linked to ACCTs. ACCTs form a complex environmental habitat in which strains are conserved over years and are exchanged between locations. WGS-based typing allows to explore this complex network, which might have public health implications on the tracing of potential sources and the interpretation of environmental findings.

Session 25 – Innovative non mammalian models (3R session) / S-89

Phenotypic heterogeneity of the honey bee gut symbiont Frischella perrara - an important trait for host colonization?

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Background
The gut microbiota of honeybees consists of a relatively simple, but highly specialized bacterial community. The Gammaproteobacterium *Frischella perrara* only colonizes a subset of bees, and induces a specific immune response resulting in localized tissue melanization and antimicrobial peptide production. Fitness costs have not been linked to *F. perrara*, neither has any molecular mechanism been elucidated underlying the specific interaction its host.
Methods
In this study we used: honey bee colonization, RNAseq, single cell reporters and microscopy to analyze molecular mechanisms of *F. perrara*colonization in the honey bee gut.
Results
F. perrara exhibits phenotypic heterogeneity when cultured *in vitro,*forming two distinct colony morphotypes: yellow and white. While white colonies typically switch back to yellow after restreaking, we managed to isolate a colony that is locked in its white state: *F. perrara white.*Strikingly, *F. perrara white*had significantly lower gut colonization success and did not induce the melanization anymore. In addition, *F. perrara* wildtype switched towards the yellow phenotype over time during colonization. Interestingly, genes known to be involved in hostmicrobe interactions were upregulated in *F. perrara yellow* compared to *white*, including Pilus-formation and Type VI secretion genes.
Conclusions
Differential expression of these genes may be needed either for specific stages during colonization or to cope with changes in the microenvironment faced by the bacterium within the host. We hypothesize that phenotypic heterogeneity might play an important role for *F. perrara* to colonize the honey bee. Future experiments will focus on the relevance of both phenotypes *in vivo* in more detail.

Session 25 – Innovative non mammalian models (3R session) / S-90*

Bacterial coexistence within species in the honey bee gut facilitated by nutritional niche differentiation

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Background

In this study, the gut microbiota of the honey bee (*Apis mellifera*) is used as a model to study natural microbial interactions. The bacteria found in this community exclusively colonise this environment, suggesting that they have co-evolved together and with their host. The most abundant member of this community belongs to the *Lactobacillus*genus. One phylotype (>97% 16S *rRNA* identity) of *Lactobacilli* includes four different sub-lineages that harbour high levels of gene content variability related to metabolic functions. Remarkably, metagenomic sequencing has revealed that these sub-lineages coexist within individual bees. We hypothesise that this coexistence is supported by ecological niche differentiation, i.e. based on the utilisation of different compounds present in the honey bee diet, which consists predominantly of pollen grains.

Methods

To test our hypothesis, we have performed untargeted *in vitro* metabolomic profiling on 14 different strains belonging to the four different sub-lineages. Moreover, we have co-cultured strains, with and without supplementation of pollen.

Results

Our preliminary results show that the *in vitro* coexistence of different sub-lineages is achieved when the carbon source in the growth medium is pollen-derived, but not when it is a simple sugar. Interestingly, our *in vitro* metabolomics experiments results suggest that even strains of the same sub-lineage present distinct metabolic
profiles.

Conclusions

Future experiments, such as random transposon mutagenesis, RNA sequencing and pairwise co-culture experiments, will allow us to determine the role of each bacterium in this context, and whether cross-feeding interactions are occurring.

**Student paper*

Session 25 – Innovative non mammalian models (3R session) / S-91

A root colonizing plant-beneficial pseudomonad persists in herbivorous insects throughout different developmental stages and can be dispersed to new host plants

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Background

Fluorescent pseudomonads possess manifold plant-beneficial activities such as plant-growth promotion and disease suppression. They are commonly found in soil, and the plant rhizo- and phyllosphere. Our discovery that the two species *Pseudomonas protegens* and *P. chlororaphis*can additionally colonize and even kill lepidopteran insect larvae after oral uptake raises new questions about the ecological significance of *Pseudomonas*-insect interactions and the potential use of these bacteria for biological insect control.

Methods

Arthropods collected from different soils were screened for *P. protegens/P. chlororaphis* based on isolation/house-keeping gene sequencing. Selected *P. protegens/chlororaphis* strains were tested for oral activity against larvae of different insect species causing plant pests by feeding larvae with bacteria-treated diet or roots. *Pseudomonas*persistence was monitored throughout the insect lifecycle.

Results

P. chlororaphis and *P. protegens* were frequently detected in soil arthropods. Oral uptake of pseudomonads caused high mortality in larvae of different lepidopteran leaf-feeders whereas certain root feeding insects were much less affected and mostly survived. Interestingly, in highly and low pathogenic interactions pseudomonads persisted in insects until the pupae, sometimes until the adult stage. We could further show that adult cabbage root flies developed from larvae feeding on roots colonized by *P. protegens* CHA0 can transfer the bacteria to new host plants.

Conclusions

Our results indicate that *P. chlororaphis* and *P. protegens* are indeed not only commonly associated with plants but also with arthropods. Their interaction with insects can be of pathogenic or rather commensal nature depending on the insect species and they even might use insects as vectors for dispersal.

Session 25 – Innovative non mammalian models (3R session) / S-92*

Predatory behavior of Vibrio cholerae towards bacteria and amoebae

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Background

Vibrio cholerae, the causative agent of cholera, is commonly found in aquatic environments, where it copes with environmental stresses like interbacterial competition and amoebal grazing. Environmental defense strategies can potentially contribute to the emergence of pathogenic strains, as they might be beneficial in a disease context. Therefore, better understanding the predatory differences between pathogenic and non-pathogenic isolates is of prime importance.

Methods

We compared the predatory behaviour of *V. cholerae*pandemic strains to pathogenic non-pandemic strains and environmental isolates. Specifically, we assessed the involvement of the type VI secretion system (T6SS) and accessory toxins in competition with other bacteria and as defense mechanisms against the amoeba*Dictyostelium discoideum*.

Results

Contrary to pandemic strains, non-pandemic pathogenic strains and environmental isolates of *V. cholerae* constitutively fire their T6SS, which allows them to efficiently eliminate bacterial competitors. These strains also show a strong defense potential against *D. discoideum*, but not all of them do so in a T6SS-dependent manner. Interestingly, these strains also constitutively produce hemolysin, although we did not observe a decreased virulence against *D. discoideum* when the pathogen lacked this toxin.

Conclusions

Our results depict a clear dinstinction in the predatory behavior of different strains of *V. cholerae*. Pandemic strains display a rather “low-profile”, which might provide a fitness advantage under transmissing conditions. Conversely, non-pandemic and environmental isolates show constitutive expression of several molecular weapons, some of which we have not yet identified. These abundant defense mechanisms might allow such strains to resist multiple selective pressures that are imposed on them in the environment.

**Student paper*

POSTERS

Clinical microbiolgy / P-01*

Inhibition of Multi-Drug Resistant Bacteria by the Fruticose Lichen Usnea Collected from Mt. Amuyao, Philippines

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Background

The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics. Hence, there is a need to search for new antibiotics from various natural sources that are either novel or express a new mechanism of action. The lichen *Usnea*, with its wide range of unique, biologically potent secondary metabolites, may provide the answer.

Methods

In this study, *Usnea*species were collected in Northern Philippines, identified through combined morphological and biochemical characterization, and tested for antibacterial activities against the multi-drug resistant ESKAPE pathogens, i.e. ***E****nterococcus faecalis,* ***S****taphylococcus aureus,* ***K****lebsiella pneumoniae,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa,*and***E****nterobacter cloacae,*and two standard test bacteria.

Results

Forty-six lichen specimens were identified as *Usnea baileyi*(10), *U. hirta*(12), *U. longissima*(4), *U. rubicunda*(10), and *U. subrubicunda*(10). Results showed that the lichen crude extracts of the *Usnea*species exhibited promising *in vitro*inhibitory activities against Gram-positive bacteria (*E. faecalis*ATCC 29212) and Gram-positive multi-drug resistant bacteria (Methicillin resistant *S. aureus*and *E. faecalis*). However, weaker inhibitory activities were observed against *S. aureus*ATCC 25923. In addition, lichen compounds in each species were identified using Thin Layer Chromatography (TLC). Subsequently, the potential bioactive metabolites included alectronic acid, connorstictic acid, consalazinic acid, diffractaic acid, echinocarpic acid, erythrin acid, galbinic acid, hypoconstictic acid, hyposalazinic acid, lobaric acid, manegazziac acid, micareic acid, pannarin, salazinic acid, stictic acid, and usnic acid. Seven bioactive lichen acids could not be identified.

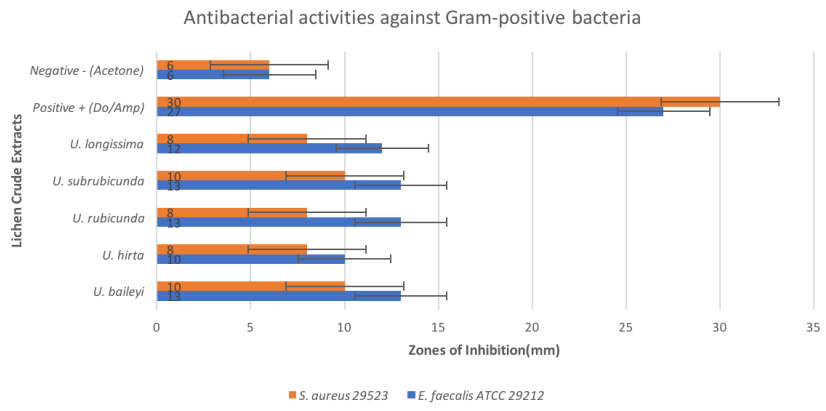
Conclusions

Our study showed the wide spectrum of opportunities for lichens for the discovery of potential antimicrobial agents.

**Student paper*

Lichen Metabolite	*Solvent Systems		
	A	C	G
Alectronic acid	+	-	-
Argopsin acid	+	-	+
Connorstictic acid	-	-	+
Consalazinic acid	+	-	+
Diffractaic acid	-	-	+
Echinocarpic acid	+	-	+
Erythrin acid	+	-	-
Fumarprotocetraric acid	+	-	-
Galbinic acid	+	+	-
Haematoventosin acid	+	-	-
Hypoconstictic acid	+	-	+
Hyposalazinic acid	+	-	+
Hypoprotocetraric acid	+	-	-
Hypothamnolic acid	-	-	+
Lobaric acid	+	-	-
Manegazziac acid	+	+	+
Micareic acid	-	+	+
Pannarin	+	+	+
Pulvinic Dilactone	+	-	-
Salazinic acid	+	+	+
Sekikaic acid	-	-	+
Stictic acid	+	-	+
Unknown PCr3	+	-	+
Usnic acid	+	+	+
TOTAL:	19	6	16

Usnea species	Test bacteria zone of inhibition (mm) at 10 mg/mL crude extract					
	Enterococcus faecalis	*MRSA*	*Kleibsiella pneumoniae*	*Acinetobacter baumanii*	*Pseudomonas aeruginosa*	*Enterobacter cloacae*
U. baileyi	10±0.23	10±0.58	0	0	0	0
U. hirta	9±1.73	9±1.15	0	0	0	0
U. longissima	9±1.37	9±1.33	0	0	0	0
U. rubicunda	13±1.82	13±0.29	0	0	0	0
U. subrubicuda	14±0.98	14±0.58	0	0	0	0
Positive Control[a]	23±0.46	28±0	0	0	0	0
Negative Control[b]	0±0	0±0	0	0	0	0



Attachments #14

Clinical microbiolgy / P-02*

Significant variation in the performances of three successive generations of a rapid test for MRSA screening

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Background
Screening to detect MRSA carriers is one of the major infection control measures in hospitals. Culture for MRSA screening have the disadvantage of a long TAT, while PCR-based reduce this time to few hours. We determined the performances of three successive generations of the Xpert® MRSA test.
Methods
From 2014 to 2017, three generations of the Xpert® MRSA test were successively used in our hospital in parallel to culture (enrichment broth and chromogenic agar). Performances of the three generations were compared to culture. In case of discordance, additional analyses were prospectively performed.
Results
A total of 9531 samples were analysed and the prevalence was 3.7%. The periods of use, the number of samples, the sensitivity, specificity, positive and negative predictive values are all shown in the figure.
The specificity and the PPV were significantly lower in the Gen3 version. Sensitivity improved, but nonsignificantly,
between the G3 and the NxG versions.
Among the 514 positive samples by PCR, 164 (32%) were culture-negative. Among these, 63 were false positive (presence of MSSA with SSC*mec*-like) and 17 were true positive as an MRSA was found by further analysis of the sample. A significant reduction of discordance was observed in the last NxG generation of the test.
Conclusions
Significant differences in performance were observed between the different versions of the Xpert® MRSA test. This was unexpected and shows the importance to evaluate new versions of commercial test. Fortunately, the worst version was used only for a year and was replaced by a version showing the best performances.

**Student paper*

Xpert® MRSA	G3 n=3512 04.2014-03.2015	Gen3 n=2794 03.2015-02.2016	NxG n=3288 06.2016-05.2017
Sensitivity	71.4 (64.0-77.9)	82.3 (74.4-88.2)	84.3 (77.0-89.7)
Specificity	98.4 (97.9-98.8)	96.8 (96.0-97.4)	99.1 (98.7-99.4)
PPV	70.6 (63.2-77.1)	55.7 (48.4-62.8)	81.4 (73.9-87.2)
NPV	98.5 (98.0-98.9)	99.1 (98.6-99.4)	99.3 (98.9-99.5)

Numbers are % (95%IC)

Clinical microbiolgy / P-03

Seminal microbiota and male infertility

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Background
Bacteriospermia was previously considered to be negatively associated with fertility. However, recent studies indicate that presence of bacteria in semen is rather frequent, including in fertile individuals with normal sperm parameters. Consequently, as for other sites of the body, semen has a specific microbiota, which is scarcely studied. We describe here the bacterial composition of semen of 96 patients from couples with fertility issues and its association with male infertility.
Methods
After DNA extraction from semen samples, total 16S rRNA copies were quantified with qPCR and sequenced using the Illumina platform. The QIIME software package was used to obtain operational taxonomic units at the genus level. Conventional bioinformatics methods were then used to analyse seminal microbiota.
Results
The majority of samples had a concentration of 10^4-10^5 bacteria per ml of semen. We were able to classify all samples within 3 major groups based on the microbiota: *Prevotella*-enriched group, *Lactobacillus*enriched group and *Corynebacterium*-enriched group. We did not observe specific association between abnormal spermiogram and the three groups. Using, LEfSe analysis we observed that presence of *Lactobacillus* had a positive impact on spermatozoa morphology, while presence of Prevotella had a negative impact on spermatozoa motility.
Conclusions
We described the seminal microbiota of 96 patients from couples with fertility issues and found three major profiles. Even if none of the three profiles was specifically associated with patients presenting semen deficiencies, specific bacterial genera showed a positive or negative impact on sperm parameters.

Clinical microbiolgy / P-04*

ISEcp1-mediated in-vitro transposition of CTX-M-14, CTX-M-15, and CTX-M-19 encoding genes

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Background

The clonal linage of *Escherichia coli*sequence type 131 (ST131) combining resistance to fluoroquinolones and producing extended-spectrum β-lactamases is endemic worldwide. Different clades of *E. coli*ST131 have been identified, in which sub-clade C2 (*fim*H30Rx) is associated with CTX-M-15. This gene is associated to ISEcp1 insertion sequence responsible for its mobility. Our aim is to decipher the dynamics of transmission of the *bla*CTX-M-15 gene in*E.coli*ST131.

Methods

An in-vitro model of transposition was elaborated using the IS*Ecp1-bla*CTX-M-15 tandem recovered from *E. coli*ST131 *fim*H30Rx as template. In addition, the IS*Ecp1B-bla*CTX-M-14 and IS*Ecp1B-bla*CTX-M-19 were also studied by cloning experiments. Transposition assays were performed under different conditions: sub-inhibitory antibiotic concentrations, temperature changes and decreased iron concentrations. RT-qPCR was performed to measure the expression of *bla*CTX-M-15, transposase (*tnpA*), and plasmid replication protein (*repA*) to determine the molecular bases of increasing rates of transposition.

Results

Transposition assays showed an increase of the transposition frequency using sub-inhibitory concentrations of cefotaxime (256 µg/ml) for (IS*Ecp1-bla*CTX-M-15, IS*Ecp1B-bla*CTX-M-14 and IS*Ecp1B-bla*CTX-M-19), ceftazidime (16 µg/ml) (IS*Ecp1B-bla*CTX-M-14 and IS*Ecp1B-bla*CTX-M-19) and ciprofloxacin (0.002 µg/ml) (IS*Ecp1B-bla*CTX-M-14). RT-qPCR results with IS*Ecp1-bla*CTX-M-15 structure showed an overexpression of *bla*CTX-M-15, *tnpA* and *repA* gene in conditions for which increased transposition rates were observed. No change in transposition frequencies were observed upon temperature changes, or iron depletion.

Conclusions

The IS*Ecp1*-related transposition frequency of *bla*CTX-M genes increased under sub-inhibitory concentrations of ceftazidime, cefotaxime and ciprofloxacin. However, this change in the transposition frequency might also be associated with an increase of the plasmid copy number, currently under evaluation.

**Student paper*

Clinical microbiolgy / P-05

A novel concept for veal production: the “outdoor veal calf” – “Freiluftkalb” (a NFP72 project)

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Background
Antimicrobials are widely used in veal calf production in Switzerland. Aims of the “outdoor calf” study are i) to reduce morbidity and antimicrobial use and ii) to describe the resistance patterns in function of antimicrobial treatments through implementation of a novel concept of veal calf management and housing.
Methods
The “outdoor calf” study includes 2 groups of 19 farms each: the intervention group implementing the new management and housing strategy and the comparison group (traditional veal fattening operations). Nasopharyngeal and rectal swabs are taken from each calf at the beginning and at the end of the fattening period (n=6600). Minimum Inhibitory Concentrations (MIC) to commonly used antimicrobials and are determined. Antimicrobial treatments are recorded prospectively for comparison with the resistance patterns observed in bacterial isolates. Disease occurrence is registered prospectively, and calf health and welfare are further assessed by a slaughterhouse survey of lung lesions (pneumonia) and abomasal ulcers (stress).
Results
Preliminary results suggest that the “outdoor calf” system allows for reduced antimicrobial use without impact on calf health (less lung lesions, less abomasal ulcers). A total of 5028 bacterial isolates (Pasteurellacae and *E. coli*) have been isolated to date from nasopharyngeal and rectal swabs (n=6600); MIC determination is ongoing.
Conclusions
Preliminary results of the “outdoor calf” project suggest that the novel management system allows for improving calf health and welfare, and reducing antimicrobial use. The impact on bacterial resistances will be determined after the end of the field phase in summer 2018.
Financed by Grant no. 407240_167083 from the SNSF within NRP72.

Clinical microbiolgy / P-06

Resistance to antibiotics and quaternary ammonium compounds of Escherichia coli from calves at the beginning of the fattening period in Switzerland

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Background

In the Swiss rearing calf production, antibiotics and quaternary ammonium compound (QAC) containing disinfectants are frequently used to control bacterial infectious diseases posing the risk of selecting for a resistant bacterial population. While prevalence of antibiotic resistance is known for *Escherichia coli* from calves at the slaughterhouse, the situation in young calves entering the fattening period is unknown.

Methods

Susceptibility to antibiotics and the QAC alkyldimethylbenzylammonium chloride (ADBAC) was determined for 100 *E. coli* from calves entering fattening farms by microbroth dilution. Resistance genes were detected by microarray, and PCR/sequencing. Genetic diversity was determined using *rep*-PCR.

Results

Thirty-six percent of the isolates were susceptible to the antimicrobials tested and 48% were susceptible to ADBAC. Isolates exhibited resistance to tetracycline (59%) (*tet*(A),(B),(E),(G)), to sulfonamides (63%) (*sul1*, *sul2*, *sul3*), ampicillin (58%) (*bla*TEM-1),trimethoprim (34%) (*dfrA*), gentamicin (29%) (*ant(2")-Ia*, *aac(3)-VIa*, *aac(3)-IVa*, *aac(3)-IIc*), streptomycin (46%) (*strA*, *strB*, *aadA*) and ceftazidime (1%) (*bla*CTX-M-15(ESBL)). Mutations in GyrA (S83L) and ParC (S80I) were found in fluoroquinolone-resistant isolates (8%). All isolates were susceptible to colistin, tigecycline and meropenem. No association between decreased susceptibility to ADBAC and presence of *qac* genes was observed. *rep*-PCR revealed a genetically diverse *E. coli* population.

Conclusions

Antibiotic- and QAC-resistant *E. coli* are present in the gut of young calves at the beginning of the fattening period, emphasizing the need for appropriate and reduced use of antibiotics and QAC-containing disinfectants in order to limit further selection and maintenance of these bacteria during the fattening period.

Financed by Grant no. 407240_167083 from the SNSF within NRP72.

Clinical microbiolgy / P-07

Evaluation of two IgM immunoassays for the diagnosis of early human leptospirosis

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1. *ADmed Microbiology,* ***2.*** *IPK*

Background
Diagnosis of leptospirosis frequently relies on specific and rapid detection of IgM, we propose to compare the performance of two kits available in Europe.
Methods
Sera from swiss and cuban patients are categorized to challenge sensitivity, specificity and correlation between the two EIA. Group I are confirmed cases, PCR positive or MAT titer >160; group II includes patients with clinical suspicion and group III patients positive in IgM for another etiologic agent (*Toxoplasma gondii*, CMV, HSV, EBV, rubella virus, malaria, dengue, …The kits tested are Panbio Leptospira IgM (Panbio) and Virion/Serion Leptospira IgM (VS)
Results
Hundred and eighty sera from 173 patients are tested. Sensitivity (n=40) of Panbio and VS is 67.5% and 50%. Specificity (N=37) is very similar for both tests with 86.5% and 83.8% respectively. In group II (n=103) reactive results are 2 times higher with Panbio, 28.1 % than VS, 14.6 % ; this indefinite group cannot be used to attest higher sensitivity or specificity, but only to test the correlation between their results.
Agreement is acceptable for specificity (83.8%) but low for sensitivity (72.5 %) and intermediate for group II (78.6%). Global correlation is moderate considering the kappa test of 0.531 (95%: 0.416 - 0.646).
Serology offers a simple and rapid diagnostic of leptospirosis. The uncertainty of the results appeals to the use of a more specific and sensitive molecular method to assess active infection.
Conclusions
These IgM assays do not correlate well and underline the need for a more liable test to rapidly confirm a leptospirosis diagnostic

Clinical microbiolgy / P-08

Performance and diagnostic accuracy of Accelerate Pheno System on clinical blood coltures in diagnosis of bacteremia

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Background
The Accelerate Pheno system (AXDX) is a fully automated test system capable of performing identification (ID) and antimicrobial susceptibility testing (AST) directly from positive blood cultures within an average of 7 hours. We have performed a pilot study on patients with bacteriemia or sepsis, in order to evaluate the performance of AXDX in comparison to routine diagnostic techniques.
Methods
During a 3-months period, 47 positive BCs corresponding to unique episodes of BSI were analyzed both by AXDX and standard laboratory testing (MALDI-TOF for ID, Phoenix BD and Kirby- Bauer for AST). Comparisons between methods were expressed as agreement (EA-CA), very major error (VME -false susceptibility), major error (ME-false resistance), or minor error (Mie-intermediate versus susceptible or resistant).
Results
An overall of 41 BCs were evaluated in the study: 22 Gram negative, 14 Gram positive and 3 off-panel microorganisms (*Salmonella napoli, Enterococcus gallinarum, Stenotrophomonas maltophilia*). 6 samples were excluded: 3 technical failures, 2 ID negative reports (*Asaia bogorensis* and *Lactobacillus sakei*), 1 ID partial report in a polimicrobic BC. The Pheno panel coverage was 87.8%. Concerning AST testing, a total of 270 microorganismantimicrobial combinations were analyzed. EA was 94.1%, CA 92,8%. Discrepancies were linked to VME (5.9%), ME (3.7%) and Mie (3.4%). The average time for AXDX to provide ID/AST for the 41 samples was h 6:21 (x±DS = 6:27 ± 0,7).
Conclusions
Accelerate Pheno System provides reliable results in a timely manner, detecting resistance phenotype of microorganisms responsible of BSI. Cost analysis and impact on antibiotic use and clinical outcomes need to be assessed.

Clinical microbiolgy / P-09*

Comparison of the phenotypic carbapenemase confirmation test and the multiplex lateral flow immunoassay NG-Test CARBA5

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Background
The alarming rise of carbapenemase-resistant-Enterobacteriaceae (CRE) represents a major public health problem. Rapid and accurate detection of carbapenemase is pivotal both for the choice of the best therapy and infection control. Here we compared the performances of a disk-diffusion-based phenotypic test and the recentlymarketed multiplex lateral flow immunoassay CARBA5.
Methods
Forty-five bacterial isolates derived from different clinical samples delivered to the IMM, University of Zürich between 2011-2018 with positive phenotypic carbapenemase screening results(Maurer et al., JCM, 2015) were subjected to the phenotypic carbapenemase confirmation test (Bloemberg et al. JAC, 2017), the CARBA5 test and PCR.
Results
Overall, the phenotypic confirmation test correctly identified 35/45 carbapenemase types and misclassified three of them (one OXA-48 as Class A, one KPC as Class B and one VIM as Class D). Moreover, the test failed to detect class A-type carbapenemases in three CPE and falsely reported the presence of carbapenemases (three class D and one class A) in four ESBL producers. Finally, two non carbapenemase-producers isolates were correctly
classified.
By constrast, the CARBA5 test correctly classified all the 45 carbapenemases and did not detect them in the seven non carbapenemase-producers.
Conclusions
The CARBA5 test exhibited a better performance than that of the disk-diffusion-based carbapenemase confirmation test and proved to be a reliable tool for rapid and accurate detection of CPE. Implementation of the CARBA5 in routine diagnostics will considerably reduce the time and costs for CPE detection. At this moment further testing is needed.

**Student paper*

Clinical microbiolgy / P-10*

An unusual colistin-resistant mcr-negative Escherichia coli strain

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Background

The colistin-MAC test is detecting very accurately *mcr*-mediated colistin resistance in *E. coli*. We recently found one colistin-resistant *E. coli* (LC-826/16) positive for this assay, but surprisingly negative for all *mcr* genes. Therefore, we studied this isolate.

Methods

LC-826/16 underwent whole genome sequencing (WGS) using both Nanopore MinION and Illumina MiSeq. Blind cloning was performed by cutting DNA in ~5kb fragments, ligating them in a pCR-Blunt vector, transforming it into colistin-susceptible *E. coli*, and plating on selective agar supplemented with colistin (2 mg/L). Colistin MICs were obtained in microdilution for all resistant colonies. Plasmids were extracted from the colistin-resistant clones and inserts underwent Sanger sequencing. Sequences were mapped to the WGS to identify the region conferring colistin resistance.

Results

Based on WGS, LC826/16 did not carry any *mcr*-like gene. In contrast, we obtained several colistin-resistant clones (all with MICs>8 mg/L) carrying vectors with ~5kb-inserts, all harboring *pmrC* and*pmrA genes*(encoding a lipid A phosphoethanolamine transferase and a regulatory protein for PmrC, respectively). No mutations were found in *pmrC*, whereas *pmrA* had two not previously reported mutations,leading to two amino acid substitutions. Moreover, all clones showed positive results with the colistin-MAC test.

Conclusions

The mutated PmrA confers colistin resistance and generates a false-positive colistin-MAC test. However, to further confirm our data, subcloning of the mutated *pmrA* alone is under progress. Moreover, using the same methodological approaches, we will analyze further colistin-resistant strains showing only substitutions in PmrA and/or PmrB. This work was supported by NRP-72 No. 177378 to AE.

**Student paper*

Clinical microbiolgy / P-11

Comparison of three commercial IgG and IgM ELISA kits for the detection of tick-borne encephalitis virus antibodies

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Background
Tick-borne encephalitis is endemic in many parts of Europe and Asia. The diagnosis of this disease is essentially based on the demonstration of specific antibodies. Enzyme-linked immunosorbent assays (ELISAs) are the method of choice for serological diagnosis of TBE.
Methods
We evaluated three commercially available anti-TBEV IgG and IgM ELISAs using 251 serum samples: the SERION ELISA *classic* FSME Virus/TBE Virus IgG and IgM kit (Virion\Serion), the RIDASCREEN® FSME/TBE IgG and IgM kit (R-Biopharm), and the anti-FSME/TBE virus ELISA "Vienna" IgG / anti-FSME/TBE virus ELISA IgM kit (Euroimmun).
Results
Discrepant test results for IgG and / or IgM were observed for 14.7 %
of tested samples. Reference values defined by serum neutralization test (SNT, n = 25) or results provided by EQA organizers (n = 2) were established for a subset of samples. In relation to these values, false-positive results were observed mainly for Euroimmun Vienna IgG and RIDASCREEN IgG, whereas false-negative results were primarily observed for Virion\Serion IgG and RIDASCREEN IgM kits.
Conclusions
We have observed significant differences for both qualitative and quantitative data of the compared ELISAs. Reference values
available for a subset of samples revealed problems in both sensitivity (Virion\Serion IgG, RIDASCREEN IgM) and specificity (Euroimmun Vienna IgG, RIDASCREEN IgG) of the evaluated kits. Whereas sensitivity problems may be overcome by testing a follow-up sample to detect seroconversion or rises in titers, specificity problems are of major relevance. In case of suspected cross-reactivity, false-positive results can be ruled out using SNT.

Clinical microbiolgy / P-12

A diagnostic decision tree to infer aminoglycoside resistance mechanisms in Escherichia coli from resistance phenotypes

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Background
The emergence and rapid expansion of multidrug resistant bacteria has led to a renewed interest in aminoglycosides. However, methods to rapidly and accurately predict resistance mechanisms are still inadequate. In Gram negative bacteria, prediction of aminoglycoside resistance genes relying on antimicrobial susceptibility tests with clinically relevant aminoglycosides, such as gentamicin, tobramycin and amikacin and based on clinical categories determined by EUCAST or CLSI clinical breakpoints (CBPs) is generally unreliable. As a consequence, molecular techniques are often necessary to confirm preliminary phenotypic findings. This study aimed to evaluate a decision tree based on phenotypic susceptibility profiles towards gentamicin, tobramycin, amikacin and kanamycin for the prediction of the corresponding resistance determinants in *Escherichia coli*.
Methods
A total of 488 clinical *E. coli* isolates were analyzed with the herewith presented aminoglycoside resistance algorithm and grouped according to their resistance profiles on the basis of epidemiological cut-offs. To evaluate the algorithm's performance the genomes of all isolates were sequenced and the concordance between phenotype and genotype was analyzed.
Results
Based on the aminoglycoside susceptibility patterns, the strains were separated into seven categories with different predicted resistance mechanisms. Overall, phenotype/genotype agreement was found in 471 isolates (96.5%), while 17 strains exhibited a discrepant genotype.
Conclusions
The presented decision tree based on resistance to gentamicin, tobramycin, amikacin and kanamycin allows a reliable prediction of the aminoglycoside resistance determinants in *E. coli*.

Clinical microbiolgy / P-13

Antibiotic susceptibility patterns of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis strains from different decades

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Background

Oral bacteria may carry resistance genes; on the other hand, they are the target of antibiotic therapy in severe cases of periodontitis. This study aimed to determine the antibiotic susceptibility patterns of 57 *Aggregatibacter actinomycetemcomitans* and 56 *Porphyromonas gingivalis* strains isolated from subgingival biofilm samples from periodontitis patients in Switzerland during the years 1980 to 2017.

Methods

The minimal inhibitory concentrations (MIC) of the most common antibiotics used either in periodontal therapy (amoxicillin, metronidazole, azithromycin, doxycycline) or for severe body infections (amoxicillin/clavulanic acid, clindamycin, ertapenem, moxifloxacin) were determined by using commercial microbroth dilution test and agar dilution technique. Strains were screened for beta-lactamases activity and for the presence of selected resistance genes (*cfxA*, *ermF* and *tetQ*).

Results

Overall, an increase of MIC values over time was not observed. *E.g.*, MIC_{90} of ampicillin were 2 µg/ml against *A. actinomycetemcomitans* and 0.5 µg/ml against *P. gingivalis* and MIC_{90} of doxycycline were 1 µg/ml against both species. Beta-lactamase positive strains and strains containing the *cfxA* gene could not be detected. Two *P. gingivalis* strains (2011, 2012) showed the highest MIC values. The first strain was *ermF* positive and had MIC values of >8 µg/ml, 2 µg/ml and 0.25 µg/ml for clindamycin, azithromycin and moxifloxacin, respectively. The second strain had a high MIC value of 4 µg/ml for moxifloxacin.

Conclusions

The absence of an increasing antibiotic resistance among the tested periodontopathogens over the last 40 years can be attributed to the restricted use of antibiotics against severe periodontitis at the dental university clinics in Basel, Bern and Zurich.

Clinical microbiolgy / P-14*

PAN-1, a metallo-beta-lactamase identified from the environmental bacterium Pseudobacteriovax antillogorgiicola

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Background

In-silico analysis identified a metallo-ß-lactamase (MBL) in *Pseudobacteriovorax antillogorgiicola* RKEM611, sharing 57% amino acid identity with the ß-lactamase SPM-1. *P. antillogorgiicola* is a Gram-negative obligate aerobe belonging to the Pseudobacteriovaraeae family that was isolated from soft coral in the Bahamas. The aim of this work was to characterize the biochemical properties of that MBL that was named PAN-1.

Methods

The gene was identified using Blastp alignement with all the clinical relevant carbapenemase genes. The *bla*PAN-1 gene was amplified by PCR and ligated into pBADb L-arabinose-inducible vector using SacI and XbaI restriction sites.

MIC of β-lactams was performed following the CLSI guidelines and were performed using broth microdilution method.

Enzymatic activity of bacterial extracts from recombinant *E. coli* expressing the PAN-1 enzyme was determined using UV spectrophotometry with different class of ß-lactams.

Results

The *P. antillogorgiicola* isolate was susceptible to most β-lactams. The *bla*PAN-1-encoded protein possesses 57% amino acid identity with the carbapenemase SPM-1. Induction of the expression of *bla*PAN-1 gene in *E. coli* conferred a resistance phenotype consistent with a class B carbapenemase. Expression of the *bla*PAN-1 gene conferred resistance to amino- and carboxy-penicillins, to narrow- and broad-spectrum cephalosporins and carbapenems, sparing aztreonam and ureido-penicillins. Enzymatic activities against ß-lactams confirmed that the PAN-1 enzyme has a hydrolytic activity similar to that of other metallo-ß-lactamases.

Conclusions

The environmental isolate *P. antillogorgiicola* harbors a chromosomal metallo-β-lactamase gene that is not expressed. The PAN-1 enzyme protein shares weak identity with other metallo-ß-lactamses, the most closelyrelated enzyme being the SPM-1 carbapenemase being widespread in Brazil.

**Student paper*

Clinical microbiolgy / P-15

ZHO-1, a metallo-beta-lactamase identified from the marine bacterium Zhongshania aliphaticivorans

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Background
In-silico analysis identified a metallo-ß-lactamase (MBL) in *Zhongshania aliphaticivorans* SM-2T, sharing 58% amino acid identity with the ß-lactamase IMP-1. *Z. aliphaticivorans* is a Gram-negative facultative aerobic bacterium belonging to the Spongiibacteraceae family that was isolated from marine sediment in the Yellow sea. The aim of this work was to characterize the biochemical properties of that MBL that was named ZHO-1.
Methods
The gene was identified using Blastp alignment with all the clinical relevant carbapenemase genes. The *bla*ZHO-1gene was amplified by PCR and ligated into pTOPO plasmid.
MIC of β-lactams was performed following the CLSI guidelines using broth microdilution method or Etest. Enzyme purification was done using an ÄKTA prime purification system using a Q-sepharose column.
Enzymatic activity of bacterial extracts from recombinant *E. coli* expressing the ZHO-1 enzyme was determined using UV spectrophotometry with different class of ß-lactams
Results
The *Z. aliphaticivorans* isolate was susceptible to all β-lactams. The *bla*ZHO-1-encoded protein possesses 58% amino acid identity with the carbapenemase IMP-1. Induction of the expression of *bla*ZHO-1gene in *E. coli* conferred a resistance phenotype consistent with a class B carbapenemase. Expression of the *bla*ZHO-1gene conferred resistance to amino- and carboxy-penicillins, to narrow- and broad-spectrum cephalosporins and carbapenems, sparing aztreonam and ureido-penicillins. Enzymatic activities against ß-lactams confirmed that the ZHO-1 enzyme has a hydrolytic activity similar to that of other metallo-ß-lactamases in particular IMP-1.
Conclusions
The environmental isolate *Z. aliphaticivorans* harbors a chromosomal metallo-β-lactamase gene, *bla*ZHO-1, that once expressed in *E. coli* confers resistance to most ß-lactams including carbapenems.

Clinical microbiolgy / P-16

Genetic characterization of an MCR-3-like producing Escherichia coli recovered from swine, Brazil

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Background

Plasmid-mediated colistin-resistance in Enterobacteriaceae is of great concern since it jeopardizes the efficacy of that last-resort antibiotic. To date, eleven *mcr-3* variants have been reported. Here we report a novel *mcr- 3* variant detected in an *Escherichia coli* isolate recovered from a post-weaning diarrhea of a pig previously treated with colistin, in Brazil.

Methods

Screening of colistin-resistant isolates was performed from 126 different pigs in the state of Minas Gerais in Brazil. Colistin minimal inhibitory concentrations (MICs) were determined using broth microdilution method using cation-adjusted MH broth. The whole genome of the MCR-3-producing isolate was sequenced using Illumina MiniSeq system.

The *mcr-3*-like gene variant was cloned into the arabinose-inducible pBADb. Induction of pBADb vector was performed in MH broth supplemented with 1% of L-arabinose. The lipopolysaccharide (LPS) of the MCR-3 producer was extracted and analyzed by mass spectrometry.

Results

Isolate I112 was positive for *mcr*-3 gene. Sequencing revealed that the corresponding protein shared 97% of amino-acid identity with the original MCR-3 variant identified. Induction of the pBADb-*mcr-3-like* plasmid conferred an MIC of colistin at 4 µg/ml. The *mcr-3 -like* gene was located onto a ca. 140kb conjugative IncA/C2 plasmid. Analysis of the LPS of the MCR-3 producer showed an addition of a phosphoethanolamine group to the lipid A the same way as MCR-1 and MCR-2.

Conclusions

This study identified the first occurrence of an MCR-3-like resistance determinant in South America.This is one of the few demonstration deciphering the impact of such phosphoethanolamine transferase in modifying the LPS and ultimately impact the susceptibility to polymyxins.

Clinical microbiolgy / P-17

Detection, isolation, and characterization of Shiga toxin-producing Escherichia coli in flour and ready-to-bake dough samples

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Background

Flour has recently been described as a novel vehicle for transmission of STEC. Very recently, an outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O121 or O26 infections was linked to flour in the USA.The aim of the present study was to generate baseline data for the occurrence of STEC in flour samples and ready-to-bake dough from different retailers in Switzerland.

Methods

A total of 86 samples, (70 flours, 16 doughs), were analysed. Following enrichment, the samples were screened for *stx*1 and *stx*2 using the Assurance GDS® Assay MPX ID. STEC strains were isolated and serotyped using the *E. coli* SeroGenoTyping AS-1 Kit. The determination of *stx* subtypes was performed by conventional PCR amplification**.** Screening for*eae*, *aggR, elt* and *estIa/Ib* was performed by real-time PCR.

Results

Nine (10.5%) of the samples tested positive for STEC by PCR. STEC were recovered from eight (88.9%) of the samples. Two isolates were STEC O11:H48 harbouring *stx1c*/*stx1d*, two were O146:H28 containing *stx2b*, one was O103:H2 containing *stx1a* and *eae,* and three were O non-typeable (Ont:H12 (*stx2a*), Ont:H14 (stx2a/stx2g), and Ont:H31 (*stx1c*/*stx1d*), respectively.

Conclusions

Although it is a low-moisture food, flour is a possible vehicle for foodborne pathogens, including STEC. Of the serogroups identified here, STEC O103 belongs to the “top five” serogroups of human pathogenic STEC in the EU, and STEC O146 is frequently isolated from diseased humans in Switzerland. Our results show that flour may be contaminated with a variety of STEC. Consumption of raw or undercooked flour may constitute a risk for STEC infection.

Clinical microbiolgy / P-18

High prevalence of Extended-Spectrum ß-Lactamase producing Enterobacteriaceae among clinical isolates from cats and dogs in Switzerland

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Background
This study aimed to identify and characterize extended-spectrum ß-lactamase (ESBL) producing Enterobacteriaceae among clinical samples of companion animals.
Methods
A total of 346 non-duplicate Enterobacteriaceae isolates were collected between 2012 and 2016 from diseased cats (n=115) and dogs (n=231). The presence of *bla*ESBL, PMQR genes and the azithromycin resistance gene *mph(A)* was confirmed by PCR and sequencing of *bla* genes. Isolates were further characterized by antimicrobial resistance profiling, multilocus sequence typing, phylogenetic grouping, identification of mutations in the QRDR of *gyrA* and *parC* and screening for virulence associated genes.
Results
Among the 346 isolates, 72 (20.8%) were confirmed ESBL producers (58 *E. coli*, 11 *Klebsiella pneumoniae*, and three *Enterobacter cloacae*). The strains were cultured from urine (n=45), skin and skin wounds (n=8), abscesses (n=6), surgical sites (n=6), bile (n=4), and other sites (n=3). ESBL genes included *bla*CTX-M-1, 14, 15, 27, 55, and *bla*SHV-12, predominantly *bla*CTX-M-15 (54.8%, 40/73), and *bla*CTX-M-1 (24.7%, 18/73). Further genes included *qnrB*(4.2%, 3/72), *qnrS*(9.7%,7/72),*aac(6')-Ib-cr* (47.2%, 34/72), and *mph(A),* (38.9%, 28/72). Seventeen (23.6%) isolates belonged to the major lineages of human pathogenic *K. pneumoniae* ST11, ST15, and ST147 and*E. coli*ST131. The most prevalent ST was *E. coli*ST410 belonging to phylogenetic group C
Conclusions
The high prevalence of ESBL producing clinical Enterobacteriaceae from cats and dogs in Switzerland and the presence of highly virulent human related *K. pneumoniae* and *E. coli* clones raises concern about transmission prevention as well as infection management and prevention in veterinary medicine.

Clinical microbiolgy / P-19

Genetic characterization of Shiga toxin producing Escherichia coli belonging to the emerging hybrid pathotype O80:H2 isolated from humans 2010-2017

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Background

Shiga toxin (Stx)-producing*Escherichia coli* (STEC) O80:H2 is an uncommon hybrid pathotype that has recently emerged in France and is associated with severe cases of the haemolytic uremic syndrome (HUS), as well as HUS associated with bacteremia. This study aimed to examine the molecular characteristics of human STEC O80:H2 isolates collected during 2010-2017 at the National Centre for Enteropathogenic Bacteria and *Listeria* (NENT) in Zürich, Switzerland.

Methods

For this study, we analysed 18 STEC O80:H2 human isolates received between 2010 and 2017 at the NENT. The O serogroup and the H type were identified by PCR. The determination of *stx* subtypes and *eae* variants were performed by conventional PCR.

Whole genome sequencing (WGS) was performed using a MiSeq Illumina platform. *In silico* genome analysis was performed using the virulence factor database (VFDB). Core genome MLST (cgMLST) was performed to assess the genetic relatedness among the isolates.

Antimicrobial susceptibility testing was performed using the disk-diffusion method and a panel of 16 antimicrobials.

Results

All isolates carried *stx2a* or *stx2d*, the rare variant *eae*-ξ variant, virulence genes associated with pS88, a extraintestinal pathogenic *E. coli* (ExPEC) plasmid. WGS identified additional chromosomal extraintestinal virulence genes encoding for type 1 fimbria (*fimA, fimC and fimH*), aerobactin (*iuc*/*iutA*) and afimbrial adhesins (*afaA/C/D/E*-VIII). cgMLST detected two closely related but distinct subclusters with different *stx2* and *iuc*/*iutA* genotypes. All isolates were multidrug resistant (MDR), but susceptible to third generation cephalosporins and azithromycin.

Conclusions

STEC/ExPEC hybrid pathotypes such as STEC O80:H2 represent a therapeutical challenge in the event of extraintestinal infection.

Clinical microbiolgy / P-20

Phenotypic and genotypic characterization of clinical isolates belonging to the Acinetobacter calcoaceticus-Acinetobacter baumannii (ACB) complex originating from different animal species

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Background

Members of the *Acinetobacter*genus are strictly aerobic Gram-negative rods ubiquitously present in the environment. A subset of *Acinetobacter*sp. has emerged as important nosocomial pathogens summarized in the *Acinetobacter-calcoaceticus-Acinetobacter-baumannii*(ACB) complex which includes *A. baumannii, A. nosocomialis, A. pittii, A. calcoaceticus, A. seifertii* and *A. dijkshoorniae*.

This study aimed to identify and further characterize members of the ACB complex from a collection of presumptive *Acinetobacter*spp.isolated between 2006-2016 from animals admitted to the University of Zürich veterinary
clinic.

Methods

Subspecies were differentiated by MALDI-TOF and *rpoB* sequencing methods. Members of the ACB complex were further characterized by antimicrobial resistance profiling, multilocus sequence typing and identification of genetic resistance determinants by micro array.

Results

Most of the isolates were identified as *A. baumannii,*followed by *A. pittii*and *A. calcoaceticus*. All except two isolates showed a multidrug resistance phenotype whereby multidrug resistance was defined as resistance against three or more antibiotic classes.

Conclusions

The high amount of multidrug resistant ACB complex isolates raises concern about transmission prevention as well as infection management and prevention in veterinary medicine since ACB complex members are known causative agents of nosocomial outbreaks.

Clinical microbiolgy / P-21

Molecular characterization of a Carbapenemase-producing Escherichia coli and an ESBL-producing Escherichia coli, both isolated from a wound infection of a dog

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Background

Carbapenem-resistant Enterobacteriaceae (CRE) are a threat to public health due to limited therapeutic treatment options. The number of CRE reports are increasing and CRE have been isolated from humans, livestock, environmental and food sources.

Here we report the molecular characterization of a carbapenem-resistant and an ESBL-producing *E. coli*, both isolated a from a wound infection of a dog with a history of a recurring wound infection.

Methods

Antimicrobial susceptibility testing was performed using the disk-diffusion method and a panel of 16 antimicrobials. Resistance genes were verified by micro array and the Carba NP-test.

Results

Microarray analysis revealed the presence of an NDM-type carbapenemase gene in one isolate. Whole genome sequencing of this strain and conjugation experiments are currently ongoing.

The ESBL producing isolate belonged to phylogenetic group F and harboured the *bla*$_{\text{CTX-M-55}}$gene. Conjugation experiments for this strain are currently ongoing.

Conclusions

CRE from companion animals constitutes major public health concern, since there is a great potential for their dissemination to humans due to the close contact between humans and companion animals.

Clinical microbiolgy / P-22

Frequency of quinolone resistance of E. coli in environmental samples from pig farms

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Background
Fluoroquinolones are critically important antimicrobials in human medicine and their use in veterinary medicine should be limited. As fluoroquinolones are little metabolised in animals and excreted in faeces and urine they enter mostly undegraded into the environment. The aim of this study was to investigate the occurrence and the spread of quinolone-resistant *E. coli* into the environment of pig farms.
Methods
Environmental samples were collected in 65 pig farms (104 dust samples, 104 swab samples from surfaces with direct animal contact and 71 samples of liquid manure). A total of 196 nalidixic acid resistant *E. coli* strains were isolated and tested by disk diffusion method for their sensitivity against 16 antimicrobial agents. The statistical comparison of the results was done with the Pearson's Chi-squared test. The significance level was set at $p<0.05$.
Results
In 45.2% of the dust samples, 51.9% of the swab samples and 70.4% of the liquid manure samples quinoloneresistant *E. coli* could be quantitatively cultivated. The liquid manure samples were significantly more often positive compared to the dust and the swab samples ($p<0.01$). The prevalence of strains showing resistance against streptomycin, tetracycline, sulfamethoxazole-trimethoprim and ampicillin was 69%, 61%, 46% and 45%, respectively. 62% of all tested strains showed resistance against 3 or more antimicrobial classes.
Conclusions
Quinolone-resistant *E. coli* are widely spread in the environment of pig farms. This is of special concern, since our data show that they are often multidrug resistant (resistant to more than 3 classes of antimicrobials).

Clinical microbiolgy / P-23

Disk diffusion test on Mueller-Hinton Rapid Medium for antibiogram susceptibility testing of resistant Enterobacteriaceae from positive blood culture

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Background
Validation of a Mueller-Hinton Rapid Medium® (MHR) to obtain rapidly a reliable antibiogram for Enterobacteriaceae strains showing resistance profiles.
Methods
Forty-two blood cultures were simulated using frozen strains of Enterobacteriaceae showing variable resistance profiles. A bacterial pellet was obtained from the positive blood culture and used to prepare a 0.5 McFarland standard. Simultaneous inoculation on MH (Axonlab) and MHR (i2a) media was performed as recommended by EUCAST. The incubation duration was 6h±0.5 and 16h±4 for MHR and 16h±4 for MH, followed by reading with a SirSCAN 2000 automatic™ instrument (i2a). Zone diameter breakpoints for each antibiotic were interpreted according to EUCAST recommendation. Each test was accompanied by an E. coli ATCC 25922 strain (n=12) as a
control.
Results
Comparing MH with MHR at 6h, 1792 antibiotic disks were analyzed and S/I/R interpretations were compared. Total agreement was observed in 1622 (90.5 %) diameters. A very major error “VME” was detected in 6 (0.3%) diameters associated with co-amoxyclav (n=1), aztreonam (n=1) aminoglycosides (tobramycin n=1, gentamicin=1) and co-trimoxazole (n=2).Major errors “ME” (n = 33, 1.84 %) were mostly observed with co-amoxyclav (n=11) followed by ceftazidime (n=7). Minimum one minor error “ME” n=131 (7.3 %) were observed in all antibiotics tested except ampicilline, cefalotine, cefuroxime and with antibiotics for which there is no intermediate zone.
Conclusions
A very good agreement rate was observed in the analysis of different combinations used in this study (Kappa=0, 83) which confirms the utility of MHR in an early adaptation of antibiotic therapy.

Clinical microbiolgy / P-24

Evaluation of DermaGenius®, a multiplex rtPCR to identify dermatophytes directly from specimens

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Background
Traditional analysis of dermatophytes is long-lasting and needs experienced technicians. Molecular analysis would be faster and more specific. Here, we assess if a multiplex rtPCR assay could replace traditional dermatophyte analysis.
Methods
Specimens were microscopically examined (200 x magnifications) and cultured on agar plates at room temperature for 4 weeks. Molecular detection of 12 dermatophytes was done with the multiplex rtPCR kit Derma- Genius®. Extracted fungal DNA was amplified on Rotor Gene Q and differentiated by melting curve Analysis.
Results
Retrospective evaluation of 309 traditionally analysed specimens showed growth of dermatophyte species (26%), possible contaminants (6%), microscope positive / culture negative (9%) and microscope / culture negative (59%) specimens. The multiplex rtPCR Derma Genius assay comprised 96% of the cultured dermatophyte species, missing *Microsporum fulvum* and *Trichosporon inkin*. In a prospective evaluation 70 specimens were analysed by traditional means and by multiplex rtPCR in parallel. No discrepancy was seen in 48 negative and 15 positive specimens. Four specimens were positive only by rtPCR (3x *T. rubrum* and 1x *C. albicans*) and three culture positive probes were negative by rtPCR (2x *Aspergillus sp.* and 1x *Fusarium sp.* both not included in the DermaGenius® kit).
Conclusions
DermaGenius® covers most of the dermatophytes, is fast, and is robust to contamination, but the interpretation of the melting curve analysis of the rtPCR can be demanding. Rare or atypical dermatophytes can only be detected by traditional analysis. If and how rtPCR can cost-effectively be implemented into the routine laboratory shall be discussed.

Clinical microbiolgy / P-25

First detection of an IMI-producing colistin-resistant Enterobacter cloacae in Switzerland

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Background

The first case of *Enterobacter cloacae*producing an IMI-type carbapenemase in Switzerland was diagnosed from a urinary specimen of a 43-years-old outpatient. This isolate was also resistant to colistin.

Methods

Antimicrobial susceptibility testing was performed by disc-diffusion (Oxoid), E-test (bioMérieux) and UMIC (Biocentric). Phenotypic detection of carbapenemases was obtained by combined disc synergy test (Rosco Diagnostic td), chromID CarbaSmart medium (bioMerieux) and Rapidec Carba NP test (bioMerieux Ltd). Genotypic detection of carbapenemases was performed by Carba-R GeneXpert (Cepheid) and sequencing. *mcr* genes (*mcr-1* to *mcr-5*) were searched by PCR.

Results

E.cloacae was isolated in pure culture from urine, in the context of a renal lithiasis. It was resistant to most betalactams and to colistin (MIC > 64 mg/L) but remained susceptible to expanded-spectrum cephalosporins. No plasmid-encoded *mcr* genes were detected. The isolate produced a carbapenemase as indicated by the growth on a ChromID CarbaSmart screening medium and the positive Rapidec Carba NP test. A synergy test demonstrated marked inhibition with meropenem and a boronic acid disc, indicating the presence of Ambler class A carbapenemase. No carbapenemase was detected by Carba-R GeneXpert. An IMI-carbapenemase was diagnosed by sequencing.

Conclusions

This first isolation of an IMI-producing colistin-resistant *E.cloacae* in Switzerland shows the diversity of *Enterobacteriaceae* producing carbapenemases. It emphasizes the value of a first screening step by using phenotypic tests to detect carbapenemase activity, and the support of a reference center for a rapid characterization of carbapenemase genes that are not identified by using commercially available kits.

Clinical microbiolgy / P-26*

Genetic diversity and antimicrobial susceptibility of Brachyspira hyodysenteriae in Switzerland

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Background

Brachyspira hyodysenteriae is an anaerobic spirochete that causes swine dysentery (SD). Today, SD is controlled by the use of antibiotics such as pleuromutilins, macrolides and lincosamides. The use of these antibiotics has selected for resistant clones worldwide raising the question whether such clones are also present in Switzerland.

Methods

Fifty-one *B. hyodysenteriae* isolates were obtained from pigs of 24 different Swiss pig herds. Identification was performed by MALDI-TOF, B*nox*-PCR and real-time PCR. Sequence type (ST) was determined by multilocus sequence analysis. Minimal inhibitory concentration was determined for six antibiotics of the pleuromutilin, macrolide, lincosamide and tetracycline classes by broth dilution. Point mutations linked to antibiotic resistance in *B. hyodysenteriae* were detected by 16S rRNA and 23S rRNA sequences analysis.

Results

Isolates belonged to ST6 (n=1), ST66 (n=9) and the novel ST196 (n=37) and ST197 (n=4). They were present in 1, 5, 17 and 2 different herds, respectively. All isolates were susceptible to tiamulin and valnemulin. Forty-one isolates of ST196 (n=36), ST66 (n=4) and ST6 (n=1) were resistant to tylosin, tylvalosin and lincomycin associated with an A2058T/G mutation in the 23S rRNA. Resistance to doxycycline was observed in three isolates of ST196 that contained a G1058C mutation in the 16SrRNA.

Conclusions

Low genetic diversity suggests a few common sources of *B. hyodysenteriae* along the pig production in Switzerland. The novel predominant Swiss clone ST196 exhibited resistance to macrolides and lincosamides, limiting therapeutical options and emphasizing prudent and targeted use of antibiotics to avoid selection of additional resistant traits in *B. hyodysenteriae* from Switzerland.

**Student paper*

Clinical microbiolgy / P-27*

Serotype epidemiology of invasive Streptococcus pneumoniae in Switzerland, 2010-2017

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Background
To describe the serotype epidemiology of invasive pneumococcal disease (IPD) in Switzerland from 2010 to 2017, i.e. since the introduction of the seven-valent (PCV7) (in 2007) and the thirteen-valent pneumococcal conjugate vaccine PCV13 (in 2011).
Methods
At the Swiss National Reference Centre for Invasive Pneumococci (NRCP) which receives >95% of all invasive isolates of Switzerland, serotypes were determined using the Quellung reaction. Descriptive analysis and logistic regression analysis were performed using the R software environment. Trend analyses of proportions were done using the chi-square test of trend.
Results
After an initial decrease in the absolute number of IPD isolates, we again observed an increase in 2017 (n=1012) as compared to 2016 (n=874). This was seen primarily in the adult population (>15 years). There has been a gradual decrease in the annual proportion of PCV7 serotypes (2010: 24.6% versus 2017: 8.40%, $p<0.05$). Non-PCV7 serotypes included in PCV13 have also declined (2010: 43.7% versus 2017: 24.8%, $p<0.05$). However, serotype 3 was the only PCV13 serotype which did not decrease. Among the non-PCV13 serotypes, serogroup 8 had the most significant increase (4.57% versus 14.8%, $p<0.05$) followed by serotype 9N (3.32% versus 6.32%, $p<0.05$).
Conclusions
There is evidence that the introduction of PCV7 and PCV13 in Switzerland has contributed to a significant decline of PCV7 and five of the additional six PCV13 serotypes regarding invasive pneumococcal disease from 2010 to 2017. Due to the increasing prevalence of non-PCV13 serotypes, continued monitoring of serotypes is crucial to understand the epidemiology of IPD.

**Student paper*

Clinical microbiolgy / P-28

Standardization of urine samples processing on Copan WASP®

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1. *Copan Italia*

Background
Urines are the largest number of specimens submitted to microbiology laboratories for bacterial culture. One of the main challenges is to ensure sample's stability during transportation to prevent overgrowth of normal bacteria flora and support pathogens viability. Copan UriSwab® (US) is a collection and transportation device for urine specimen compatible with WASP®.
The study objectives are:

- Compare US performance with BD Vacutainer (VC) at time 0 and after 24h storage.
- Evaluate the compatibility of both devices with WASPTM using a new streaking pattern

Methods
Clean catch urines (n=84) were used for this study. Vacutainer and US were filled using the manufacturer's indications. Both devices were processed on WASP® at time 0 and after 24h storage at RT using 10µL loop and SST8 on chromID CPSElite plates. Inoculated plates were incubated in WASPLab® incubator (35°C, O2 for 18h). Samples with a concentration ≥10.000 CFU/mL were defined as positive and a semiquantitative indication was assigned using an "abacus" obtained by streaking *E. coli* 10-fold dilutions. Colonies were identified using MALDI-TOF technology.
Results
Forty positive, 37 negative and 7 contaminated urines samples were reported. No discrepant results were observed between the interpretation of both culture plates from samples stored in US and VC devices at time 0. Samples maintained in transport devices for 24h provided an agreement respectively of 96% and of 92% compared to time 0.
Conclusions
Both preservation systems guarantee a good performance in maintaining bacterial viability and the new streaking pattern allows to obtain enough isolated colonies to perform ID and AST.

Clinical microbiolgy / P-29

Automatic preparation of samples for MALDI-TOF Identification

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Background
Microbiology laboratories rely on MALDI-TOF Technology for bacteria identification. In order to standardize the process of the target preparation and overcome the repetitive operations potentially affected by technician's skills, Copan introduced the Colibrì™, a new system able to pick and spot colonies from the culture plates to the target and automatically deposit the matrix.
Methods
In this study, Gram-negative clinical isolates were selected from positive urine samples and streaked on CPSElite (bioMerieux) by WASP®. Plates were incubated in WASPLab® for 18 hours at 35°C in O2. At the end of the incubation time, plates were digitalized and analyzed on WASPLab® working station to select the designed colonies with the aid of WASPLab® Imaging Plug-In. As reference method, colonies were also processed manually. Clinical isolates (n=382) were included: *E. coli* (n=96), *E. aerogenes* (n=16), *C. koseri* (n=16), *C. freundii* (n=8), *A. baumanii* (n=48), *K. oxitoca* (n=48), *K. pneumoniae* (n=66), *P. aeruginosa* (n=4), *M. morganii* (n=24), *P. mirabilis* (n=56). Identification obtained with VitekMS (bioMerieux) from target prepared manually and automatically were compared.
Results
All the designated colonies were correctly picked producing 100% of identification agreement between Colibrì ™ and manual method with *Good discrimination*. Sample number and isolate information were successfully transmitted for all the spots from WASPLab® and Colibrì™ to VitekMS.
Conclusions
Colibrì™ is a fully automated preparator to standardize the MALDI-TOF identification process in clinical microbiology laboratories, improving the samples traceability and supporting the daily routine leading to a higher efficiency.

Clinical microbiolgy / P-30

Evaluation of PARASITEMIA an imaging software for the screening of parasites infected erythrocytes in Plasmodium falciparum malaria

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Background

In case of Malaria caused by *Plasmodium falciparum*, the determination of parasitemia, the percentage of erythrocytes containing parasites, is mandatory to estimate the severity of the infection and to guide the therapy. Parasitemia is time-consuming and may vary according to the experimenter. PARASITEMIA is an automatized imaging software capable of detecting erythrocytes and classifying parasitized erythrocytes. In this study, we evaluated this software, which could facilitate the determination of parasitemia and reduce the interexperimenter variability.

Methods

Using the software PARASITEMIA we analysed thin stained smears (May-Grünwald-Giemsa), corresponding to peripheral blood samples positive for rapid malaria diagnostic tests. For each slide, 3 sets of 15 images were taken with a Leica microscope and a camera (Leica DFC420). The results obtained with PARASITEMIA before and after reclassification by biomedical technicians were compared with the results obtained in routine by conventional parasitemia carried out by biomedical technicians and with real-time PCR.

Results

Results obtained on 32 cases suggest that the overall results of PARASITEMIA after reclassification by biomedical technicians correlated with microscopy (R2 = 0.971). For each case, we reported a mean of 4 elements reclassified as parasitized erythrocytes and 3,1 elements reclassified as non-parasitized erythrocytes.

Conclusions

These preliminary results suggest that the imaging software PARASITEMIA has the potential to facilitate and standardize parasitemia analysis. Increasing the number of case will increase the statistic power of the study. The results will also be compared to those of PCR. This study support a very soon introduction of such approach in diagnostic laboratories.

Clinical microbiolgy / P-31

Analysis of Pseudomonas aeruginosa virulence in human burn wound exudates

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Background

Burn wound sepsis is currently the main cause of morbidity and mortality after severe burn injury. Bacterial pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter baumannii* impair patient recovery and can be fatal. The specialized medical care required after burn injury faces major challenges to prevent bacterial development on burn wounds. Our overall objectives were to develop an *in vitro* burn wound infection model in order to improve evaluations of new biological bandages formulations used to treat burn wounds, and to better understand the bacterial pathogenesis in a wound environment.

Methods

In this study, we used different experimental approaches including: i) collection and chemical composition analysis of human burn wound exudates (BWE) from 11 burn patients, ii) characterization of *P. aeruginosa* pathogenicity by measuring virulence factors production, and iii) analysis of *P. aeruginosa* transcriptomic profile using RNA-seq technology.

Results

Growth assay in BWE showed that *P. aeruginosa* species were the only pathogens able to proliferate. Moreover, synthesis of typical virulence factors was strongly enhanced in *P. aeruginosa* compared to standard laboratory growth conditions. These results were confirmed by whole genome expression analysis revealing the activation of a broad range of virulence factors including lipases, toxins and proteases. Activation of specific pathways such as iron-acquisition mechanisms appeared to be essential for *P. aeruginosa* proliferation in BWE.

Conclusions

Description of *P. aeruginosa*physiology and genome expression in BWE provides new insights for the clinical understanding of burn wound infections and appears to be essential to develop new and efficient strategies against pathogenic microorganisms.

Clinical microbiolgy / P-32

Antimicrobial susceptibility testing of neisseria gonorrhoeae in the region of bern (june 2013 to february 2018)

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Background
In the last few years, the awareness has risen for antimicrobial resistance (AMR) of *Neisseria gonorrhoeae*(NG), especially against first line treatment options azithromycin and ceftriaxone. Nevertheless, current data for AMR are lacking for the region of Bern. Here, we retrospectively analyzed the antimicrobial susceptibility of NG for ceftriaxone, ciprofloxacin and azithromycin.
Methods
In this study, 60 clinical isolates were collected from June 2013 to February 2018, of which 60 were tested for ceftriaxone and 59 for ciprofloxacin. Since June 2015, 39 isolates were also tested for azithromycin. Antimicrobial susceptibility testing (AST) was performed on Mueller-Hinton agar (MH-F) for fastidious microorganisms, using Etest (Biomérieux) of ceftriaxone (0.002-32 µg/mL) and (0.016-526 µg/mL), ciprofloxacin (0.002-32 µg/mL) and azithromycin (0.016-256 µg/mL). Results were interpreted according to EUCAST.
Results
All isolates (60/60) tested were susceptible to ceftriaxone. In 2015 and 2016, two isolates demonstrated a decreased susceptibility to ceftriaxone (DSC; MIC ≥0.125 mg/L). For azithromycin, the resistance rate was 15.38 (6/39). So far, one isolate (2.56 %, 1/39) showed DSC combined with an elevated MIC-value for azithromycin (MIC = 0.5 mg/L). Ciprofloxacin had the highest resistance rate, being 33.9 % (20/59). A clear distinction between wild type and non-wild type phenotype was observable.
Conclusions
The ceftriaxone resistance rate has been relatively stable, whereas the increasing azithromycin resistance rate indicates a potential source for treatment failure. This study provides an outlook on the current AMR situation: A nation-wide, collaborative approach is needed, to fully determine the AST on genetic and phenotypic levels.

Clinical microbiolgy / P-33

LAMP assay optimization for rapid diagnostic purpose

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Background
Rapid diagnostic tests are long-awaited to speed up the time-to-result in the clinics. A distinct advantage of isothermal amplification over PCR is the amenability to simple implementation in a microfluidic format and to complete automation. These two hallmarks are anticipated to reduce both the instrumental and product costs and to extend the range of applications. Amongst isothermal techniques, Loop-mediated isothermal amplification (LAMP) technique amplifies DNA with high specificity, efficiency and rapidity. The goal of this study was to develop a simple protocol based on LAMP technology for the rapid analysis of spiked blood culture samples.
Methods
LAMP experiments were performed in 96-wells microplate format. The fluorescence was monitored on a generic real-time qPCR system. Four lysis protocols were evaluated based on gDNA extracts and using representative Gram-positive bacteria. The impact of heat, the best dilution and the interest of filtration/centrifugation steps were studied using spiked blood samples in 10-fold dilution series.
Results
Osmotic and thermal shocks, and glass-bead based lysis gave similar results for *S. pneumoniae* and *E. faecalis*. For *S. aureus,* the results were slightly delayed for the osmotic shock. Heat had detrimental impact on LAMP in spiked blood cultures. The centrifugation step had no impact. The filtration step delayed the amplification only for *S. pneumoniae* and not for *S. aureus* and *E. faecalis*.
Conclusions
The detection of microorganisms from blood culture medium usually requires extensive sample purification and removal of inhibitors. The results showed that heating, centrifugation and filtration were dispensable allowing a simple implementation in a microfluidic device.

Clinical microbiolgy / P-34

A comparison of Sensititre™ Anaerobe MIC Plate with ATB ANA® test for the routine susceptibility testing of common anaerobe pathogens

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Background
The accuracy of the Thermo Scientific™ Sensititre™ Anaerobe MIC plate was assessed against the ATB ANA® test (bioMérieux) on 56 clinically relevant anaerobic strains collected at Geneva University Hospitals. Anaerobic Gram negative bacilli represented 70% (n=39) of the clinical isolates included in this study. Gram positive anaerobic bacilli and cocci represented 30% (n=17).
Methods
Antimicrobial susceptibility testing was performed in parallel by using the Sensititre™ Anaerobe MIC plate (ANO2B or ANAERO3) and ATB ANA® test according to the manufacturers' instructions. When both methods agreed, we considered the susceptibility category correct and no further determination was performed. When the methods gave discordant results, we performed the gradient strip method to address this uncertainty.
Results
The overall categorical agreement between both methods reached 95%. The Sensititre™ Anaerobe MIC plate had excellent accuracy for most antibiotics tested. When the Sensititre™ Anaerobe MIC plate disagreed with ATB ANA® test, the gradient strip method resolved the antimicrobial susceptibility categories of all the antibiotics tested, except for piperacillin-tazobactam and penicillin, in favor of the Sensititre™ Anaerobe MIC plate (65% [15/23]). Several very major errors were observed for piperacillin-tazobactam (12.5% [7/56]) and penicillin (2% [1/56]). The gradient strip method revealed that the categorical differences for piperacillin tazobactam and penicillin were at least partly explained by the presence of distinct colonies growing within the inhibition zone, thus clearly demonstrating the presence of antibiotic-resistant subpopulations.
Conclusions
The Sensititre™ Anaerobe MIC plate offers a useful alternative to the ATB ANA®test for the routine antimicrobial susceptibility testing of anaerobes.

Clinical microbiolgy / P-35

Comparison of analytical performances of the Roche Cobas 6800 CT/NG assay with the Abbott m2000 Real Time CT/NG assay for detecting Chlamydia trachomatis and Neisseria gonorrhoeae

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Background

The objective of this study was to assess the analytical performances of the Cobas 6800 CT/NG assay (C6800) against the Abbott m2000 Real Time CT/NG (m2000) assay for the detection of *C. trachomatis*and *N. gonorrhoeae*.

Methods

The evaluation of C6800 was performed retrospectively on 714 specimens (19.6% were positive for *C. trachomatis*, 4.7% for *N. gonorrhoeae*, and 0.6% for both pathogens), and prospectively on 9 external quality controls. Discordant specimens were retested by an independent set of real-time PCR assays. Urine and swab specimens were collected using the Abbott multi-Collect Specimens Collection KIT. After m2000 analysis, the transport tubes were stored at 4°C until testing by C6800. For C6800 analysis, all the stored specimens were analyzed by spiking 2mL of urine and 600µL of swabs specimens into cobas® PCR Media tube.

Results

For *C. trachomatis*, the sensitivity of C6800 compared to m2000 was 100%, the specificity was 99.1%. For *N. gonorrhoeae*, the sensitivity of C6800 compared to m2000 was 100%, the specificity was 99.7%. One of 6 discrepant C6800 specimens (m2000 negative or indeterminate/C6800 positive) tested by an independent real-time PCR assay was confirmed positive for *C. trachomatis*. Two discrepant specimens (m2000 negative or indeterminate/C6800 positive) were considered positive because the two patients had, at the time of testing, another specimen positive with the same pathogen. The discordant quality control (QCMD) Glasgow/CTDNA 18C1-05 (m2000 indeterminate/C6800 positive) was confirmed positive for *C. trachomatis*.

Conclusions

After discordance analysis, the specificities for C6800 for *C. trachomatis* and *N. gonorrhoeae* increased to 99.5% and 99.9% respectively.

Clinical microbiolgy / P-36

Mechanisms of Staphylococcus aureus leukotoxins inducing ETosis

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Background

ETosis is a form of cell death, where immune cells release extracellular traps (ETs). ETs are formed in response to various stimuli, to trap/kill invading pathogens. The bi-component leukotoxin Panton-Valentine leukocidin (PVL), secreted by *Staphylococcus aureus,* has yet to be confirmed as an effector of ETosis. ETosis can be beneficial for pathogenic infections, but may also present negative effects such as tissue damage. Thus, it is essential to understand the signaling mechanisms of ETosis and identify specific signaling pathways in response to PVL.

Methods

We used primary human monocytes for ET observation, triggered by PVL. Cell culture assays, immunofluorescence and microscopy analyses were performed in various conditions, with variance analyses followed by Bonferroni's corrections for significance testing. Transcriptomics were then applied to identify differential gene expression levels of PVL exposed vs. non-exposed cells with cufflinks/cuffdiff.

Results

ET formation was observed *in vitro* in response to a broad spectrum of PVL concentrations. Differential expression analyses, showed a significant alteration in specific genes (*p*-values< 0.001), with either upregulations during PVL exposure or downregulations. Specific genes are identified as key elements of several signaling pathways, including pathways known to be essential for ET formation.

Conclusions

We provide evidence that PVL may induce ET formation, which seems to alter the expression of specific genes of well-known signaling pathways, enabling the identification of adapted ETosis inhibitors. Findings, may lead towards the development of a therapy favoring bacterial clearance in severe infections, as this virulence factor is known to be involved in cell death resulting in extensive tissue damage.

Clinical microbiolgy / P-37*

Staphylococcus aureus Panton-Valentine Leukocidin causes alternative NETosis in human neutrophils

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Background

Staphylococcus (S.) aureus is a commensal Gram+ bacterium, but can become pathogenic by causing a variety of community and nosocomial infections. In fact, this bacterium can produce many virulence factors enabling innate immune evasion. More precisely, Panton-Valentine Leukocidin (PVL), one of several bi-component toxins (BCTs), has previously been demonstrated to cause neutrophil death by an accelerated apoptosis in 40% of tested cells rather than rapid membrane pore formation. However, a greater percentage of cells were positive for membrane permeabilization in the presence of PVL at 6h post-exposition.

Methods

We thus analysed another type of cell death called NETosis (Neutrophil Extracellular Trap release) with regard to some known elements involved in the classical pathway described for a NETosis inductor Phorbol-12-myristate-13-acetate (PMA): phosphorylation of PI3K, Akt and ERK, autophagy, the increased production of Reactive Oxygen Species (ROS) from NADPH oxidase and the spread of extracellular chromatin fibres decorated with Neutrophil Elastase (NE).

Results

Our experiments demonstrate that after 6h, PVL induces NETosis following an alternative pathway, resulting in specific differences compared to the classical process: ERK and NADPH oxidase are not involved, while the mitochondria seem to be the main source of ROS. Furthermore, PVL-induced NETs also present a post-translational modification called citrullination on the histone H3, a typical marker of autoimmune processes caused by Peptidylarginine deiminases (PADs), which are absent during classical NETosis.

Conclusions

Our results indicate an alternative outcome for PVL on human neutrophils compared to PMA and may suggest a new link between *S. aureus* infections and some autoimmune diseases.

**Student paper*

Clinical microbiolgy / P-38 => see CM-09

Clinical microbiolgy / P-39* => see S-56*

Clinical microbiolgy / P-40* => see S-61*

Clinical microbiolgy / P-41* => see S-74*

Clinical microbiolgy / P-42 => see S-75

Environmental microbiolgy / P-43

Grassland ecosystems in lahar-areas as habitats for slime molds: assessment of myxomycete diversity in taal, pinatubo and mayon volcanoes

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1. *University of Santo Tomas*

Background
Natural disturbances made by volcanoes such as eruptions and lahar flows have negative effect on the original vegetation of the affected areas. These may lead to the re-establishment of other vegetation, primarily of pantropical grasses. Recently, grassland ecosystems have been reported as habitat for myxomycetes in the Philippines, but none so far in areas affected by volcanic activities. Does volcanic eruption affects also the diversity of myxomycetes?
Methods
In this study, the grasslands in areas affected by lahar from the previous eruptions of Taal (42 years ago), Pinatubo (26 years ago), and Mayon (11 years ago) served as study sites. Aerial and ground litter of grass were collected from six sampling localities within these areas. A total of 1,080 moist chambers were then set up from grass substrata collected in these sites.
Results
From the 912 moist chambers positive for myxomycetes, a total of 40 species belonging to 11 genera were identified. Taal Volcano reported a higher species diversity & richness than Mt. Pinatubo and Mt. Mayon. Interestingly, more than 4 decades has passed since the last eruption of Taal and the grassland communities were already well-established. Taal is also dominated by at least two species of grasses as opposed to the other collection sites. Comparing the myxomycetes assemblages, a high CC value (70%) was computed between Taal and Mayon indicating similarity in terms of species composition.
Conclusions
Our results showed that volcanic eruption can alter the vegetation of the area and in turn, also affects the diversity of grassland-inhabiting myxomycetes.

Environmental microbiolgy / P-44*

Development of new strategies to produce and characterize PceC, a membrane-bound flavoprotein involved in organohalide respiration

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1. *École polytechnique fédérale de Lausanne (EPFL)*

Background
Halogenated organic compounds (so-called organohalides) represent one of the major widespread groundwater pollutants. Organohalide respiration (OHR) is a bacterial anaerobic process in which chlorinated compound, *e.g.* tetrachloroethene (PCE), is used as terminal electron acceptor. *Desulfitobacterium* and *Dehalobacter*, paradigmatic organohalide-respiring bacteria (OHRB), harbour the *pceABCT* gene cluster, representing one model system for the study of PCE respiration. To date, the function of PceA, the key enzyme in the process, and PceT, the dedicated molecular chaperone for PceA maturation, are well defined. However, the role of PceB and PceC are still not elucidated. Analysis of the sequence of PceC (and other members of the RdhC family) revealed the presence of multiple transmembrane segments, a flavin mononucleotide (FMN) binding motif and two conserved CX3CP motifs.

Methods
The experiments conducted so far, permitted to express in *E. coli*the FMN-binding domain (FBD) of PceC. However, FBD expression resulted in the formation of inclusion bodies. After denaturation with urea, a strategy was developed to reconstitute FBD in a soluble form by inserting FMN with the help of Ftp1, a flavin-transferase from *D. hafniense*.

Results
Moreover, preliminary experiments with the recombinant FBD protein showed redox activity, indicating that PceC may play a role in electron transfer in the metabolism of organohalide respiration.

Conclusions
These results invite to conduct the following investigations: *i*) a thorough analysis of the redox properties of FBD using redox titration and UV-vis spectrophotometry and, *ii*) the development of a dedicated strategy for the heterologous production and characterization of the full-length membrane-bound PceC protein.

**Student paper*

Environmental microbiolgy / P-45

Unexpected discovery of an insecticidal toxin gene cluster in a bacterial fish pathogen

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1. *University of Lausanne / Department of Fundamental Microbiology*

Background
The Fit toxin gene cluster is required for the pathogenicity of the rhizobacteria *Pseudomonas protegens* and *Pseudomonas chlororaphis*towards certain plant pest insects. Genomic analyses so far suggested that the occurrence of the *fit* gene cluster is restricted to these two *Pseudomonas* taxa. Here, we investigated whether the *fit* gene cluster occurs in phylogenetically distant pseudomonads and potentially is involved in the interaction with other eukaryotic hosts.
Methods
We inspected the genomes of 120 *Pseudomonas* strains for the presence of the *fit* gene-like clusters and generated a *fit*cluster-based phylogenetic tree that we compared with the one inferred from housekeeping genes. Additionally, we explored the insecticidal potential and the expression conditions for *fit* gene clusters in selected strains.
Results
We report here on the presence of the *fit* cluster in a *Pseudomonas* strain that is phylogenetically distant from the previously described *fit* carrying taxa. Moreover, we demonstrate that this pseudomonad, a fish pathogen, does not exhibit toxicity towards insects. Sequence comparison with the *P. protegens fit* gene cluster identified remarkable differences, which could be responsible for the lack of insecticidal properties in the fish pathogen. We also noticed that the different *fit* clusters are induced upon contrasting growth conditions.
Conclusions
To our best knowledge, this is the first report on a *fit*-like cluster that is not correlated with the ability of the bacterium to kill insects. The presence of *fit*-like genes in a fish pathogenic bacterium is surprising and raises questions about the host specificity (insect versus fish) of the different Fit toxins.

Environmental microbiolgy / P-46*

Nitrogen cycling microbial community dynamics in freshwater ecosystems

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Background

Nitrogen is an essential element in all living organisms and a limiting nutrient to phototrophic organisms in terrestrial and aquatic ecosystems. Due to increasing anthropogenic nitrogen input, many aquatic systems face changes in the nitrogen cycle that may result in eutrophication of coastal ecosytems. Lakes reduce the nitrogen load through several processes, such as denitrification, DNRA, anammox, and nitrogen burial. The nitrogen removal depends on the activity of microorganisms and several environmental parameters.

Methods

This study, which is part of a multidisciplinary effort involving biogeochemistry and modelling, aims to better understand the factors influencing nitrogen cycling dynamics including the key microbial players involved in freshwater ecosystems using metagenomics and metatranscriptomics. A detailed seasonal and spatial resolution of nitrogen removal rates, the microbial community, its nitrogen gene expression activity and environmental drivers in two lakes with different trophic state will be measured.

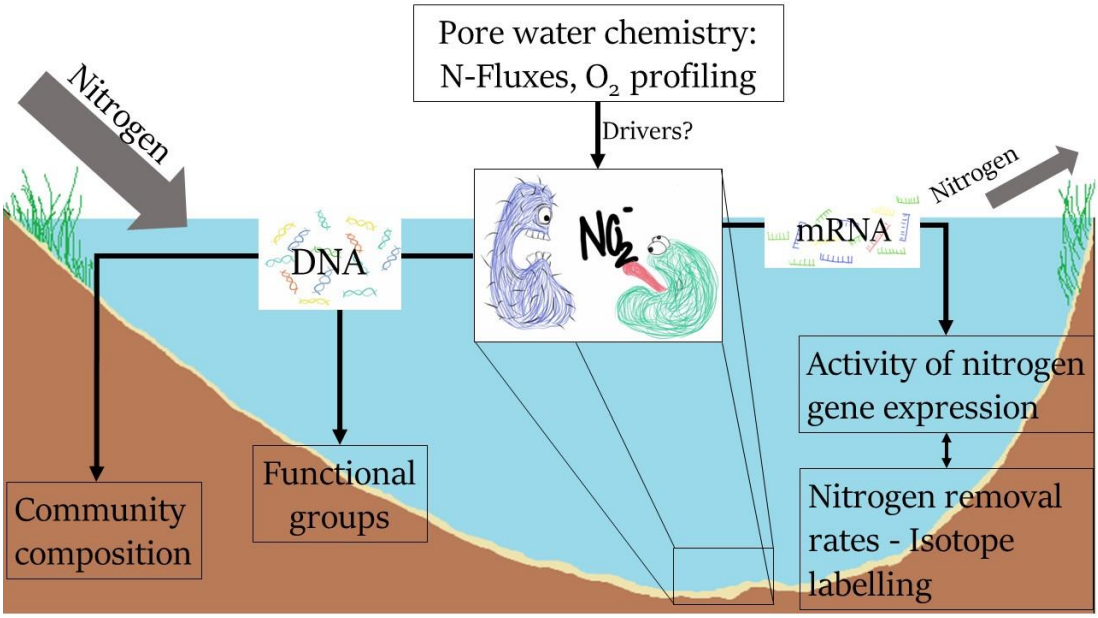
Results

As the project is in its early stages, this poster discusses our study approach and first results on the biochemistry and microbial community composition of the study system.

Conclusions

Ultimately, the data will contribute to better understanding of the main populations and pathways that drive the nitrogen cycle in Swiss lakes and show how environmental conditions influence biological nitrogen removal.

**Student paper*



Attachment #148

Environmental microbiolgy / P-47

In-silico evaluation of hybrid transmembrane sensory proteins

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Background
transmembrane sensory proteins are difficult to overexpress, isolate and crystallize. Here we propose an insilico approach using homology modelling to evaluate their properties.
Methods
Homology modelling was performed using Swiss-Model.
Results
Functionality of different transmembrane proteins can be estimated depending on the quality of the 3D model
Conclusions
Homologie modelling offers a feasible way of evaluating transmembrane proteins.

Environmental microbiolgy / P-48

Type VI secretion-mediated competition of the root-colonizing biocontrol agent Pseudomonas protegens CHA0 with phylogenetically related rhizosphere pseudomonads

Dr. Maria Péchyné-Tarr [1], Mrs. Silvia Brochet [1], Dr. Jordan Vacheron [1], Dr. Monika Maurhofer [2], Dr. Christoph Keel [1]

1. *University of Lausanne / Department of Fundamental Microbiology,* ***2.*** *Swiss Federal Institute of Technology Zurich / Plant Pathology, Institute of Integrative Biology*

Background

Pseudomonas protegens CHA0 is representative of a group of plant-associated bacteria known for their capacities of pest suppression, plant growth promotion and defense priming. Various antimicrobial, lytic and toxic compounds contribute to the pest control abilities of *P. protegens* CHA0. The strain is a highly competitive rootcolonizer that following field crop inoculation successfully establishes over several months in the rhizosphere, an environmental niche that is densely populated by competing bacteria. The molecular basis of the niche competitiveness of *P. protegens* is largely unexplored.

Methods

We investigated whether the type VI secretion system (T6SS) of *P. protegens* CHA0 contributes to its competitiveness, notably during exposure to phylogenetically related pseudomonads exhibiting similar niche preferences. The T6SS, a phage tail-like contractile transmembrane apparatus, functions like a puncturing device to deliver toxic and lytic effectors into neighboring cells. We used interbacterial confrontation assays combined with mutational analyses and time-lapse microscopy to investigate the role of the *P. protegens* T6SS.

Results

The genome of *P. protegens* CHA0 harbors gene clusters encoding a T6SS core apparatus and two distinct VgrG modules with spike, effector and immunity proteins. We demonstrate that the core apparatus and one of the VgrG spikes plus associated effector are essential in the competition with related rhizosphere pseudomonads. We visualized T6SS-mediated killing of a competitor pseudomonad by CHA0 by monitoring the activity of a T6SS sheath protein-sfGFP fusion encoded in the native genomic context with time-lapse microscopy.

Conclusions

Our results highlight the importance of T6SS in inter-bacterial competition of *P. protegens* with related rhizosphere pseudomonads.

Environmental microbiolgy / P-49*

In vivo regulation of organohalide respiration in Desulfitobacterium hafniense DCB-2

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Background
Organohalide respiration (OHR) is a bacterial anaerobic respiratory metabolism which takes advantage of halogenated compounds (= organohalides) as terminal electron acceptors. While its environmental interest does not have to be demonstrated anymore, many aspects of this process remain poorly characterized. We have decided to explore the regulatory network of OHR in *Desulfitobacterium hafniense* strain DCB-2, a typical OHR bacterium showing a versatile energy metabolism which harbours seven distinct reductive dehalogenase (*rdh*) gene clusters potentially involved in OHR.
Methods
Strain DCB-2 was cultivated anaerobically with lactate and fumarate (under respiration conditions) or with pyruvate (under fermentation conditions) and exposed to various chlorinated phenol derivatives, after which RNA was extracted from samples collected at different time intervals. A combination of reverse transcription, qPCR and transcriptomic analysis targeting genes belonging to *rdh* gene clusters will allow a quantitative analysis of the activity of selected RdhK proteins which are involved in transcriptional regulation.
Results
This study aims at deciphering the role and cross-talk of characterized and uncharacterized RdhK regulatory proteins of strain DCB-2 *in vivo*. The activation of transcription of *rdh* gene clusters after addition of selected chlorophenols will be presented and discussed.
Conclusions
These data will be confronted to results obtained from *in vitro* experiments describing the tripartite interaction of purified RdhK proteins with the chlorophenols and the targeted DNA promoters (see also the abstract by M. Willemin *et al*.).

**Student paper*

Environmental microbiolgy / P-50*

Enhance the metal recycling in urban wastes through bacterial-fungal interactions

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Background
Anthropogenic waste such as e-waste is known to contain high concentrations of precious metals (Au, Ag, Pd) which are in the same range than the concentrations in natural ores. Urban mining is a solution proposed to tackle over-exploitation raw materials. Waste recycling is about a great interest for ethical, environmental and economic reasons. Both prokaryotes and fungi are known to be important actors in the biogeochemical cycling of elements. Indeed, bacterial-fungal (BF) consortia can be used for mobilize and immobilize metals in order to recover them. The use of BF consortia is a consequence of the synergistic interactions (geochemistry and coexistence) between bacterial and fungi. Therefore, the aim of this study is to recover metals from anthropogenic waste through BF consortia.
Methods
Organic and inorganic characterization of anthropogenic waste was carried out to assess the most appropriate conditions to trigger metals by bioremediation. Metal-resistant BF couples were isolated from a metal-polluted site and urban waste. Several experiments were designed to investigate their bioremediation capabilities of BF comparing cocultures to monocultures.
Results
The isolation of microbes in their natural environment seems to be an effective strategy to select metal tolerant organisms. Moreover, model organisms can be acclimatized on specific anthropogenic waste to apply a specific metal-microbe interaction. Specific BF coculture are more tolerant as compared to single species cultivated alone.
Conclusions
Microbial consortia may be a good strategy for urban mining. Nevertheless, additional work is still required to understand the conditions that lead to effective metal biorecovery in function of the type of waste.

**Student paper*

Environmental microbiolgy / P-51*

Identification of microorganisms in aerobic granular sludge actively involved in biological phosphorus removal

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Background

One of the aims of wastewater treatment is the removal of phosphorus prior to discharge into the environment. The biological phosphorus removal is based on the ability of a group of microorganisms, named "Polyphosphate Accumulating Organisms" (PAO), to store large quantities of intracellular polyphosphate.

In this study, we are focusing on the PAO actively involved in bioreactors operated with Aerobic Granular Sludge (AGS) technology. This process based on dense microbial biofilms is a cost-effective and land-saving alternative to the conventional biological wastewater treatment with activated sludge. This promising technology has received a significant commercial interest, but questions regarding the system performances remain in the context of the full scale application.

Indeed, the knowledge acquired on AGS has been mainly obtained with model laboratory-scale experiment using artificial wastewater. Therefore, the PAO responsible for the biological phosphorus removal might differ for a full scale wastewater treatment operated with AGS.

Methods

The studies were performed on AGS-reactors operated at Eawag, Dübendorf with different wastewater compositions. The identification of the PAO relies on their distinct phenotype revealed with a fluorescent probe targeting the intracellular polyphosphate structures.

Results

Sequencing of the 16S rRNA gene amplicons of PAO selected with flow-cytometry was used to uncover the phylogenetic affiliation of the different actors involved in the phosphorus removal.

Conclusions

We hypothesize that the maintenance of different populations involved in the same functional process is explained by multiple ecological niches created by the nutrient complexity present in the wastewater together with the microbial structuration of the AGS.

**Student paper*

Environmental microbiolgy / P-52*

A historical legacy of antibiotic utilization on bacterial seed banks in sediments

Mr. Christophe Paul [1], Dr. Laura Madueño [1], Dr. Thomas Junier [2], Mrs. Zhanna Bayrychenko [1], Dr. Sevasti Filippidou [1], Ms. Karin Beck [3], Prof. Gilbert Greub [4], Dr. Helmut Bürgmann [3], Prof. Pilar Junier [1]

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Background

The introduction of antibiotics for both medical and non-medical purposes has had a positive effect on human welfare and agricultural output in the past century. However, there is also an important ecological legacy regarding the use of antibiotics and the consequences of increased levels of these compounds in the environment as a consequence of their use and disposal.

Methods

This legacy was investigated in Lake Geneva using a sediment core previously used for the paleoecological reconstruction of the last 100 years of the lake. Two antibiotic resistance genes (ARG) were quantified in bacterial seed bank DNA. These genes confer resistance to tetracycline (*tet*(W)) and sulfonamide (*sul*1).

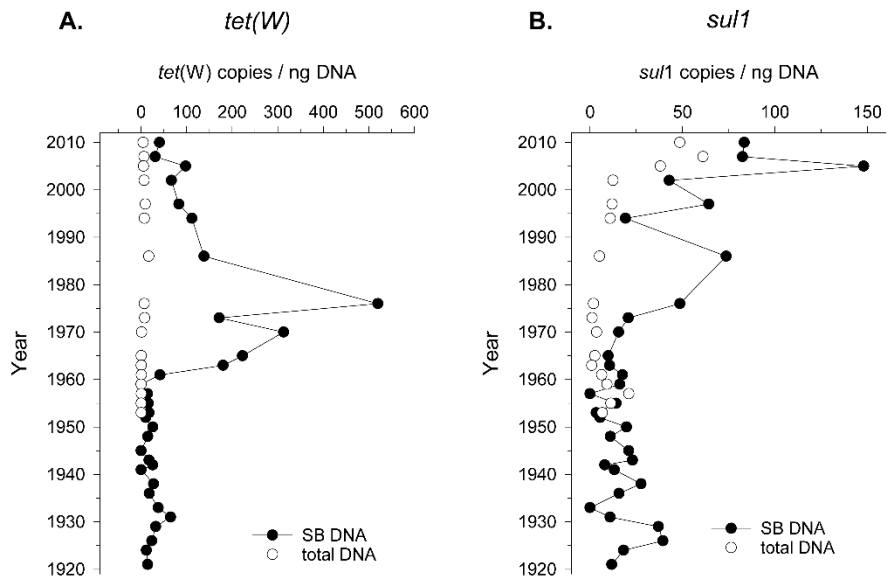
Results

The industrial introduction of antibiotics caused an abrupt increase in the total abundance of tet(W) and a steady increase in sul1. The abrupt change in tet(W) corresponded to an increase in relative abundance from ca. 1960 that peaked around 1976. This pattern of accumulation was highly correlated with the abundance of specific members of the seed bank community belonging to the phylum Firmicutes. In contrast, the relative abundance of sul1 increased after 1976. This correlated with a taxonomically broad spectrum of bacteria, reflecting sul1 dissemination through horizontal gene transfer. The accumulation patterns of both ARGs correspond broadly to the temporal scale of medical antibiotic use.

Conclusions

Our results show that the bacterial seed bank can be used to look back at the historical usage of antibiotics and resistance prevalence.

**Student paper*



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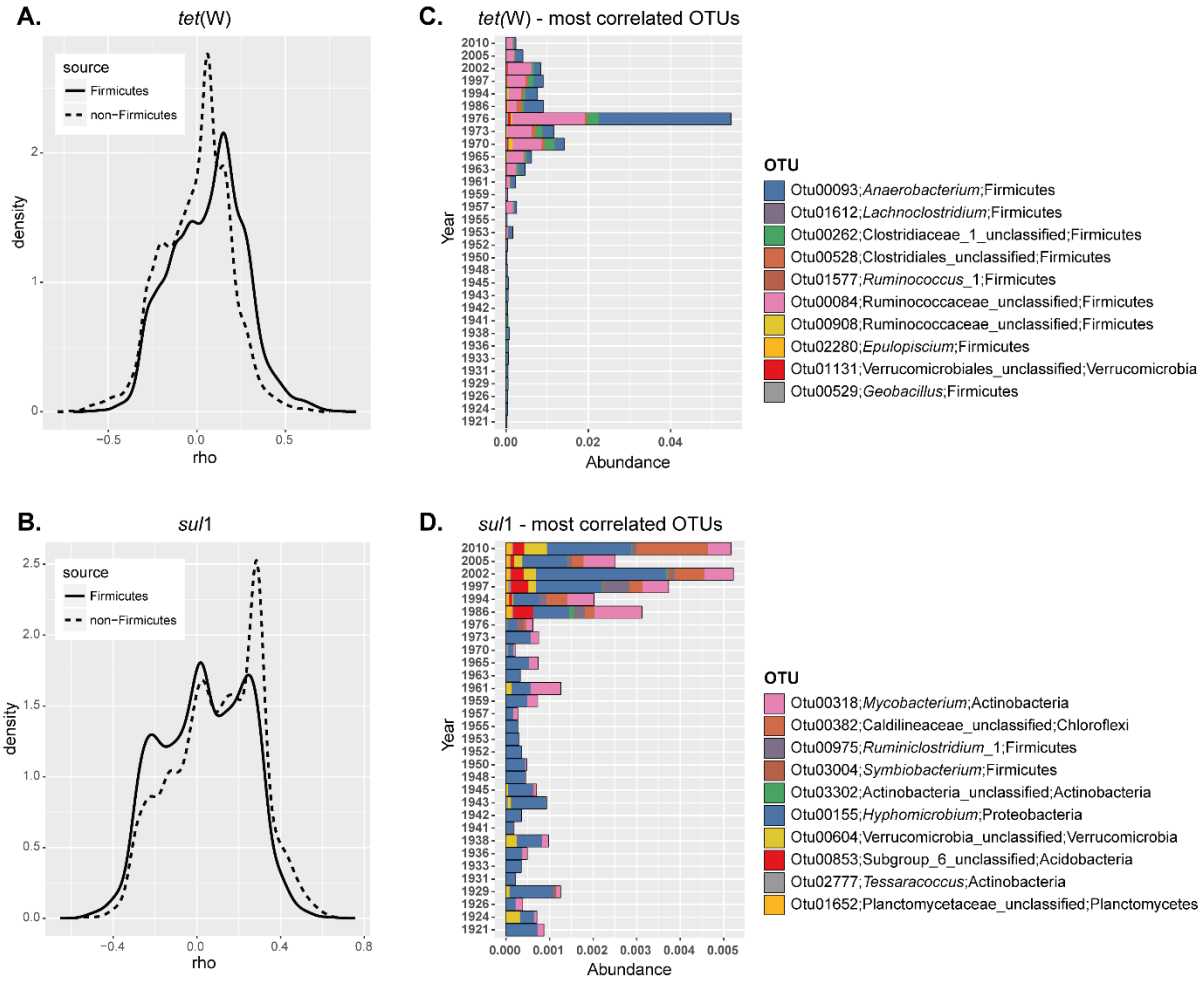

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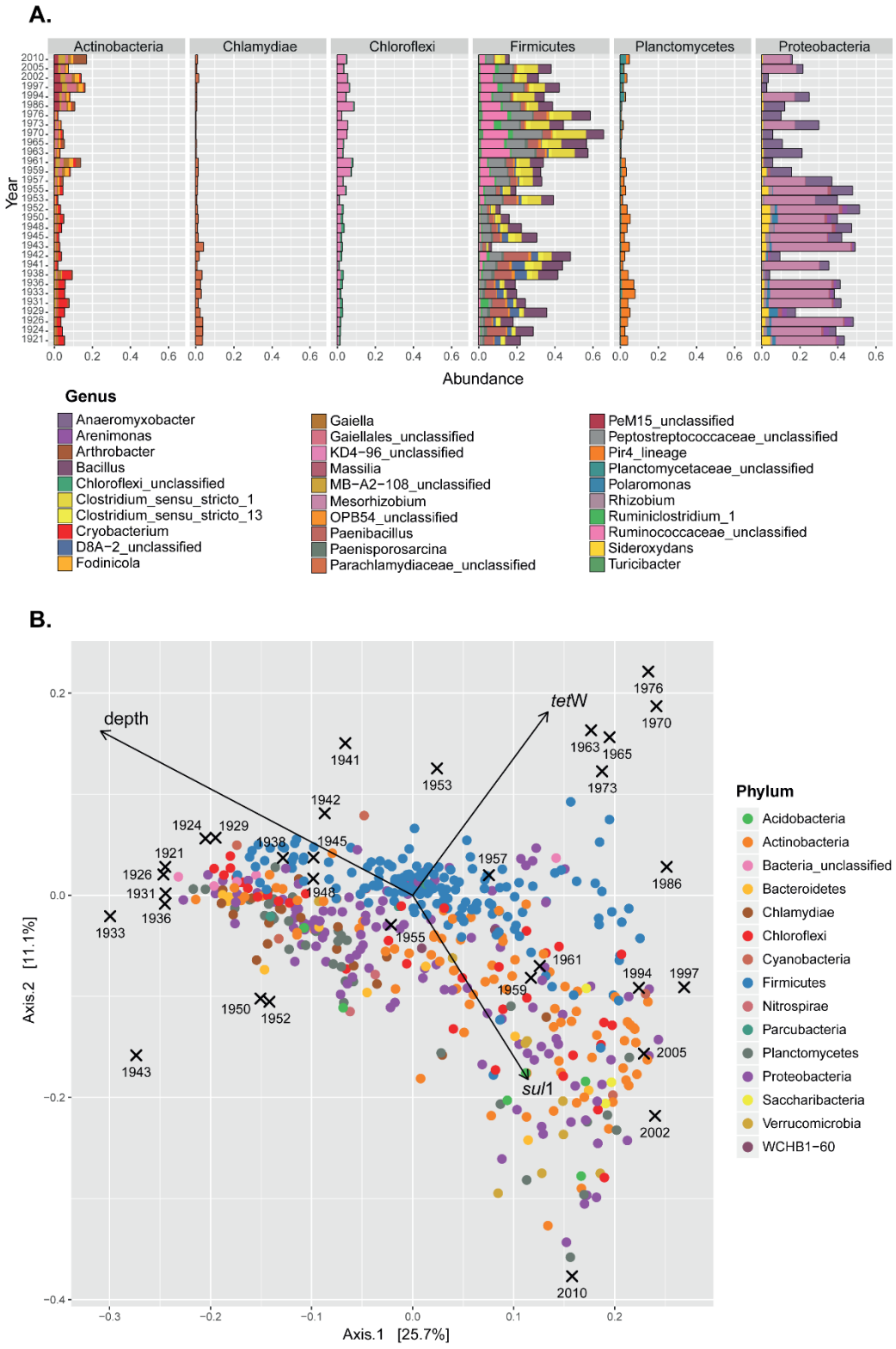

Attachments #243

Environmental microbiolgy / P-53

Characterization of the predicted membrane anchor of reductive dehalogenases

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1. *École polytechnique fédérale de Lausanne (EPFL),* ***2.*** *EPFL*

Background
Organohalide respiration (OHR) is a process that couples the reduction of halogenated organic compounds to energy conservation in phylogenetically diverse anaerobic bacteria. The key enzyme in OHR is well characterised and is encoded by the reductive dehalogenase (*rdhA*) gene, which is always accompanied by a short second gene (*rdhB*). While RdhB was proposed to be the cytoplasmic membrane anchor of RdhA, no clear evidence has yet been published. *Dehalobacter restrictus*, a strictly anaerobic and obligate OHR bacterium is used as model organism to study organohalide respiration of tetrachloroethene (PCE), a major groundwater pollutant. Here, we aim at understanding the role of PceB as membrane anchor for the PCE reductive dehalogenase (PceA).
Methods
First PceB or parts thereof are produced heterologously in *E. coli*. These proteins are used in pull-down experiments with cell extracts of *D. restrictus* to trap PceB interacting proteins, among which PceA should be clearly identified. Direct protein interactions between PceB and PceA will be addressed by producing a soluble version of PceA in *E. coli* and by applying a combination of size-exclusion chromatography and other techniques.
Results
As PceB harbours 3 transmembrane helices, its production as soluble protein is challenging. Therefore loops in-between helices were produced in fusion with GST. A preliminary pull-down experiment showed that loop-2 is interacting with PceA in cell extract. A soluble PceA protein is obtained in *E. coli* with help of PceT (1) and used for direct protein interaction analysis with GST-PceB proteins.
Conclusions
The function of PceB as membrane anchor will be discussed.

Environmental microbiolgy / P-54

Sulfur cycling in the terrestrial deep subsurface

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1. *EPFL,* ***2.*** *Posiva Oy,* ***3.*** *Envi*

Background
Olkiluoto, an island in south-west Finland, has been selected as the site for a deep geological repository for the final storage of spent nuclear fuel. It is therefore important to understand the geomicrobial processes underway at this site to ensure long-term, safe storage of the fuel. Of particular concern is the generation of sulfide, as it can induce corrosion of the waste-bearing copper canisters. Groundwater at Olkiluoto is geochemically stratified with depth, and sulfide production is observed when shallower sulfate-rich groundwater mixes with deeper methane-rich groundwater. To constrain the electron donor(s) driving the sulfidogenesis, we investigated three groundwaters from different depths with varying concentrations of sulfide.
Methods
Genome-resolved metaproteogenomics revealed distinct metabolic processes mediating sulfur cycling in each groundwater. To confirm the activity of these metabolisms, we performed groundwater incubations with isotopically labelled substrates and conducted fluorescence *in situ* hybridization (FISH) coupled to nanoscale secondary ion mass spectrometry (nanoSIMS).
Results
In the groundwater with the least sulfide, groundwater was amended with nitrate and sulfide to demonstrate nitrate-dependent sulfide oxidation by *Epsilonproteobacteria*. With groundwater from the transition between sulfate-rich and methane-rich groundwaters, we amended incubations with ^{13}C-labelled methane to probe whether methane-oxidation by anaerobic methane oxidising archaea was coupled to sulfate- or nitratereduction. In the groundwater with the highest sulfide concentration, ^{13}C-labelled acetate and bicarbonate were added to demonstrate autotrophic and heterotrophic sulfate reduction in deep methane rich groundwater.
Conclusions
The results contribute towards our understanding of microorganisms in deep terrestrial subsurface ecosystems and their role in geochemical cycling.

Environmental microbiolgy / P-55* => see S-17*

Environmental microbiolgy / P-56* => see S-77*

Prokaryotic biology / P-57

Zinc homeostasis in Pseudomonas aeruginosa: deciphering the role of Zur as a master regulator of metal import and export

Ms. Verena Ducret [1]***, Dr. Manuel Gonzalez*** [1]***, Ms. Sara Leoni*** [1]***, Dr. Perron Karl*** [1]

1. *Microbiology Unit, Department of Botany and Plant Biology, University of Geneva, Geneva*

Background
P. aeruginosa possesses a wide range of systems involved in zinc (Zn) homeostasis. In addition to the conventional transporter ZnuABC, it owns an arsenal of other proteins that contribute to its survival even in high Zn deficiency. All these uptake systems are regulated by the Zur protein.
This bacterium is also able to withstand high concentrations of Zn thanks to the CzcCBA efflux system, which is able to effectively expel the cytoplasmic or periplasmic metal out of the cell. This pump is under the control of the Zn-inducible two-component system CzcRS.
Methods
In order to study the ability of P. aeruginosa to adapt to variations in Zn concentrations, we followed the expression of all genes involved in Zn homeostasis during a transition from a deficiency to an excess of metal. This kinetic experiment was carried out in a wild-type (wt) strain but also in the Dzur and DczcRS mutants.
Results
Results showed a defect in the induction of the export system in the Dzur strain. Indeed, in this mutant, expression of CzcRS and the CzcCBA efflux pump was weaker and delayed, as measured by qRT-PCR, western blot and czcC-GFP fusion. Moreover, the Dzur strain was more sensitive to Zn excess compare to the wt.
Conclusions
Taken together, these results confirm the involvement of Zur in Zn export systems and making it the major regulator of Zn homeostasis in Pseudomonas aeruginosa.

Prokaryotic biology / P-58*

Interplay between β-lactam antibiotic resistance determinants and Caulobacter crescentus

Mr. Jordan COSTAFROLAZ *1*, ***Prof. Viollier Patrick*** *1*

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Background
β-lactam antibiotics are the most frequently used antimicrobial molecules in the treatment of infectious (bacterial) diseases. These agents kill bacteria by perturbing the synthesis of the peptidoglycan layer of bacteria. Resistance to β-lactams can be acquired or due to intrinsic mechanisms. We choose to explore intrinsic mechanisms of resistance in the bacterium *Caulobacter crescentus* that exhibits intrinsic resistance to all major classes of β-lactams (carbapenems, penicillins, cephalosporins and monobactams) as well as to certain quinolone antibiotics that inhibit DNA replication by targeting DNA gyrase including nalidixic acid (Nal).
Methods
To identify genes that protect *C. crescentus* from β-lactams, we launched a series of transposon insertion sequencing (Tn-Seq) experiments with wild-type cells plated on a different of β-lactams after transposon (Tn) mutagenesis. We sought Tn insertions that were synthetically lethal, i.e. no longer recovered, when cells were grown on β-lactams and found genes required to protect against β-lactams.
Results
Including that a chromosomally encoded β-lactamase (BL). Again using Tn-Seq, we then asked if protection to certain β-lactams could be restored in cells lacking the BL gene (ΔBL) and fortuitously discovered that Nal (an antibiotic used to eliminate *E. coli*cells that deliver the Tn to *C. crescentus*) protects the ΔBL mutant against β-lactams.
Conclusions
These experiments reveal a double-layered intrinsic mechanism for β-lactam resistance in *C. crescentus*, including one that is inducible by a different type of antibiotic. We are now using forward genetic methods to dissect the molecular mechanism(s) this inducible intrinsic resistance and will explore the range of action towards other antibiotics.

**Student paper*

Prokaryotic biology / P-59* => see PB-11*

Prokaryotic biology / P-60* => see S-48*

Prokaryotic biology / P-61 => see S-55

Mycology / P-62

Identification of positive and negative mediators of antifungal tolerance in Candida albicans

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Background
Antifungal tolerance can be defined as the ability of *C. albicans* cells to survive at high antifungal drug concentrations but without acquiring mutations associated with resistance. The mechanisms mediating drug tolerance are still not well understood and their study may reveal factors contributing to treatment failures and facilitate the design of improved therapies. In this study we aimed to identify mediators of tolerance to fluconazole (FLC)
Methods
We used a collection of 582 tetracycline-inducible overexpression barcoded strains constructed in the background of BWP17 (SC5314 derivative), which were pooled and maintained under FLC pressure for five days of repeated subcultures. This strategy was used to enrich and/or deplete the pool in strains with increased and/or decreased tolerance. After amplification and Myseq sequencing of all barcodes from the cultures, the fractional index of each population was calculated to identify positive (enriched strains) and negative (depleted strains) regulators of tolerance.
Results
Two potential positive tolerance mediators (the transcription factors *CRZ1* and *GZF3*), when overexpressed, were confirmed by single culture assays in BWP17. This strain background was found not suitable for testing individual negative tolerance mediator due to intrinsic low tolerance levels. We therefore challenged potential negative tolerance mediators in different *C. albicans* clinical isolates with high FLC tolerance levels. Thus, we identified at least 3 potential negative regulators (*SFL2*, *CPH1*, *CSK1*).
Conclusions
Our approach based on the use barcoded strains revealed several tolerance mediators which are still needing further characterization. Given that only 10% of the ORFome was used here, additional tolerance mediators may be identified.

Mycology / P-63*

Site-directed mutagenesis of the 1,3-β glucan synthase catalytic subunit of the human pathogenic fungus Pneumocystis jirovecii suggests its sensitivity to caspofungin

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Background
Echinocandins inhibit the catalytic subunit Gsc1 of the enzymatic complex responsible for the synthesis of 1,3-β glucan, an essential constituent of the fungal cell wall. Studies in rodent models demonstrated the efficacy of the echinocandin caspofungin to treat *Pneumocystis* pneumonia. However, its efficacy against *Pneumocystis jirovecii* infecting specifically humans remains controversial. Our aim was to study the sensitivity of the *P. jirovecii*Gsc1 to caspofungin.
Methods
In absence of an established *in vitro* culture method for *Pneumocystis*, we used functional complementation of the *Saccharomyces cerevisiae gsc1*deletant. In the fungal pathogen *Candida albicans,*resistance to echinocandins is conferred by point mutations leading to two amino acid substitutions within the hotspot no. 1 of mutations of Gsc1. We used site-directed mutagenesis to introduce the corresponding mutations within the *gsc1*gene of *P. jirovecii*as well as of the *Pneumocystis* species infecting rats and mice. Sensitivity to caspofungin was assessed using spot test on solid medium, E-test, and YeastOne®.
Results
The *S. cerevisiae gsc1*deletant showed an increased sensitivity to caspofungin compared to the wild type. Upon expression of a *Pneumocystis gsc1* gene on plasmid, a partial restoration of the wild type growth was observed. The latter restoration increased in presence of one substitution, and increased more in presence of two substitutions. Sensitivity levels were similar for Gsc1 from all three *Pneumocystis* species.
Conclusions
The *P. jirovecii*Gsc1 catalytic subunit is sensitive to caspofungin as those of *Pneumocystis* species infecting rodents, suggesting the usefulness of this drug in human infections.

**Student paper*

Mycology / P-64

First Detection of TR34/L98H mutation in Aspergillus fumigatus isolates from clinical and environment in Switzerland

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Background

*Aspergillus fumgatus*isolates resistant to azole drugs have been reported in several countries, however no recent data exist in Switzerland. Snelders and colleagues have described azole resistance in *A. fumigatus* isolated from clinical samples for the first time in 2008. The mutations found in the azole-resistant isolates was associated with the gene *CYP51A*, whose product is involved in ergosterol biosynthesis. Different studies have suggested an environmental origin of the TR_{34}/L98H and TR_{46}/Y121F/T289A mutations with a predominance of TR_{34}/L98H.

Methods

Phenotypic analyses were used to screen the presence of *A. fumigatus*both in clinical and environmental samples. Gene sequencing confirmed the presence of the azole resistance mutations and CSP1 typing allowed a categorization of the resistant isolates.

Results

Here, we describe the first clinical and environmental *A. fumigatus*isolates, which harbored *CYP51A*mutations that confer azole resistance. Over the year 2016, we screened, from clinical samples, every *A. fumigatus*strain for azole resistance. We identified two isolates harboring the TR_{34}/L98H, from cystic fibrosis patients. Prior to the screening procedure, sixty-nine environment samples were collected in the Geneva Lake area and five of them showed resistance to azoles drugs. Four isolates possessed the TR_{34}/L98H mutation and one isolate had a single substitution at the position 54 (G54) of *CYP51A*.

Conclusions

In conclusion, the occurrence of azole resistance from clinical and environmental origin in Switzerland is in agreement with reports from other countries. This situation needs further systematic surveillance, since transmission of azole-resistant isolates to patients is possible.

Virology / P-65*

Atypical porcine pestivirus (APPV) in Switzerland: An emerging virus?

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Background
In 2015, a new pestivirus was identified in pig sera in the US. This new ”atypical porcine pestivirus” (APPV) was later linked to piglets with congenital tremor. Since then, APPV has been reported in several European, American and Asian countries. The prevalence appears to be rather high, but the lack of knowledge on virus diversity and the use of various diagnostic tests prevents direct comparisons.
Methods
Sera and tissue specimens were collected from samples obtained between 2006 and today. To determine the presence of APPV, we developed a real-time RT-PCR assay for the detection of viral RNA.
Results
For 2006, we identified 31 samples from 11 cantons to be positive for APPV out of a representative set of 184 sera samples from 26 cantons, which corresponds to a prevalence of 16.8%. In addition, various samples collected in 2011, 2013, 2017, and 2018 were positive for viral RNA, including samples from piglets with congenital tremor. Sequencing of positive samples and phylogenetic analysis revealed a broad diversity of APPV strains in Switzerland that are clearly distinct from sequences reported from other isolates in Europe and overseas.
Conclusions
This study indicates that APPV has been widely distributed in Switzerland for many years and provides new data on the epidemiology of this pestivirus in the Swiss pig population. In addition, it became evident that it is essential to develop sensitive and specific diagnostic tests based on the virus strains present in Switzerland, particularly for differential diagnosis from other pestivirus infections that are to be eradicated in Switzerland.

**Student paper*

Virology / P-66*

Characterization of coronavirus spike – receptor interactions among different host species

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Background
SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome) coronavirus (CoV) caused severe outbreaks in humans in the past, SARS-CoV first in China (2002) and MERS-CoV in the Middle East (2012). Both are zoonotic viruses, with the animal sources of SARS-CoV traced back to horseshoe bats as the primary reservoir and MERS-CoV transmitted via dromedary camels, with bats as a possible vector for transmission to humans and a possible viral reservoir.
Methods
We used the Vesicular Stomatitis Virus pseudovirus system to express spike proteins of different species and to evaluate the permissiveness to either cell lines originating from human, bat, alpaca or llama sources or upon overexpression of the recombinant receptor in HEK293T target cells.
Results
We could show that camel and bat spike pseudoviruses enter HEK293T target cells if the respective receptor is expressed. As previously reported, viruses pseudotyped with camel spike are able to enter the human hepatoma cell line Huh7, whereas bat spike pseudoviruses are not. Both alpaca and llama cell lines are susceptible to camel spike pseudovirus and to the human MERS-CoV, SARS-CoV and HCoV-229E pseudovirus. Some bat cell lines can be infected with pseudovirus expressing the bat spike protein as well as the human MERS-CoV, SARS-CoV or HCoV 229E spike.
Conclusions
In conclusion, we could show that spike-receptor interaction represents a crucial species barrier that can be overcome by zoonotic viruses. A detailed understanding of CoV spike receptor interaction will be crucial in understanding transmission pathways to prepare for future zoonotic virus outbreaks.

**Student paper*

Lay communication / P-67*

Pedagogic board games: which impact on which audiences?

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Background

Emerging and imported pathogens remain mostly unknown to the general public and often overlooked by health professionals due to their rarity and diversity. This is an issue considering that long-haul flights, outdoor hobbies and climate changes expose an increasing number of people to exotic environments and disease vectors. Communication supports should help to educate on prevention and detection of these diseases. However, passive reading of texts or infographics can be insufficient to generate a durable knowledge. Accordingly, our group developed a pedagogic board game, "*KROBS*", introducing agents of arthropods, water and food-borne diseases as well as related preventive measures.

Methods

A questionnaire with 25 questions covered by *"KROBS"* and with 25 questions not covered by "*KROBS"* was designed. Three groups of interest were identified: (i) academic researchers of our microbiology institute, (ii) technicians of our microbiology diagnostic laboratory and (iii) lay public exposed to the game in toy libraries.

Results

Submission of this questionnaire before and after exposition to the game will allow to assess its pedagogic impact on groups with different backgrounds and interests.

Conclusions

Board games represent an alternative to diffuse knowledge on our field of interest to the general public. However, considering the resources needed for their development, their impact should be established. Positive results could motivate further developments to cover broader microbiology topics and to reach other audiences such as medical students.

**Student paper*

Lay communication / P-68 => see S-44

Anti-bioresistance / P-69*

Mode of action and resistance determinants of dendrimer antimicrobial peptides in Gram-negative bacteria

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Background

Antimicrobial peptides (AMPs) are one of the most diverse classes of antibiotics. They are most often polycationic and act by disrupting the bacterial membrane. A prominent example is colistin, used as last-resort drug against MDR Gram-negative bacteria. Among explored synthetic peptides, cyclic and dendrimeric AMPs display promising pharmacokinetic properties. G3KL is a dendrimer AMP, which shows potent activity against MDR Gram-negative pathogens like *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Methods

To unravel the mode of action and potential resistance mechanisms of G3KL, we selected spontaneous resistant mutants on polymyxin B (Pmx-B) and on G3KL in *P. aeruginosa, A. baumannii* and *Escherichia coli*. We analyzed the mutants by MIC determinations and whole genome sequencing.

Results

We obtained Pmx-B resistant mutants in*E. coli, A. baumannii* and *P. aeruginosa,*while G3KL resistant mutants were obtained only with *E. coli* and*A. baumannii*. The Pmx-B-selected mutants of *E. coli* and *A. baumannii* were not cross-resistant to G3KL and the G3KL-selected mutants were not cross-resistant to Pmx-B. Two classes of Pmx-B-selected mutants were obtained in *P. aeruginosa*: one class remained susceptible, while the other class was cross-resistant to G3KL.

Whole genome sequencing of G3KL-resistant mutants from *E. coli* and*A. baumannii* identified mutations in genes involved in lipopolysaccharide transport, while Pmx-B-resistant mutants showed alterations in genes regulating lipopolysaccharide modification. Importantly, G3KL-selected mutants in all three organisms remained susceptible to other dendrimer AMPs.

Conclusions

Pmx-B and G3KL show both specific and common mutational targets, suggesting similar but distinct mode of actions, which is encouraging for potential therapeutic applications.

**Student paper*

Anti-bioresistance / P-70*

The impact of wastewater treatment plants on the prevalence of antibiotic resistant bacteria in Swiss rivers

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Background
River systems have been pointed out to be a major route for spreading antibiotic resistance from Wastewater treatment plants (WWTPs) effluents. Therefore, the impact of WWTPs on receiving rivers, and the downstream fate of antibiotic resistant bacteria (ARB) should be investigated.
Methods
Eleven sampling campaigns were performed at 9 WWTPs and their receiving rivers during July and October 2017. Three campaigns were performed during or after precipitation events, the rest of campaigns were performed under dry conditions. Water samples were collected from four locations – Upstream (US), wastewater effluents (EF), and downstream D1 (0.5 km), and D2 (1 km from discharge point). Conductivity, Cl^-, and SO_4^{2-} were measured for estimating dilution factors (DF), and following types of bacteria were cultivated: 1) total heterotrophs on R2A; 2) extended spectrum β-lactamase producing organisms; 3) carbapenem resistant Enterobacteriaceae; vancomycin resistant Enterococci.
Results
The differences in total heterotrophs and ARB abundances were not significant between US and D1. The abundances of total heterotrophs from US, D1, and D2 were significantly higher for the samples under rainy conditions. DF varied significantly by site which may have lowered the statistical significance. Furthermore, the decay of resistant bacteria from D1 to D2 was not significant within the given distance of this study.
Conclusions
The effects of rainwater event and DF will be considered for identifying the impact of WWTPs. Metagenomic and qPCR based analyses of resistance genes are under way. Furthermore, future studies will be performed over longer downstream distance (~10km) to confirm the fate of ARB.

**Student paper*

Anti-bioresistance / P-71*

Antibiotic potential of the Arabidopsis leaf microbiome

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Background
The increasing number of multi-resistant pathogens is a threat to human health around the world. Over the past years, only a few novel antimicrobial drugs have been brought to market. The identification of novel antibiotics is therefore critical for the appropriate treatment of pathogens in the context of rapidly evolving drug resistance.
Methods
We employed activity and genome guided approaches to identify promising antimicrobial producer strains from a genome sequenced bacterial collection. The bacteria were isolated from the phyllosphere of *Arabidopsis* and tested in binary interactions. As a complementary approach, genomic information of the strains was evaluated for the presence of biosynthetic gene clusters. Promising strains were then subjected to activity-guided isolation of active compounds followed by structure elucidation.
Results
A binary interaction screen with 224 bacterial leaf isolates revealed 725 inhibitory interactions. A large proportion of observed inhibitions were caused by only a few bacterial isolates mainly belonging to the orders of *Bacillales* and *Pseudomonodales*. In-depth analysis of the most potent producer-strain, a *Brevibacillus* strain, revealed the production of four active small molecule compounds. Structure analyses showed that two of these compounds are novel. Notably, one of these showed activity against Gram-negative bacteria. The biosynthetic gene clusters were validated by mutagenesis.
Conclusions
Plant-colonizing bacteria are a rich source for the isolation of antimicrobial compounds. Therefore, screening of more isolates is a promising approach to discover novel therapeutics to combat resistant pathogens. Moreover, this study provides a basis to examine the relevance of antibiotic production for shaping the bacterial community structure *in planta*.

**Student paper*

Anti-bioresistance / P-72 => see EM-04

Anti-bioresistance / P-73* => see EM-12

Anti-bioresistance / P-74 => see S-15

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