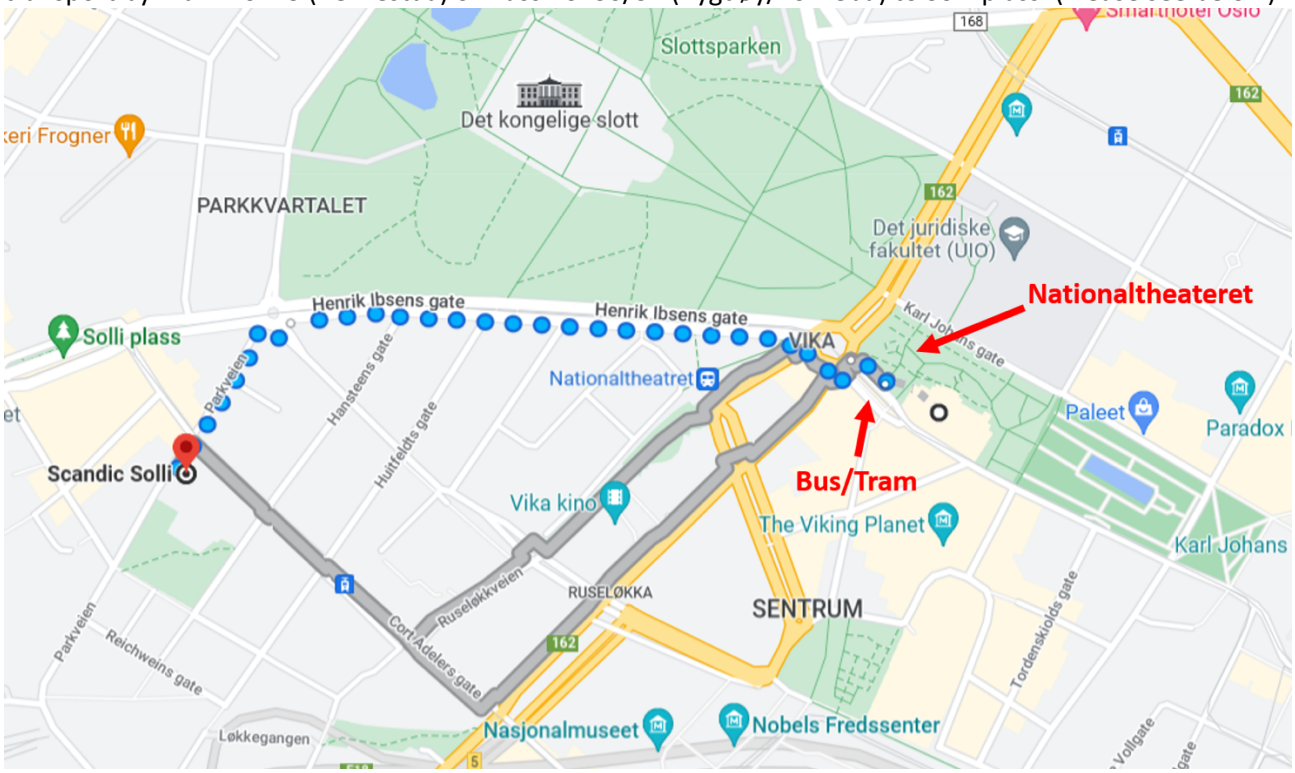
IBA Annual Meeting 2022, Oslo

Date: October 27th and 28nd 2022

Meeting location: Vika room at Scandic Solli, Parkveien 68, Oslo

Meeting dinner (for everyone): Vika room at Scandic Solli, Oslo
Accommodation (for registered attendees): Scandic Solli, Oslo

You can take train from the Oslo airport for coming directly to Nationaltheatret train station (takes around 30 min), which is located 650 m away from Scandic Solli, Parkveien 68. You can reach the hotel by public transport by Tram no. 13 (Bekkestua) or Buss no. 30/31 (Bygdøy/Fornebu) to Solli plass. (Please see below).



Organizing committee:

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Tone Tønjum, University of Oslo (UiO), Faculty of Medicine, Institute of Clinical Medicine, Dept. of Microbiology

Ole Andreas Løchen Økstad, University of Oslo (UiO), The Faculty of Mathematics and Natural Sciences, Dept. of Pharmacy

Thursday	October 27th	
10:00	Registration and Poster set up	
11:00	Welcome and opening remarks	
Session 1	Chair: Tone Tonjum & Milda Kaniusaitê	
11:15	Evolution of virulence - The *Legionella* paradigm	Carmen Buchreiser, *Pasteur Institute*
12:00	Genomic K-capsular antigen (Group 2) typing and prevalence in *E. coli* from bloodstream infections in Norway	Rebecca A. Gladstone, *UiO*
12:15	Sanitary Condition and Hygienic Practice of Street Food Vendors in Selected Towns of Ethiopia: a Cross-sectional Study Addressing Public Health Concern	Moges Mathewos, *Jimma University*
12:30	Evolution of *Vibrio cholerae* biofilms	Øyvind M. Lorentzen, *UiT*
12:45	Early life probiotic treatment reshapes antibiotic-impaired gut microbiota, resistome, and mobilome networks in extremely preterm infants	Ahmed Bargheet, *UiT*
13:00	Lunch	
Session 2	Chair: Dominique A. Y. Caugant & Srijana Bastakoti	
14:00	Molecular genomic analyses of decreased beta-lactam susceptibility in *Streptococcus pyogenes*: Is it 5 minutes before midnight?	James M. Musser, *Houston Methodist Institute*
14:45	The transcription of the *Staphylococcus aureus* peptidoglycan hydrolases SAOUHSC_00671 and *ssaA* is interconnected	Danae Morales Angeles, *NMBU*
15:00	Characterisation of putative virulence factors in *Enterococcus faecium*	Ingeborg Mathiesen, *UiT*
15:15	BK Polyomavirus successfully infects a polarized renal proximal tubule epithelial cell model	Elias M. Lorentzen, *UiT*
15:30	Commensal E*nterobacteriaceae* isolated from human stools are capable of out-competing enterotoxigenic *Escherichia coli* in vitro	Oda B. Vedøy, *UiB*
15:45	Break (15 min)	
16:00	Poster session (1.5 hrs)	
19:15	Aperitif	
19:30	Dinner	

Friday	October 28th	
8:30	IBA board meeting (board, SAB and PhD reps only)	
Session 3	Chair: Ole A. Økstad & Rebekka R. Rolfsnes	
09:30	Sensing and adapting with RNA in M*ycobacterium tuberculosis*	Kristine Arnvig, *UCL*
10.15	Novel mycobacterial tolerance inhibitors (MTIs) against multidrug-resistant tuberculosis	Tahira Riaz, *UiO*
10:30	Can insight from bacteriocin:receptor interaction studies help design novel antimirobial peptides?	Sofie Kristensen, *NMBU*
10:45	Break (15 min)	
11:00	Can`t touch this – Improving catalysis is useless if substrate doesn't bind	Christopher Fröhlich, *UiT*
11:15	Spatiotemporal distribution and diversity of airborne resistant bacteria: An exploratory observational study in the urban and rural environments of Bangladesh	Muhammad Asaduzzaman, *UiO*
Session 4	Chair: Mike Koomey & Daniel Hatlem	
11:30	The dynamic trio – Species definition by mobile DNA	Ole A. Økstad, *UiO*
12:15	Mucosal antibody response targeting glycosylated epitopes of E*scherichia coli* mucinase YghJ (SslE)	Saman Riaz, *UiB*
12:30	Proteins condensing DNA during the SOS response identified by a machine learning-assisted, genome-wide screening	Krister Vikedal, *UiO*
12:45	Closing session	
13:00	Lunch	

Keynote speakers

Session 1

Carmen Buchreiser, Pasteur Institute

Carmen Buchrieser is Professor at the Institut Pasteur, France. She obtained her PhD from the University of Salzburg, Austria, continued as a postdoc researcher in the USA and the Institut Pasteur, and was then appointed Director of the Biology of Intracellular Bacteria Unit at the Institut Pasteur in 2008.

Her major interest is to understand how bacteria cause disease: what are the genetic factors conferring bacterial virulence, how do they evolve, how to they allow subverting host functions, how do human pathogens emerge. After conducting postdoctoral trainings in the USA and the Institut Pasteur in Paris, she was appointed in 2008 director of the Unit "Biology of Intracellular Bacteria" at the Institut Pasteur, Paris, France. The research activity of her group focuses on investigating the genetic bases of virulence of *L. pneumophila* and *L. longbeachae*, The projects of her laboratory are focused on the identification and study of bacterial virulence factors, with particular emphasis on their functions, the mechanisms leading to their acquisition and their evolutionary origin.

Her work has been recognized through a number of membership elections including: German Academy of Science « Leopoldina », EMBO, American Academy of Microbiology, European Academy of Microbiology, Academia Europea.

Session 2

James M. Musser, M.D., Ph.D., Houston Methodist Institute

James M. Musser, M.D., Ph.D. is the Fondren Distinguished Presidential Endowed Chair of the Department of Pathology and Genomic Medicine for the Houston Methodist Hospital System in Texas. He also is Director, Center for Infectious Diseases at the Houston Methodist Research Institute. His laboratory uses genome-wide integrative analysis of *Streptococcus pyogenes* as a model to decipher the molecular events underpinning pathogen-host interactions and pandemics. He is an elected member of many professional societies and has received many awards for his research contributions fusing bacterial molecular pathogenesis, population genomics, and infectious diseases. He also has had a decades-long interest in developing a group A streptococcus vaccine. Some of his group's recent efforts have focused on understanding the molecular genetic basis of decreased susceptibility of group A streptococcus to beta-lactam antibiotics. He has published 450 papers and book chapters in the field of bacterial pathogenesis, bacterial population genetics, and infectious diseases, and has an h-index of 111.

Session 3

Kristine Arnvig, University College London (UCL)

Kristine Arnvig is an associate Professor at University College London, United Kingdom. She received her scientific training at The University of Copenhagen, Denmark and at the National Institute for Medical Research, UK, where she started her work with M. tuberculosis. In 2013 she set up her own lab at UCL, where her research is focused on M. tuberculosis regulatory RNAs.

Mycobacterium tuberculosis (*Mtb*) remains one of the biggest killers throughout human history. Yet, very little is known about the RNA biology of this very successful pathogen. Research over the last two decades has demonstrated that RNA is much more than an intermediary between genes and proteins. This multi-functional molecule has turned out to perform regulatory roles that affect all levels of gene expression in all domains of life. Bacterial RNA-based regulators (riboregulators) come in different shapes and sizes including small RNAs (sRNAs), antisense RNA, 5' leaders and riboswitches, and they form an extensive network of co- and post-transcriptional regulators that modulate and fine-tune the protein-based regulation of gene expression mediated by e.g. transcription factors. Our aim is to obtain a deeper understanding of the basic (RNA) biology of *Mtb*, which in time will support the development of novel diagnostics, drugs and vaccines. By combining high-throughput methods with a range of genetic and molecular biology approaches, we aim to characterize the expression, processing, degradation and regulatory role of different *Mtb* riboregulators.

Sesssion 4

Ole Andreas Økstad, Dept. of Pharmacy, University of Oslo

Ole Andreas Økstad is professor of pharmaceutical microbiology at the Department of Pharmacy, University of Oslo. He graduated with a PhD in microbiology at the Department of Pharmacy in 2000. Following a one year at the Norwegian Defense Research Establishment (2000), Økstad was a visiting scientist in David Sherratt's group at the Department of Biochemistry, University of Oxford, studying the molecular biology of DNA replication and recombination in *Escherichia coli* (2001). In 2005, Økstad started as Associate professor of microbiology at the Department of Pharmacy, University of Oslo, before being promoted to full professor in 2015. Økstad has in this period spent two sabbatical periods abroad, in professor Ian Paulsen's group, Macquarie University, Sydney, Australia studying multidrug resistance proteins and in Didier Lereclus' group at INRA, Joey-en-Josas, France studying *B. cereus* and *Bacillus thuringiensis* virulence.

His research interests are focused on 3 main areas: 1) Investigating the regulatory mechanisms and the molecular basis for microbial biofilm formation, employing models such as *Acinetobacter baumannii* and *Bacillus cereus*. 2) Mapping resistance mechanisms toward novel antimicrobials, including adjuvant substances used as inhibitors of resistance. 3) Microbial genome studies, primarily of pathogenic (disease-causing) bacteria belonging to the *Bacillus cereus* group, including repeated and mobile elements and global transcriptional profiling.

Abstracts for oral presentations

THE TRASCRIPTION OF THE *STAPHYLOCOCCUS AUREUS* PEPTIDOGLYCAN HYDROLASES *SAOUHSC_00671* AND *ssaA IS INTERCONNECTED*

Danae Morales Angeles and Morten Kjos

Norwegian University of Life Science, Faculty of Chemistry, Biotechnology and Food Science, Molecular Microbiology group

Peptidoglycan hydrolases (PHs) play a critical role during bacterial cell wall synthesis and biofilm formation. PH digest the cell wall to allow cell expansion and daughter cell splitting during division, and lysis of cells to release extracellular DNA during biofilm formation. Bacteria often encode many PHs, but their functions and regulation are not well understood.

Introduction and objectives: *Staphylococcus aureus* has 11 PHs that are regulated by the WalRK two-component system. This project aim is to understand how these PHs are regulated and coordinated during cell division and biofilm formation.

Methods: Knockout strains in combination with gene reporter systems was used to follow transcription of PH genes. A luciferase reporter was used to analyse transcription at the population level, while fluorescent reporters combined with confocal and epifluorescence microscopy were used for single-cell analysis and biofilm assays.

Results: We found that the uncharacterized PH *SAOUHSC_00671* has a role in cell splitting and biofilm formation in *S. aureus.* A regulatory interplay between SAOUHSC_00671 and the homologous PH *ssaA* was a revealed where transcription of *SAOUHSC_00671* is upregulated in the absence of SsaA and vice versa.

Using GFP and mCherry reporters for *SAOUHSC_00671* and *ssaA*, respectively, we show that the two PH are upregulated at different stages of growth and biofilm formation. For example, *ssaA* transcription increases with time in biofilms, but *SAOUHSC_00671* is upregulated during stationary phase of growth. Our results suggest that the regulatory interplay is mediated by the GraRS two-component system, which may sense structural changes in the staphylococcal cell wall resulting from the activities of PHs.

Conclusion: We demonstrate the two PH *SAOUHSC_0067*1 and *ssaA* inversely regulated during biofilm formation and growth. This is the first time such a regulatory connection has been demonstrated for PH in *S. aureus* and showcases the complex functional and regulatory interplay between PH in Gram-positive bacteria.

SPATIOTEMPORAL DISTRIBUTION AND DIVERSITY OF AIRBORNE RESISTANT BACTERIA: AN EXPLORATORY OBSERVATIONAL STUDY IN THE URBAN AND RURAL ENVIRONMENTS OF BANGLADESH

Muhammad Asaduzzaman[1]

[1]*Department of Community Medicine & Global Health, Institute of Health and Society, University of Oslo, Oslo, Norway.*

Introduction and objectives: Antimicrobial resistance (AMR) is a widespread planetary health issue. However, there is dearth of knowledge and scientific evidences on the magnitude of resistant bacteria in air and their transmission pathway. Therefore, we conducted an exploratory observational study in Bangladesh to quantify the clinically significant drug resistant bacteria in air with spatial diversity.

Methods: We collected air samples from both urban and rural settings in four distinct environments – i) Urban live bird markets(LBM) ii) Urban residential area(URA) iii) Commercial poultry farms(CPF) and iv) Rural households(RHH). We used MacConkey agar supplemented with 3rd generation cephalosporin (3GC) and meropenem respectively to obtain 3GC resistant (3GCr) and carbapenem resistant *Enterobacteriaceae* (CRE). Mannitol Salt agar supplemented with oxacillin and Slanetz-Bartley medium supplemented with vancomycin were utilized to obtain Methicillin (Oxacillin) resistant Staphylococci (MRS) and Vancomycin resistant Enterococci (VRE). The bacterial identification and susceptibility testing were conducted by VITEK 2 system.

Results: We found the presence of 3GCr, CRE, MRS and VRE in 85%, 60%, 100% and 80% air samples respectively. 3GCr, CRE and MRS were highest in CPFs and VRE in LBMs. The abundance (>90%) of MRS, VRE and 3GCr in URA is alarming whereas the air samples from RHHs were heavily burdened with 3GCr and MRS (60-100%).The CRE in poultry environment also establishes the threat added by current farm practice. The diversity and richness of resistant organisms were measured by Shannon diversity index, which was higher in both seasons at LBMs and CPFs (H-2.17-2.21 and H-1.99-2.03 respectively). Considering the organism family, the major bacteria were Staphylococcaceae (35%), Pseudomonadaceae (20%), Enterobacteriaceae (15%), Moraxellaceae (10%), Lactobacillaceae (7%) and Enterococcaceae (6%).

Conclusion: Our study findings emphasize on the inclusion of air in the system approach and surveillance to tackle AMR due to its high potential for acting as both reservoir and medium of spread of resistance.

EARLY LIFE PROBIOTIC TREATMENT RESHAPES ANTIBIOTIC-IMPAIRED GUT MICROBIOTA, RESISTOME, AND MOBILOME NETWORKS IN EXTREMELY PRETERM INFANTS

Ahmed Bargheet[1,2], Eirin Esaiassen[3], Erik Hjerde[4], Jorunn Pauline Cavanagh[1,3], Johan Bengtsson-Palme[5,6], Claus Klingenberg[1,3], Veronika K Pettersen[1,2]

[1]Host-Microbe Interactions Research Group, Department of Medical Biology, UiT The Arctic University of Norway, Tromsø, Norway [2]Paediatric Research Group, Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsø, Norway [3]Department of Paediatrics, University Hospital of North Norway, Tromsø, Norway [4]Department of Chemistry, Norstruct, UiT The Arctic University of Norway, Tromsø, Norway [5]Department of Infectious Diseases, Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, Guldhedsgatan 10A, SE-413 46 Gothenburg, Sweden [6]Centre for Antibiotic Resistance Research (CARe) at University of Gothenburg, Gothenburg, Sweden

Introduction and objectives: Prematurely born infants generally have greater health risks of infection and sepsis. Antibiotics are often given in neonatal intensive care units (NICUs) to prevent bacterial infection in vulnerable preterm infants. However, such treatment damages the gut microbial ecosystem and expands the gut antibiotic resistance genes collection (resistome). Routinely, infants born extremely prematurely (gestational age GA < 28 weeks) receive probiotic supplements in Norway to prevent necrotising enterocolitis and sepsis. The administration of probiotics to this high-risk group is based on empirical evidence; however, the long-term effect of probiotic strains on the gut microbial community is poorly understood.

Methods: We conducted a longitudinal multi-centre study in six Norwegian NICUs to study the influence of probiotics containing *Bifidobacterium longum* and *Lactobacillus acidophilus* on the gut microbiota, resistome, and mobilome of antibiotic-treated extremely preterm infants ($n = 29$) and compared them to antibiotics-exposed very preterm ($n = 25$), antibiotic-naïve very preterm ($n = 8$), and antibiotic-naïve full-term infants ($n = 10$). Stool samples were collected at four time points (on days 7, 28, 120, and 365), extracted DNA was sequenced, and we analysed the resulting metagenome data.

Results: The administration of probiotics modified the gut microbiota of extremely preterm infants at day 7, rendering them more similar to full-term babies. Whereas GA is a known factor modifying microbiota composition, probiotic therapy appeared to diminish its influence and accelerate microbiota maturation, boost microbial community interconnectedness, and enhance community stability. Furthermore, probiotic therapy appeared to reduce resistome expansion caused by prolonged antibiotic use and hospitalisation. Interestingly, probiotic treatment diversified mobile genetic elements and potentially promoted long-term horizontal gene transfer.

Conclusion: Probiotic supplementation may accelerate microbiota development, alleviate harmful effects of antibiotics on the gut microbiota, and resistome; nevertheless potentially promotes resistome dissemination.

CAN`T TOUCH THIS – IMPROVING CATALYSIS IS USELES IF SUBSTRATE DOESN'T BIND

Christopher Fröhlich[1], H. Adrian Bunzel[2,3,4], Adrian J. Mulholland[3], Marc W. Van der Kamp[3,4], Hanna-Kirsti S. Leiros[1], Pål Jarle Johnsen[5] and Nobuhiko Tokuriki[6]

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[2]*Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland*
[3]*Centre for Computational Chemistry, School of Chemistry, University of Bristol, Bristol, UK*
[4]*School of Biochemistry, University of Bristol, Bristol, UK*
[5]*Department of Pharmacy, UiT The Arctic University of Norway, Tromsø, Norway*
[6]*Michael Smith Laboratories, University of British Columbia, Vancouver, Canada*

Antibiotic resistance often develops gradually by the acquisition of mutations with non-additive effects, a phenomenon called epistasis. The molecular mechanisms allowing for such effects are poorly understood, limiting our ability to predict resistance development. Using the serine b-lactamase OXA-48, we studied the molecular mechanisms of how epistasis drives resistance development towards the b-lactam ceftazidime.

Five cycles of directed evolution were performed to gradually enhance the ceftazidimase activity of OXA-48. Fitness landscapes of mutational combinations were built and characterised with respect to their ceftazidime susceptibility. Steady-state and pre-steady state enzyme kinetics were performed to shed light on the molecular action of selected of mutations. Structural changes were investigated by X-ray crystallography and molecular dynamics (MD) simulations.

In total, a set of 5 mutations were acquired leading to increments in ceftazidime resistance up to 40-fold. Characterisation of a fitness landscape with 16 reconstructed mutants showed that positive epistatic drove resistance development. For example, F72L increased resistance by 2-fold, while other mutations, such as S212A and T213A, did not increase resistance as single mutants. However, these alanine mutants demonstrated great synergy in combination with F72L, leading to an overall 40-fold increase in resistance. Kinetic characterisations showed that the non-additive effect mediated by F72L and the alanine mutants was caused by the interplay between improved substrate binding and optimisation of the catalytic machinery, respectively. Structural investigations and MD simulation support these observations, demonstrating that the epistatic evolution was driven by both tighter dynamical substrate binding and catalytically superior loop conformations.

Our data underline the importance of epistasis during the evolution of antibiotic resistance enzymes. In particular, we showed how improvements in enzyme dynamics can shape the evolution of non-additive phenotypes due to synergistic effects on binding and catalysis. Understanding these molecular mechanisms is pivotal for the predictability of emergent antibiotic resistance and the development of novel drugs.

GENOMIC K-CAPSULAR ANTIGEN (GROUP 2) TYPING AND PREVALENCE IN *E. COLI* FROM BLOODSTREAM INFECTIONS IN NORWAY

R.A. Gladstone[1], S. Arredondo-Alonso[1], A.K. Pöntinen[1,2], A. McNally[3], Ø. Samuelsen[2,4], P.J Johnsen[2], J. Corander[1]

1. Department of Biostatistics, University of Oslo, Oslo, Norway. 2. Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway. 3. Institute of Microbiology and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK. 4. Department of Pharmacy, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway

Background: Capsules are an important virulence determinant in many pathogens. K capsular antigens in *Escherichia coli* are known to be associated with different manifestations; K1 with neonatal sepsis and K2 with urinary pathogenicity. Phenotypic K-typing is a laborious and expensive method, while no genomic tool for K-typing from genomic data yet exists. This leaves the current prevalence of K-types in disease unknown.

Methods: 3254 *E. coli* genomes from bloodstream infections (BSI) in Norway 2002-2017 were combined with other published collections and 118 genomes with a reported K-type, representing 48 different K-designations. Regions 1-3 of group 2 capsule loci (*kpsF-kpsM*), were annotated and clustered into gene groups using Panaroo. The presence and absence of K-locus gene groups were overlaid on a phylogeny of a K-locus alignment to determine unique patterns of K-locus gene content. Where available the presence of a reference K-type was used to assign a putative K-type.

Results: There were 56 distinct K-locus gene content patterns, 22 of which had a phenotypic K-type reference. Four K-types accounted for over 50% of Norwegian BSIs. K1 was the most prevalent (777/3254, 24%) and was spread across phylogroups B2, F, A and D, predominating in ST95 (B2), ST59 and ST62 (both F). K5 was detected in 16% of the collection whilst K2 and K52 were detected in 6% and 5% respectively. ST131 (B2) represented a capsular recombination hotspot with multiple different K-types.

Conclusions: This database of K-genotypes can be used to infer K-type in additional collections and generates new data on the prevalence of K-types in *E. coli* from BSIs. Like O/H-types, K-types may represent important therapeutic or vaccine targets. By applying this methodology to other niches, comparisons of *E. coli* frequency in gut carriage or urinary tract infections would allow the association of different K-types with disease to be measured.

CAN INSIGHT FROM BACTERIOCIN:RECEPTOR INTERACTION STUDIES HELP DESIGN NOVEL ANTIMIROBIAL PEPTIDES?

S.S. Kristensen[1], T.F. Oftedal[1], C. Kranjec, G. Mathiesen[1] and D.B. Diep[1].

[1] *Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science*

Enterocin K1 (EntK1), a bacteriocin that is highly potent against vancomycin-resistant enterococci, dependents on specific binding to an intramembrane protease of the site-2-protease family, RseP, for its antimicrobial activity. RseP homologs are highly conserved in multiple species and play an important role in physiology and virulence in multiple human pathogens, including *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*, thereby making RseP an attractive antimicrobial target.

Here, we present a mutational study of RseP to unravel the nature behind the interaction between the bacteriocin and its receptor. A systematic set of mutations on *rseP* were performed and the resulting mutants were assessed by bacteriocin binding assay and sensitivity assay as well as by structural studies using the AI-based prediction program AlphaFold. The results provide strong evidence that the extracellular PDZ domain and the extended LDG domain of RseP are key determinants of EntK1 binding and sensitivity. These regions are highly conserved in several RseP homologs, making RseP as a very attractive drug target.

EntK1 belongs to the LsbB-family of bacteriocins, which are attractive, not only because they are potent against vancomycin resistant Enterococci, but also because they are short, synthetized without an N-terminal leader sequence, and contain no post-translational modifications. These qualities make these peptides ideal of starting point for bioengineering of novel bacteriocins. As a proof-of-concept, we constructed a novel hybrid bacteriocin, H1, which showed superior antimicrobial activity against *Staphylococcus haemolyticus* compared to its parental bacteriocins. In a bacteriocin based formulation, H1 contributed to completely eradicating *S. haemolyticus* biofilms in vitro. The construction of H1 confirms that the LsbB-family of bacteriocins can be bioengineered to improve both potency and alter activity spectrum.

Taken together, the data presented provide valuable insight for guided construction of novel bacteriocins and highlight that RseP can serve as a drug target for infection treatments.

BK POLYOMAVIRUS SUCCESSFULLY INFECTS A POLARIZED RENAL PROXIMAL TUBULE EPITHELIAL CELL MODEL

E. M. Lorentzen[1,2] and C. H. Rinaldo[1,2]

[1]*Department of Microbiology and Infection Control, University Hospital of North Norway*
[2]*Department of Clinical Medicine, UiT - The Arctic University of Norway*

Introduction: BK Polyomavirus (BKPyV) is a ubiquitous virus that establish a lifelong infection in the reno-urinary tract. Although renal epithelial cells are polarized *in vivo*, BKPyV replication has almost exclusively been studied in non-polarized cell cultures. The aim of this study was therefore to establish a polarized renal epithelial cell model to study BKPyV replication in a more in-vivo like situation.

Methods: Primary human renal proximal tubule epithelial cells (RPTECs) were cultured on collagen-coated cell culture inserts. Polarity was assessed by immunofluorescence microscopy (IF), electron microscopy (EM), barrier integrity assay, measurement of transepithelial resistance and P-glycoprotein assay. Viral infection was assessed by IF, quantitative PCR (qPCR) and infectivity assay. Cell death and viability was examined by LDH Glo Cytotoxicity assay, CellTox Green-staining and live-cell imaging.

Results: IF and EM demonstrated that RPTECs developed tight junctions, microvilli and apico-basal polarity on cell culture inserts. Additionally, an intact barrier function and a functional P-glycoprotein pump was found. IF confirmed uptake of BKPyV and expression of BKPyV proteins in polarized RPTECs. Infections performed from either the apical or the basolateral side, demonstrated that apical infection yielded 5x more infected cells. Viral release was examined with qPCR and infectivity assay. Up to 72 hpi, 96-99% of BKPyV-DNA was in the apical compartment and progeny virus was exclusively detected apically, indicating apical release of BKPyV. Minimal cell lysis was measured up to 58 hpi. At 120 hpi, cell lysis and basolateral levels of BKPyV increased concomitantly.

Conclusions: Our study demonstrates that RPTECs polarize on cell culture inserts and that polarized RPTECs support BKPyV infection. BKPyV display polarized entry and release through the apical membrane. Insignificant cell lysis was observed early in the first replication cycle. After the first replication cycle, lysis increased markedly, indicating both non-lytic and lytic release of BKPyV.

EVOLUTION OF V*IBRIO CHOLERAE* BIOFILMS

Øyvind M. Lorentzen[1], Sören Abel[1, 2], Pål J. Johnsen[1]

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Introduction: Many bacteria form biofilms, aggregates of bacterial cells covered in a self-produced extracellular matrix. Biofilms are an effective defense against external threats and are therefore a widespread survival strategy among bacteria. It is well-known that evolving bacterial biofilm populations undergo phenotypic changes, but many of the regulatory details are still poorly understood. The second messenger c-di-GMP is an important regulator of biofilm formation. C-di- GMP is metabolized by conserved enzymatic domains that either synthesize (GGDEF-domains) or degrade (EAL/HD-GYP-domains) it. These domains can occur alone or as bi-functional enzymes that can both degrade and synthesize c-di-GMP. In this study, we experimentally evolved *V. cholerae* under conditions selecting for biofilm formation to better understand how biofilm formation is regulated.

Methods: We performed experimental evolution by serially passaging biofilms or planktonic cultures to obtain biofilm-evolved or planktonic-evolved *V. cholerae* populations. We whole-genome sequenced the evolved populations to map mutations. Phenotypic assays were performed to quantify biofilm formation. We conducted mutant analysis to assess how individual mutations affected biofilm formation.

Results: All of the biofilm-evolved populations displayed greatly increased biofilm formation compared to wild-type and planktonic-evolved populations. We observed multiple independent mutations in the same bi-functional c-di-GMP-metabolizing protein (mbaA) in all biofilm-evolved populations. A particular mutation (mbaA E576K), lead to a hyper-biofilm phenotype. Recreating the mutation in the wild-type was necessary and sufficient to reproduce the phenotype. Δ*mbaA* or ΔEAL- *mbaA* mutants produced a different phenotype with only intermediate levels of biofilm formation. Inactivation of the mbaA GGDEF-domain also abolished the observed hyper-biofilm phenotype.

Conclusions: This study demonstrates that *V. cholerae* rapidly acquire novel mutations that increases biofilm formation during biofilm evolution. The observed hyper-biofilm phenotype seems to be due to *mbaA* gain-of-function mutations altering the protein function from c-di-GMP degradation to synthesis. Our data suggest that domain crosstalk regulates the enzymatic function of mbaA.

CHARACTERISATION OF PUTATIVE VIRULENCE FACTORS IN *ENTEROCOCCUS FAECIUM*

I. Mathiesen[1], M. Johannessen[1], K. Hegstad[1,2], T. Wagner[1]

[1] *Research group for Host-Microbe Interactions, Department of Medical Biology, Faculty of Health Sciences, UiT the Arctic University of Norway*

[2] *Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway*

Increasing antibiotic resistance hampers treatment options and thus new ways of treatment are needed. Therefore, it is important to understand how resistant bacteria cause disease and use this knowledge to find new targets for intervention. Multi-resistant *Enterococcus faecium* is posing a great threat to public health and needs to be studied closer. Clinical strains of *E. faecium* are increasingly invasive, and their ability to acquire new resistance and virulence genes stands out. Discovery of new virulence factors and novel knowledge of bacterial expression upon meeting with host, will provide future targets for infection prevention and/or treatment.

In this project, bioinformatic methods comparing whole genome sequencing data of nosocomial and commensal *E. faecium* isolates were utilized to point out putative new virulence factors. Potential candidate genes will be characterized regarding their prevalence and genetic context. Their expression profile will be studied through RT-qPCR in standard bacterial growth medium and thereafter in conditions which are relevant in an infection context. These may include different components mimicking body or infection sites. Further functional assays will be designed depending on potential functional domains of the putative virulence factor, which domains can be predicted based on the genomic sequence of the candidate gene.

The characterization of novel virulence factors will help the understanding of the invasive properties of *E. faecium* and may reveal interesting targets for therapeutics discovery using anti-virulence strategies.

HYGIENIC PRACTICE OF STREET FOOD VENDORS AND SANITARY CONDITIONS OF VENDING SITES IN SELECTED TOWNS OF ETHIOPIA

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Background: Street foods are ready-to-eat foods prepared and/or sold by vendors and hawkers, especially in streets and other similar public places. The objective of this study is to assess the hygienic practices street food vendors and sanitary condition of their stalls.

Methods Community based Cross-sectional study design used from April to August 2022 in four selected major cities of Ethiopia. The cities selected purposively by considering the number of population, their location and the availability of industrial parks inside them. Sample size calculated based on single population proportion formula to select 1168 street food vending stalls in which one vendor selected randomly from it. Proportional allocation to the number of total population of the town used to assign the number of street food vending stalls to each city then after the vending stalls again allocated to the number of total population of each sub cities. Finally, the sub cities divided in to five-zones and took vending stalls purposively. Interviewer administered questioner used to collect data. Besides, observational checklist used to assess the sanitary condition of the vending stalls. Data analyzed by using SPSS-23.

Result the overall good hygienic practice of the street food vendors and sanitary status of the vending stalls were 16% and 6.8 % respectively. Sex (male) (AOR=1.6, 95% CI=1.1–2.6), work experience (AOR=3.6, 95% CI=1.5-8.6) receiving training on food safety (AOR=2.4, 95% CI=1.6–3.5), having medical checkup (AOR=1.5, 95% CI=1.1–2.3). Availability of hand washing facilities (AOR = 2.3, 95% CI: 1.4–3) were predictors of good hygienic practice.

Conclusion: the hygienic practice of street food vendors and the sanitary conditions of the vending stalls were poor. Hence, the comprehensive activities expected from the owners, vendors, health professionals and other stakeholders to reach the desired food safety and hygienic standards.

MUCOSAL ANTIBODY RESPONSE TARGETING GLYCOSYLATED EPITOPES OF *ESCHERICHIA COLI* MUCINASE YghJ (SslE)

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To reduce the incidence, severity, and deaths from *Escherichia coli* (*E. coli*) infections, there is an ongoing effort to develop efficient cross-protective vaccines against pathogenic *E. coli*. The conserved secreted mucinase YghJ, which is needed for successful colonization by both intestinal and extraintestinal pathogenic *E. coli*, is currently being evaluated for use in new vaccines. This study examines the role that glycosylation may play for YghJ antigenicity, including to what extent antibody responses to *E. coli* infections target glycosylated YghJ epitopes, which is relevant when deciding on vaccine formulations.

We used multiplex bead flow cytometric immunoassays based on natively secreted glycosylated YghJ and recombinant non-glycosylated YghJ. Proportions of IgA and IgG antibodies targeting the glycosylated epitopes of YghJ were estimated in antibody in lymphocyte supernatant (ALS) and IgA antibodies in saliva in 21 volunteers experimentally infected with wild type enterotoxigenic *E. coli* (ETEC) strain TW10722.

Defining responders as volunteers who had ≥ 5-fold increase in ALS anti-YghJ IgA or IgG 7 days after infection and ≥ 2-fold increase in saliva anti-YghJ IgA 10 days after infection, the number of responders were 19 (90%) for ALS IgA, 7 (58%) for ALS IgG, and 11 (52%) for saliva IgA, respectively. The corresponding median fold increase was 30.6 (interquartile range [IQR]: 12.8, 90.7), 5.0 (IQR: 2.0, 9.0), and 2.03 (IQR: 1.16, 3.68). The median proportion of antibodies specifically targeting glycosylated YghJ epitopes was 0.39 (IQR: 0.20, 0.59), 0.11 (IQR: 0.06, 0.21), and 0.87 (IQR: 0.71, 0.97), respectively.

The strong and predominantly glycosylation-specific anti-YghJ mucosal IgA antibody responses seen in saliva and in redistributed plasma cell populations are of relevance to vaccine development efforts since development of subunit vaccine candidates have traditionally utilized recombinant, non-glycosylated antigens.

NOVEL MYCOBACTERIAL TOLERANCE INHIBITORS (MTIS) AGAINST MULTIDRUG-RESISTANT TUBERCULOSIS

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Introduction and objectives: *Mycobacterium tuberculosis* (Mtb) causes tuberculosis (TB), which was declared a global health emergency more than two decades ago, but still ranks among the top ten causes of mortality. In 2021, 10M people fell ill with TB and 1.5M TB deaths were recorded. New therapeutics and preventive strategies are therefore urgently needed to fight TB. Novel insight into the mechanisms that drive drug susceptibility and resistance are required to improve treatment. The focus of this project is to discover the mechanism of action of a new class of drugs, named Mycobacterial Tolerance Inhibitors (MTIs), recently discovered by partners in our consortium.

Methods: A combination of culture, molecular biology and –omics approaches in both Mtb reference strains and clinical isolates was employed in testing MTIs. Drug activity were tested by resazurin microtiter assay (REMA) and drug cytotoxicity was assessed in human cell lines. Cultures of clinical Mtb strains were treated with MTIs and compared to non-treated cultures. Lysed Mtb cells were subjected to proteomic analysis by in-gel digestion and high-end mass spectrometry (MS). The proteomics data sets were analysed by statistical and bioinformatics tools for molecular annotations.

Results: Here, we have shown that clinical isolates with isoniazid resistance due to mutation in the *inhA* promoter or *katG* gene are sensitive to MTIs, alone and in combination with isoniazid. IC_{50} values of novel MTIs were low and exhibited no cytotoxicity in human cell lines. Proteome analyses reveal decrease in total protein count in Mtb treated with MTIs compared to non-treated. MTIs sensitize *Mtb* to the frontline antibiotic isoniazid and re-sensitize isoniazid-resistant Mtb.

Conclusion: The use of combined approaches has enabled the discovery of pathways involved in Mtb MTI drug tolerance and resistance, representing a novel basis for discovering how the MTIs work, in order to optimize TB treatment. Through collateral sensitivity action, MTIs thus hold great potential in the treatment against TB.

COMMENSAL *ENTEROBACTERIACEAE* ISOLATED FROM HUMAN STOOLS ARE CAPABLE OF OUT-COMPETING ENTEROTOXIGENIC ESCHERICHIA COLI IN VITRO

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Background: In an experimental infection study with the enterotoxigenic *Escherichia coli* (ETEC) strain TW10722, 11 out of 21 volunteers never developed diarrhea. This apparent protection against diarrhea was strongly associated with low levels of intestinal TW10722 colonization, possibly as a result of colonization resistance by the host's resident gut bacteria. In the present pilot study, we explore the potential role of commensal *Enterobacteriaceae* in protecting its host against TW10722 colonization.

Methods: Isolates of commensal *Enterobacteriaceae* from before, during and after experimental infection with TW10722 were isolated from frozen stool samples after culturing on MacConkey agar. Based on all the isolates for each volunteer, unique clones were identified by using antibiotic selection and *ompC* DNA sequencing. For the competition assay, these unique clones were individually mixed 1:1 with fluorescently labelled TW10722 and spotted onto M9 minimal media. After 24 hours of co-culture incubation, the ratio between TW10722 and commensal was estimated by flow cytometry and used as a measure of competition efficiency.

Results: A total of 63 unique *E. coli* clones and 21 other non-*E. coli* isolates were isolated from the stools from these 21 volunteers, with a median 3 (range: 1–6) unique *E. coli* clones per volunteer. Fifteen isolates were included in this pilot study, and we found that 53% (8/15) competed well against TW10722. Of these, two (13%) appeared to completely out-compete TW10722.

Conclusions: Preliminary competition results indicate that commensal *Enterobacteriaceae* isolated from the stools from 21 human volunteers commonly compete well against ETEC strain TW10722 in vitro, with some commensals being capable of completely out-competing TW10722. So far, however, the presence of these competitive clones does not appear to be associated with protecting their hosts against TW10722-induced diarrhea. These experiments are ongoing, and more data will be presented at the meeting.

PROTEINS CONDENSING DNA DURING THE SOS RESPONSE IDENTIFIED BY A MACHINE LEARNING-ASSISTED GENOME-WIDE SCREENING

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When bacteria experience DNA double-strand breaks following treatment with certain classes of antibiotics they activate the SOS response. During the early stages of this response the chromosome condenses to a central position in the cell, eliciting the start of a program to repair DNA. Several of the activated repair proteins in the SOS response increase mutagenicity and thus facilitate development of antimicrobial resistance. However, little is known about which proteins are involved in DNA condensation and if this condensation is crucial for the increased mutagenicity. In this study, we have identified key proteins involved in SOS-induced DNA condensation. We performed a genome-wide screening of all available *E. coli K-12* single gene-deletion mutants in the Keio collection. This was achieved by high-content imaging of strains to identify DNA condensation after treatment with the DNA-damaging antibiotic ciprofloxacin. We then applied machine learning to identify mutants that did not condense their DNA following treatment. Our screening was able to identify eight proteins involved in DNA condensation, in addition to a set of recombinational repair proteins important for the SOS response. These eight proteins have not previously been associated with DNA condensation. Our findings lay the foundation for further investigations into how these proteins are interacting with the *E. coli* chromosome and into the mechanism of SOS induced DNA condensation.

Abstracts for poster presentations

ULTRAFAST AND COST-EFFECTIVE PATHOGEN IDENTIFICATION AND RESISTANCE GENE DETECTION IN A CLINICAL SETTING USING NANOPORE FLONGLE SEQUENCING

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Introduction and objectives: Rapid bacterial identification and antimicrobial resistance gene (ARG) detection are crucial for fast prescription of antibiotics, especially for septic patients where each hour of delayed antibiotic prescription might have lethal consequences. This work investigates whether the Oxford Nanopore Technology's (ONT) Flongle sequencing platform is suitable for sequencing directly from blood cultures and real-time analyses to identify bacteria and detect resistance-encoding genes. The aim was to see how fast can we get the information on the bacterial taxonomy, ARGs, and plasmids using Flongle sequencing.

Methods: For the analysis, we used pure bacterial cultures of four clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* and two blood samples spiked with either *E. coli* or *K. pneumoniae* that had been cultured overnight. We sequenced both the whole bacterial genome and plasmids isolated from pure culture and spiked samples using two different sequencing kits (SQK-LSK110 Ligation kit and SQK-RBK004 Rapid Barcoding kit).

Results: Flongle data allow rapid bacterial ID and resistome detection based on the first 1,000–3,000 generated sequences (10 min to 3 h from the sequencing start), albeit ARG variant identification did not always correspond to ONT MinION and Illumina sequencing-based data. Flongle data are sufficient for 99.9% genome coverage within at most 20,000 (clinical isolates) or 50,000 (positive blood cultures) sequences generated. The SQK-LSK110 Ligation kit resulted in higher genome coverage and more accurate bacterial identification than the SQK-RBK004 Rapid Barcode kit.

Conclusion: Flongle can be successfully used for the identification of bacteria and the detection of antibiotic resistance genes both in clinical isolates and when using direct sequencing of blood spiked with these clinical isolates. Although the Flongle showed higher error in ARG variant detection than MinION, it still displays comparable performance in real-time for pathogen ID and ARG detection. This, together with its cost- effectiveness, could lead to Flongle potential future use in clinical microbiology.

NANOPARTICULATE DRUG CARRIERS FOR DELIVERY OF CUTTING-EDGE ANTIMICROBIALS TO BIOFILM

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Project background: Bacterial biofilm is a cluster of bacterial cells attached to living or inert surfaces. It is considered a critical health threat as it enables the bacteria of evading the host immune response and it makes the bacteria 1000-fold more resistant to antibiotic treatment compared to planktonic cells. These outcomes are attributed to the presence of a thick layer of extracellular polymeric matrix surrounding the bacteria inside the biofilm and existence of bacterial cells in metabolically inactive dormant state. The currently available strategies are mainly prophylactic to prevent the biofilm formation. However, once a biofilm is formed it is challenging to resolve with the current treatments.

Project aim: The project aims to develop and test in-vitro efficacy of nanoparticulate delivery systems loaded with newly developed peptide-based antibiotics. Teixobactin analogues are used as a model drugs which resemble a new class of antibiotics. We hypothesize that the formulation will enhance the antibacterial activity of the drug and minimize the host toxicity. The prepared nanoparticles should enhance the drug penetration into the biofilm and do not have any negative effect on the drug chemical stability and microbiological activity.

Methods: Biomaterials such as phospholipids will be used in preparation of the nanoparticles to minimize any possible toxicity to the host. Physicochemical characterization of the nanoparticles will be conducted.

The antibacterial activity of the prepared nanoparticles will be evaluated by determining the minimum inhibitory concentrations and minimum bactericidal concentrations. The antibacterial activity will be

tested on bacterial strains associated with health concerns. Moreover, the nanoparticles ability to prevent the formation of biofilm will be evaluated. In addition to the activity on already established biofilm clusters of different bacterial strains. Finally, toxicity studies of the developed nanoparticles on mammalian cell lines will be performed to assess the possible side effects on the host.

UNRAVELLING THE ROLE OF BACTERIOCINS IN SHAPING ORAL BIOFILMS

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Introduction and objectives: Biofilms are the most common form of microbial life in nature, but also an important pathogenic mechanism. Understanding how bacterial biofilms are formed is key to controlling the infection process. In these structures, microorganisms aggregate in proximity which enables substrate exchange and distribution of metabolites. Among these metabolites, bacteriocins are ubiquitous antimicrobial peptides primarily considered as a bacterial defense system. Here, we explore their social role in shaping biofilm communities and their influence on the microbe-microbe dialogue. The main goal is to study how bacteriocin genes, and particularly, bacteriocin immunity genes, affect oral streptococcal biofilm formation.

Methods: *Streptococcus mutans* will be used to optimize a biofilm model to study bacteriocin-related interactions. From the wild-type strain (strain A), a bacteriocin-sensitive (strain B), and an immune non-producing strain (strain C) will be constructed and labelled with unique fluorescence and antibiotics markers. In strain B, markerless deletions of the functional bacteriocin clusters will be made, while in strain C only the bacteriocin structural genes will be deleted. Biofilm assays will be performed with different combinations of the designed strains, and different biofilm models will be tested, including biofilms on saliva-coated hydroxyapatite disks. Biofilms will be imaged with confocal microscopy to evaluate bacterial interactions.

Results: Even if the strains form biofilms individually, we hypothesize that bacteriocins and bacteriocins immunity genes will have major impact on the mixed biofilms, where the wild-type strain would be expected outcompete the sensitive one. By further co-incubation of strain A and/or B with strain C, we will get initial insights into how unpaired immunity genes affect the biofilm formation dynamics and whether such genes contribute to establishing stable, mixed biofilms. The better-performing biofilm model will be chosen for further analysis.

Conclusion: We expect to establish a model to study competitive biofilm-formation for oral streptococci in relation to bacteriocin production.

UNCOVERING A NOVEL MECHANISM CONTROLLING LIPOTEICHOIC ACID BIOSYNTHESIS IN *STAPHYLOCOCCUS AUREUS*

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Teichoic acids are anionic polymers attached to the cell wall (wall teichoic acids, WTAs) or cell membrane (lipoteichoic acids, LTAs) of Gram-positive bacteria. While WTAs are covalently attached to peptidoglycan, LTAs are normally tethered to the membrane via glycolipids. In *Staphylococcus aureus*, these polymers are important virulence factors and modulators of antibiotic susceptibility, in addition to being critical for cell integrity and proper cell division. The teichoic acid biosynthetic pathways are therefore attractive as potential antibiotic- and anti-virulence targets. The paralogous membrane proteins CozEa and CozEb have previously been reported to have essential roles during cell division in *S. aureus*. In this work, the functions of the conserved membrane proteins known as CozE were further investigated in methicillin-sensitive- and methicillin-resistant *S. aureus* (MSSA and MRSA) using different genetic and molecular approaches. Construction and characterization of single and double *cozE* mutants, confirmed that *cozEa* and *cozEb* constitute a synthetic lethal gene pair in *S. aureus*. Florescence microscopy also revealed that the two proteins have a spotty, dynamic localization in the cell membrane. By performing genome-wide synthetic lethal screens, we showed that CozE proteins are genetically linked to different cell surface biosynthetic pathways. Based on these results combined with phenotypic characterizations, we hypothesized that the functions of CozEa and CozEb were linked to biosynthesis of teichoic acids. Indeed, we found that proteins involved in synthesis and flipping of the glycolipid anchor of LTA, modulate the essentiality of CozE proteins. While the essentiality of CozE proteins was increased in cells devoid of glycolipid synthesis (Δ*ugtP*), the essentiality was alleviated in cells lacking the flippase activity (Δ*ltaA*). Furthermore, anti-LTA immunoblot assays revealed that CozEb play a unique role in controlling LTA polymer length and stability. Together, the results demonstrate that the CozE proteins directly or indirectly control LTA biosynthesis in *S. aureus*.

TRANSCRIPTOMIC PROFILING OF *STAPHYLOCOCCUS AUREUS* CO-CULTURED WITH *STREPTOCOCCUS ANGINOSUS* AND HUMAN TONSILLAR EPITHELIAL CELLS

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Colonization with *Staphylococcus aureus* is a risk factor for infection. Nasal colonization is well described; however, we have limited knowledge about *S. aureus* throat colonization. Recently, we investigated the transcriptional response exhibited by *Staphylococcus aureus* during *in vitro* co-culture with human tonsillar epithelial cells (HTEpiC). This enabled us to identify bacterial determinants for *S. aureus* influenced during throat colonization. We aim to explore the responses triggered by the encounter of two throat pathogens to identify transcripts in *S. aureus* that are important when facing a potential competitor in presence of HTEpiC. Further, identifying bacterial interactions that influence the expression of virulence factors and antimicrobial resistance genes.

The current study involved co-culturing of *S. aureus* with another throat bacteria, *Streptococcus anginosus*, and both being exposed with/without HTEpiC. We performed RNA sequencing to identify interaction-induced transcriptional alterations in surface-associated populations. *S. aureus* and *S. anginosus* were monocultured and co-cultured without and with host cells, referred to as control samples and test samples, respectively. Two different time points (1h and 3h) were selected for triplicate *in-vitro* experiments in 6-well plates. Following incubation, bacteria were collected from all the conditions. Adhesion and cytotoxicity assays were performed, and total RNA subjected to RNA-seq.

S. aureus indicated higher adherence capacity than *S. anginosus* with tonsillar cells after 1h and 3h of coculturing with tonsillar cells. In further analysis, we will explore the transcriptomic profiling of *S. aureus* during *in vitro* co-culture with *S. anginosus* in presence/absence of epithelial cells and further compare this transcriptome with the genes expressed by *S. aureus* during monoculture.

In vitro co-culturing of *S. anginosus* may alter the *S. aureus* transcriptome during colonization with tonsillar cells.

LATE-STAGE FUNCTIONALIZATION FOR ANTIBIOTIC CONJUGATION

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Antibiotic resistance (ABR) represents one of today's greatest threats to global health. While the discovery and development of fundamentally novel antibiotic scaffolds is highly challenging and time consuming, one proposed fast-track method to access new drugs with improved resilience towards ABR is conjugation of known antibiotic scaffolds to other molecular units which confer improved properties to the new molecule as a whole. In order to be conjugated, two molecules need to contain a pair of compatible molecular groups, the most common being alkynes and azides, which can be directly introduced onto the molecules by late-stage functionalization. This project aims to develop a novel synthetic procedure for the introduction of flexible terminal alkynes onto complex molecules to enable antibiotic conjugation through 'click' chemistry.

Radical chemistry is an interesting approach for late-stage functionalization as radicals have different reactivities than charged molecules and therefore open up a new range of possible chemical reactions and products. Among the mild methods developed to generate radicals, photoredox catalysis is of particular interest and is the main method used in this project.

The synthetic methodology is currently being developed on simple alkyl iodide model substrates with tethered silane chains. Terminal alkene and alkyne products were successfully synthesized using the oxidative quenching catalytic cycle of an iridium photocatalyst under irradiation with blue light.

These early results suggest that the approach is a promising way to introduce 'click'able handles in molecules, and will be a useful addition to the late-stage functionalization field. In the longer term, the late-stage modification and 'click' synthesis of drug conjugates will contribute to the discovery of new viable antibiotics options.

QUANTIFICATION OF SHORT-CHAIN FATTY ACIDS AND ORGANIC ACIDS IN MECONIUM USING QUANTITATIVE MASS SECTROMETRY-BASED METABOLOMICS

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The gut microbiome contributes to human physiology by producing beneficial metabolites. Among these metabolites are short-chain fatty acids (SCFA), which are produced by bacterial fermentation of dietary components non-digestible by human enzymes. Evidence shows that these metabolites have important roles in early life; from inhibiting colonization of pathogenic bacteria to the maturation of the host immune system. SCFA and associated metabolites thus represent promising fecal biomarkers for clinical applications. However, standardized quantitative measurements of SCFA are lacking, as well as an overview of their range and concentrations in feces of newborns.

SCFA have traditionally been analyzed using gas chromatography-tandem mass spectrometry (GC-MS/MS) methodology. However, through a derivatization procedure, these acids can readily be analyzed by liquid chromatography (LC)-MS/MS. Here, we have adapted an LC-MS/MS method for the absolute quantification of 13 SCFA and two organic acids using 3-nitrophenylhydrazine (3NPH) as a derivatization reagent. We applied this method to determine SCFA profiles in samples of meconium (the first stool after birth, <48 hours of life) from 40 healthy, vaginally delivered infants born between 37-42 gestational weeks. Of the 15 metabolites targeted in our method, acetate had the highest concentration (average of 12.0 µmol/g dry weight), followed by lactate (9.7 µmol/g), propionate (4.2 µmol/g), succinate (1.9 µmol/g), and butyrate (0.8 µmol/g), respectively. The other 10 SCFA had relatively low quantity (<3%).

This method is a step toward establishing standard metabolomic protocols in human microbiome research. In addition to elucidating the concentration ranges of SCFA in meconium, we will also profile bile acids and other metabolite classes by non-targeted metabolomics, with the aim to obtain a more extensive insight into the metabolome of meconium. Finally, we will explore how the fecal microbiome and metabolome changes in response to antibiotics and probiotics used in early life by an integrative multi-omics approach.

COHABITATION OF SALMON PATHOGEN *PISCIRICKETTSIA SALMONIS* GENOGROUPS: NEW INSIGHTS INTO THE DEVELOPMENT OF SRS DISEASE

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Piscirickettsia salmonis is a facultative intracellular bacterium, biological agent of SRS (Salmon Rickettsial Syndrome), and although it has been described in many salmon farming countries, only in Chile (the second largest producer of salmon after Norway) is the most critical disease for Atlantic salmon (*Salmo salar*). *P. salmonis* evades the salmon immune system by replicating within cytoplasmic vacuoles of macrophages. That is why vaccines still do not provide efficient control. There are two genogroups identified for this bacterium (LF-89 and EM-90) that cohabit just in Chile, and recently after intensive genomics analyses, proposed as different species. This project study if this multiple- genotype infection could be related to the high fish mortality there, as the implications of within-host competitive interactions are linked to virulence, development and persistence of diseases, and changes in pathogens population dynamics. Thus, the aim of this work is to evaluate how the cohabitation of both LF-89 and EM-90, can affect their growth performance and virulence factors expression.

To accomplish this, first five different strains were co-cultured in broth medium to compare bacterial growth and biofilm formation. Later, one strain of each genogroup was chosen to perform *in vivo* cultures. Briefly semi-permeable dialysis tubes containing single and co-cultures of *P. salmonis* were surgically placed into the abdominal cavity of 12 Atlantic salmon. Six days after implantation, fish were euthanized and dissected for removal of the implants, and RNA-Seq was performed on total RNA extracted.

The results show growth and biofilm differences between isolates where all EM-90 strains exhibited improved growth at expenses of LF-89. Virulence markers, communication and chemotaxis indicators showed differential expression in co-cultures and during *in vivo* co-cultures, suggesting a response to the cohabitation.

Additional *in vivo* co-infections challenges in Atlantic salmon are expected to show the relevance of this pathogenicity biomarkers in SRS disease dynamic.

THE ROLE OF CAPSULE VARIANTS IN *STAPHYLOCOCCUS HAEMOLYTICUS* ON IMMUNE RESPONSE AND BIOFILM PRODUCTION

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Introduction and objectives: *Staphylococcus haemolyticus* is a common cause of sepsis in premature babies and children with cancer. *S. haemolyticus* is part of our normal microbiome and has only a few virulence factors. However, clinical *S. haemolyticus* isolates are highly antibiotic-resistant and form biofilms on invasive catheters and medical implants. Such infections are difficult to treat; the biofilm matrix prevents sufficient diffusion of antimicrobial substances into the biofilm, cells are dormant in addition to a high degree of antibiotic resistance in clinical *S. haemolyticus* isolates. Biofilm is an important virulence factor that also reduces phagocytosis. Little is known about the strategies *S. haemolyticus* uses to establish an infection or evade the host immune system. Recently, four different capsule types were found in *S. haemolyticus.* Expression of capsule in *S. aureus* inhibits expression of several adhesion proteins, and factors involved in biofilm formation. Since biofilm is a known virulence factor in *S. haemolyticus,* we want to investigate the interaction between capsule production and biofilm and how this is regulated under different growth conditions for clinical isolates versus commensal isolators.

Methods: We will investigate whether the different capsule types are associated with different biofilm-producing abilities and if capsule expression will contribute to reduced biofilm formation. This will be examined by a combination of molecular manipulation (such as knockouts and heterologous expression), negative staining techniques followed by microscopy, biofilm assays and RT-PCR to examine capsule expression during different growth conditions. Depending on the findings, animal models may also be included.

Results: In progress: We believe these results and knowledge will help identify new therapeutic targets and better treatment strategies to treat *Staphylococcus haemolyticus* infections in immune compromised patients.

SARS-COV-2 IN CHILDREN AND ADOLESCENTS: PERSISTING SYMPTOMS AND ANTIBODY RESPONSES

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Background: While long COVID after SARS-CoV-2 is a major health concern among the adult population, limited evidence exists about long COVID in children and adolescents. However, long-term sequelae among younger age groups are rising with the increasing number of infected cases after each pandemic wave. Data thus far indicates adolescents have a heightened risk of prolonged illness compared to younger children. Moreover, how vaccination impact persisting symptoms and the duration of protection against reinfection remain unknown. We aimed to explore persisting symptoms after SARS-CoV-2 delta infection, the impact and protective duration of vaccination in children and adolescents 10-20 years old against omicron infection.

Methods: 276 SARS-CoV-2 PCR-positive participants 10-20 years were recruited and answered questionnaires about acute and persistent symptoms at 2-4 (n=89) and 6-8 months (n=204) post delta infection. Blood samples were collected 2-4- and 8-months post-infection. A hemagglutination test (HAT) that we have previously shown correlated well with neutralising antibodies was used to measure antibodies directed against the receptor binding domain of the spike protein of the Wuhan, delta, and omicron variants.

Results: We found increased symptoms of dyspnea, memory and concentration problems at 6-8 months compared to 2-4 months post-delta infection in adolescents (16-20 years old). COVID vaccination did not reduce long-term symptoms in this age group at 2-4- and 6-8-months post delta infection. However, vaccinated cases had broader and higher antibody responses to variants of concern. Fifty-five percent of our cohort of delta infected individuals were reinfected with omicron 100 days post-vaccination. Vaccination provided 90% protection from reinfection up to 30 days.

Conclusion: A large proportion of children and adolescents reported persisting symptoms at 6 months post delta infection, irrespective of vaccination status. Due to the short duration of vaccine protection, half of our cohort was reinfected 6 months after vaccination.

EX VIVO LUNG MODEL TO ELUCIDATE HOST-PATHOGEN INTERACTIONS IN TUBERCULOSIS

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Introduction and objectives: Tuberculosis (TB) is one of the world's deadliest diseases, causing approximately 1.5 million deaths every year. It is caused by *Mycobacterium tuberculosis* (Mtb), which initially enters the alveolar space where it encounters alveolar macrophages (AMs), and alveolar epithelial cells type 1 (AEC1s) and type 2 (AEC2s). AMs play a key role in TB pathogenesis, but little is known about the role of AECs, which we will explore. Our objective is to create a co-culture model with AMs and AEC2s derived from induced Pluripotent Stem Cells (iPSCs) to study how Mtb interacts with AMs and AECs as well as how crosstalk between these cells shapes the infection outcome.

Methods: We have established iPSC derived macrophages and we are currently establishing protocols for AECs, which grow as alveospheres for a year. These will be validated during differentiation using flow cytometry and gene expression panels, as well as RNA sequencing comparing them to primary AEC2s. Next, we will expose the obtained AEC2s to 2 different auxotrophic strains of Mtb that can be used in BSL2 settings and/or various ligands to learn how the alveolar epithelium responds to infection. We will then establish the co-culture model of AEC2s and AMs by setting up ALI models in regular trans-well systems, thereby creating a model of the alveoli. Lastly, this co-culture model will be used to explore various questions regarding Mtb-induced inflammation, host cell death and spread.

Results and Conclusion: We expect this model to provide novel understanding of how crosstalk between alveolar cells influence Mtb infection outcome. In the long run, results from this project can guide host-directed therapeutic efforts in TB, and the model can be used to study other airway viral and bacterial infections.

DENTAL EDUCATION ON ANTIBIOTIC RESISTANCE: A QUALITATIVE ANALYSIS OF THE EDUCATORS' PERSPECTIVES

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Antimicrobial resistance (AMR) is a public health emergency, which can be responsible for 10 million deaths every year by 2050. One of the main drivers of this crisis is the overuse and the misuse of antibiotics, and evidence shows that dentists are responsible for ~10% of antibiotic prescriptions given in primary care globally. More importantly, recent studies indicate that knowledge and prescription practices in the dental profession can be improved.

With the goal of optimizing the use of antibiotics in Dentistry, we will focus on gathering the experiences from dental educators to explore how they perceive the educational development of dental students regarding antibiotic resistance and prescription practices.

To address our aim, we will employ qualitative research in the form of individual in-depth interviews. With this approach, we will get a better understanding of dental educators' point of views regarding the topic encompassing their lived experiences, what their opinions are on effectiveness of the current curricula, and what they think might be beneficial for dental students. The first steps we have worked with focused on conducting a thorough literature review to identify what studies have been done, and which questions should be prioritized. The overarching research question of the study is to explore how prepared dental students and graduates are to prescribe antibiotics. We have anchored our research questions into an educational framework based in three different elements: (i) assessing the students' learning process through the eyes of the interviewee; (ii) identifying challenges in teaching/learning and areas that should be addressed; (iii) identifying curricular modifications/interventions that could be helpful. It should be noted that the aim of qualitative research is not to confirm or reject a hypothesis, but rather explore the topic to give us a better understanding. Such approach will be employed in three different countries – Norway, Canada, and Brazil – to gather valuable information across different cultural settings.

As the goal of this project is to lead to an optimized use of antibiotics in Dentistry by focusing on engaging students through interventions in dental curricula, we expect that our findings can lead to the development of innovative pedagogical tools and assessment methodologies. The control of bacterial infections remains a cornerstone for the maintenance of a healthy global population; thus, we need to ensure that curricula in health care professions are well equipped to address the AMR crisis.

OVEREXPRESSION OF THE PENICILLIN RESISTANT DETERMINANT MURM IS TOXIC IN MURN DEFICIENT PNEUMOCOCCI

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Streptococcus pneumoniae is an opportunistic pathogen that causes serious infections of the lower respiratory tract as well as bacteremia and meningitis. It kills around one million individuals annually. Historically, penicillin have been the preferred treatment of respiratory tract infections. However, during the past decades, increased numbers of infections are caused by penicillin non-susceptible pneumococci. Resistant isolates have mainly three characteristics; expression of low affinity penicillin binding proteins (PBPs), a branched cell wall structure and an altered MurM version. MurM and MurN are responsible for adding Ser-Ala or Ala-Ala to the lipid II cell wall precursor causing the branched peptide structure of the pneumococcal cell wall. MurM is known as a resistant determinant in highly resistant isolates, since its removal resensitizes many isolates to penicillin. In this study, we examined phenotypical traits of mutants overexpressing a *murM* version from either the sensitive *S. pneumoniae* R6 strain or from a resistant *Streptococcus oralis* Uo5 strain in a background of both high and low affinity PBPs. We found that overexpression of *murM* was toxic in Δ*murN* mutants. When overexpressing *murM* in Δ*murN* mutants, the cells displayed slower growth and abnormal cell morphology. In addition, decreased penicillin tolerance was seen when *murM* was overexpressed in Δ*murN* mutant having low-affinity PBPs. We hypothesize that the deletion of *murN* combined with the overexpression of *murM* cause the pneumococci to incorporate high levels of single amino acid-branched muropeptides. High levels of single-branched muropeptides are probably not well tolerated in *S. pneumoniae*, explaining the lethal effect of *murM* overexpression in Δ*murN* mutants. We also suggest that low affinity PBPs interact with these single-branched muropeptides in a different manner causing an alteration in the competition between transpeptidation and peptide binding, leading the PBPs to have higher affinity towards penicillin. The overall results suggest that *S. pneumoniae* depends on MurN for the penicillin resistant determinant MurM to produce a branched structure cell wall compatible with a resistant phenotype.

CHANGES IN THE SKIN MICROBIOME IN RESPONSE TO ANTISEPTIC USE DURING A NATO EXERCISE: A METAGENOMICS APPROACH

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The skin is home to diverse populations of microorganisms and constitutes a first-line defense against pathogenic invaders. Most skin microorganisms are beneficial, but temporary changes in their environment can transition some of the species into more harmful ones. As the global use of antimicrobial agents increases, it is crucial to understand how these affect the microbial communities on the skin, especially for members of military forces where the risk of infection and wounds are higher.

In connection with the development of a novel antiseptic product for military purposes, this study will investigate the effect of stabilized hypochlorous acid (HOCl) at low concentrations on the microbial populations of human skin. HOCl has a broad-spectrum antimicrobial effect, without showing induced resistance. As the product is aimed at military forces, the sampled subjects are soldiers participating in real military training operations, with all the hygienic challenges such exercises entail. We have "swabbed" skin from Norwegian soldiers using the antiseptic product during the NATO exercise "*Cold response 2022"*. Through "shotgun" sequencing, all DNA extracted from these skin samples will be sequenced directly to capture the complete *metagenome* (DNA from *all* species), including bacteria, fungi, and viruses. Taxonomic classification will identify which microbes are present on the soldiers' skin. Functional analysis will show what potential their genomes encode, whether beneficial or pathogenic; and identify any presence of antimicrobial resistance genes. This will provide knowledge about the natural skin microbiota before and after field exercises, as well as whether short-term use of the antiseptic product has an effect on the skin microbiome. Overall, the results can contribute to improved routines for hygienic practice and wound cleaning in the armed forces, and simultaneously serve a part in the global community to fight increasingly resistant populations of microbial pathogens and the spread of infectious agents.

THE ROLE OF DRUGS USED IN CANCER CHEMOTHERAPY AS DRIVERS OF ANTIBIOTIC RESISTACE EVOLUTION IN ESCHERICHIA COLI

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Background: Many chemotherapeutic agents used in cancer treatment confer antimicrobial effects. How bacterial populations respond to selective and co-selective pressures exerted by individual cancer drugs and the implications for antimicrobial resistance (AMR) evolution, is however, to a large extent unknown. Here we screen through a chemical library of oncology drugs, searching for those where conferring AMR will give bacteria growth advantages.

Methods: Ten different pairs of fluorescently tagged *E. coli* MG1655 Δ*tolC* were constructed, each expressing a different resistance gene (*aac(6')-lb-cr, arm*A, *cat*A1*, dfrA1, qnrS1, tet*(A), *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{TEM-1,}}$ or *ble*$_{\text{MBL}}$). The different strains were competed against a susceptible strain over 30 generations of growth in the presence of one of 72 drugs belonging to the therapeutic drug class oncology (Prestwick Chemical Library®). Fluorescence signal was measured, and possible hits identified where expressing a resistance gene gave the strains growth advantages when compared to the susceptible parent strains in the presence of any of the drugs tested.

Results: Of the 720 combinations tested in the initial screen, 133 were identified as potential hits. Strong hits (69 in total) were identified for combinations of 22 cancer drugs and 6 resistance genes. The majority of hits were identified for resistance genes known to play a role in DNA and protein synthesis. Notably, those genes were positively selected for by cancer drugs targeting DNA synthesis, suggesting cross-resistance between multiple common AMR genes and a range of cancer drugs.

Conclusions: The preliminary screening results presented clearly indicate that different drugs used in cancer chemotherapy have a potentially major role in AMR evolution. Here we identify 133 hits in a screen of 720 cancer drug/AMR-gene combinations (~18.5%). The effects observed seem to be to some extent mechanistically dependent, with a large part of positive hits identified being for AMR-determinants/oncology drugs with joint molecular targets.

IDENTIFYING IMMUNOLOGICAL BIOMARKERS FOLLOWING LIVE ATTENUATED INFLUENZA VACCINATION IN CHILDREN

L. Hoen, K.Brokstad

Lower respiratory tract infections and pneumonia continue to be the leading cause of death in children, primarily mediated by viral agents such as Influenza and Respiratory Syncytial Virus. As a vaccine preventable disease, efficacious Influenza vaccine regimes are critical to protect vulnerable populations. Inactivated influenza vaccines have been widely used for the latter half of the century, with reliable assays for biomarkers predictive of protection. Yet, poor protective efficacy at ~60% in children, has pushed for the use of other vaccine types. Recently an intranasal Live Attenuated Influenza Vaccine (LAIV) has been approved for children older than than 2, demonstrating higher efficacy than inactivated vaccines. However, biomarkers predictive of protection are lacking as well as a complete understanding of the immunological events taking place following successful vaccination with LAIV. We have set out to fully elucidate the immunological underpinnings of LAIV vaccination using tonsil tissues from both vaccinated and unvaccinated individuals. Using multiparametric flow cytometry, immunhistochemistry, *In situ* hybridization, and RNA-seq we aim to identify novel biomarkers indicative of protection. This project will use the unique biobank of tonsillar and adenoid samples from clinical trials conducted at the Influenza Centre. We suspect the higher efficacy stems from the cross protection of T cells, as well as an immune response that more closely mirrors that of a natural infection, due to the intranasal delivery and nature of the vaccine.

EFFECT OF THE DYNAMIN INHIBITOR DYNASORE ON MURID BETAHERPESVIRUS 1 INFECTION IN SCAVENGER ENDOTHELIAL CELLS IN MOUSE LIVER

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Introduction: Liver sinusoidal endothelial cells (LSECs) are highly specialized scavenger cells with an unsurpassed capacity to endocytose and degrade many blood-borne soluble macromolecules and colloids, including several viruses. Dynasore is a small-molecule inhibitor of dynamin and used in studies of dynamin-dependent endocytosis. Although the inhibitory effect of dynasore on endocytosis in cell lines is well established, its effect on primary cells like LSECs has been little explored. The aim of the study was to examine dose- and time-dependent effects of dynasore on LSEC morphology, viability, and endocytosis and evaluate the use of dynasore as a tool in studies of viral uptake mechanisms in the cells.

Methods: LSECs from C57BL/6J mice were isolated by CD146-MACS of non-parenchymal liver cells obtained by collagenase perfusion of liver, and cells maintained in serum-free medium. Functional assays included endocytosis studies of model ligands for the mannose and scavenger receptors in LSECs (i.e. formaldehyde treated serum albumin (FSA), and ribonuclease B), and murid betaherpesvirus 1 (MuHV-1). LDH and apoptosis assays, live/dead cell imaging, immunofluorescence, and scanning electron microscopy were used to study cell toxicity, viability and morphology.

Results: Toxicity of dynasore was significantly affected by the LSEC density in culture, and time of treatment. Dynasore in doses and time of treatment that were well tolerated by the cells was able to effectively inhibit MuHV-1 infection of primary mouse LSECs, suggesting a dynamin-dependent uptake in the cells. Endocytosis of FSA (^{125}I- or FITC-labeled), and RibB (^{125}I-labeled) was also significantly inhibited by dynasore.

Conclusion: We found that dynasore is effectively inhibiting dynamin-dependent endocytosis, making it a powerful tool to study the mechanisms of endocytosis in LSECs. However, careful considerations should be given to time of treatment and dose of dynasore.

PLASMIDS PROMOTE INTRAGENOMIC MICROINDEL MUTATIONS IN *A. BAYLYI.*

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Short-Patch Double Illegitimate Recombination (SPDIR) is a recently discovered spontaneous mutation mechanism during which intragenomic ssDNA molecules hybridize at microhomologous sites and create a small (2 to >100 bp) combined insertion/deletion (microindel). SPDIR mutations are extremely rare, (approximately $5x10^{-13}$ per locus and generation, which is about 200-fold lower than spontaneous single nucleotide changes) but occur at exponentially higher rates under conditions that increase intracellular ssDNA concentration, such as lack of ssDNA exonucleases, under genotoxic stress, or during natural transformation. Recently we discovered that presence of extracellular genetic elements (plasmids) can result in significant increases in microindel frequencies.

Acinetobacter baylyi ADP1 strains with an insertion-inactivated *hisC* (histidine biosynthesis) gene were grown to stationary phase, washed and plated on medium without histidine. His^+ Colonies were counted, and the *hisC* genes were sequenced to determine the His^+ mutation. Presence of plasmid pQLICE (RSF1010 derivative) increased frequency of SPDIR mutations approximately tenfold (about $4.6x10^{-12}$). In ssDNA-exonuclease-deficient strains, the median SPDIR-frequency was increased fivefold. DNA sequencing confirmed that the ectopic DNA in His^+ microindel mutants frequently originated from pQLICE. However, the majority of SPDIR events still occurred with chromosomal DNA molecules, suggesting that pQLICE also promotes SPDIR mutations indirectly. Preliminary results with separate broad-host-range plasmids pBBR1 or pRK415 have also shown increased His^+ frequencies, but with less consistent increases in SPDIR frequencies.

Earlier experiments have demonstrated that increased cytoplasmic ssDNA levels result in elevated microindel mutation frequencies. It can be speculated that plasmids produce some ssDNA in the course of DNA replication, e.g. through strand displacement (pQLICE) or as rolling circle, though whether this ssDNA is the sole cause of the mutational increase is presently unknown.

IMAGING MITOCHONDRIA IN MYOCARDIOCYTES FROM ATLANTIC SALMON INFECTED WITH PISCINE MYOCARDITIS VIRUS

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Introduction and objectives: In this study we imaged myocardiocyte cells from Atlantic salmon infected with piscine myocarditis virus (PMCV). In order to study the effects of PMCV infection on cardiomyocyte mitochondria, tissues were digested and resulting myocardiocyte cells were cultured and stained with a mitochondria-specific dye. This investigation is part of VirtualStain, an inter-departmental project at UiT which brings together expertise from biology, imaging, and machine learning to foster advances in all three fields.

Methods: Post-smolt Atlantic salmon were infected via intramuscular injection with heart homogenate from infected fish. At weekly or biweekly intervals 6 fish from each group were humanely euthanized and processed for sampling. The heart tissues were enzymatically digested using a langendorff-free protocol and plated onto microscopy culture dishes. Following overnight culture in a cooled incubator the cells were stained with mitochondrial dyes for volume imaging using a widefield fluorescent microscope. Cells were then fixed for downstream electron microscopy. Images were processed using ImageJ and analyzed using ImageJ and python.

Results: The preliminary analysis indicates that mitochondria in infected animals are smaller at 1 and 4 weeks post-infection, with a similar trend at week 2. Following this, no changes were observed until the end of the experiment at 16 weeks post infection. Analysis is ongoing, with plans for electron microscopy and graph analysis of skeletonized mitochondrial network.

Conclusion: Our study indicates that PMCV infection initially affects development of mitochondria in post-smolt Atlantic salmon. We plan to investigate cytokine profiles to reveal whether the mitochondria effects were the result of viral pathology or the immune response. The investigation also brings to light the capabilities and limitations of fluorescent imaging in non-model animal immunology research.

NOVEL PRINCIPLES OF DRUG RESISTANCE IN THE WHO PRIORITY 1 PATHOGEN *ACINETOBACTER BAUMANNII*

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Treatment options for carbapenem resistant *Acinetobacter baumannii* (CRAb) are limited. *A. baumannii* is notorious for its ability to persist in the hospital environment, and constitutes a frequent cause of multidrug resistant nosocomial infections. The bacterium has developed an extraordinary ability to survive on surfaces, and is strongly linked to development of resistance toward common disinfectants. This project aims to identify novel factors and mechanisms in *A. baumannii* that are linked to resistance toward antimicrobials and disinfectants. This will be done by exposure of cells in planktonic growth to subinhibitory concentrations of commonly used antibiotics to *Acinetobacter* infections and to common disinfectants. Also, evolved isolates obtained by serial passaging will be subjected to whole genome sequencing to map mutations and possibly epigenetic changes linked to the development of increased resistance. Fitness of evolved strains will be compared to wild type. Also, membrane vesicles in *A. baumannii* are known to be linked to horizontal gene transfer, antimicrobial resistance development, and virulence. Furthermore, it is known that carbapenem-resistant *A. baumannii* (CRAb) can shelter cohabiting carbapenem-susceptible bacteria from carbapenem killing via extracellular release of carbapenem-hydrolyzing class D β-lactamases, including OXA-58. We will investigate whether strains evolved to resistance release modified amounts of MVs. Furthermore, the vesicles will be characterized by protein and potentially nucleic acid content relative to the mother cell (in evolved strains compared to wild type), to investigate whether the selection for antimicrobial resistance also leads to selective packaging of cargo in MVs, and characterize whether vesicles (MVs) from evolved strains are more virulent to host cell cultures compared to MVs from wild type mother cells.

TURNING STREPTOCOCCI'S FIGHT AGAINST ANTIBIOTICS TO OUR ADVANTAGE

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Several species in the *Streptococcus* genus frequently colonize the oral cavity, nasopharynx, and skin of humans and animals. Here they can infect the host leading to both mild and life threatening diseases. Today the healthcare systems and veterinarians are challenged by an increasing number of patients infected with antibiotic resistant streptococci. These infections deplete the treatment options available. New methods to treat and prevent streptococcal disease are therefore required. Streptococci's ability to readily acquire antimicrobial resistance and evade vaccines, as seen in pneumococci, is due to a mechanism known as natural competence for genetic transformation, which allows competent streptococci to acquire new traits by taking up naked DNA from the surrounding environment and incorporate it into their genome. In addition, to gain access to genes from other bacteria, competent streptococci secrete enzymes, known as fratricins, that efficiently kill and lyse closely related streptococcal species, releasing their DNA for uptake. Since fratricins are highly specific and efficient lytic enzymes we aim to explore the use of fratricins as a potential antimicrobial for prevention and/or treatment of skin and soft tissue infections caused by streptococci.

We plan to screen for the most efficient enzymes and by combining data on activity and target range with structural information, rational design will be used to improve their target spectrum, efficiency and stability. This includes characterization of fratricins mode of action, identify which bond(s) in the cell wall they cleave and solve their 3D structure. Furthermore, promising candidates will be tested for antimicrobial potential in mouse- and biofilm assays.

STUDYING THE MOLECULAR INTERACTIONS OF BACTERIAL TOXINS WITH HOST CHAPERONES

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Vibrio cholerae and enterotoxigenic *Escherichia coli* (ETEC) are two bacterial pathogens responsible for millions of diarrhea cases each year. These pathogens release two similar AB_5 toxins that are directly responsible for the severe diarrhea: the cholera toxin (CT) and the heat-labile enterotoxin (LT), respectively. They consist of a catalytically active A subunit anchored to five copies of a cell-binding B-subunit. After cell uptake, the toxins are transported to the endoplasmic reticulum (ER), where the A subunit dissociates from the rest of the toxin, unfolds, and is finally exported to the cytosol with the help of several host chaperones. These steps are essential for cholera and ETEC intoxication, however, they are still poorly understood at the structural level.

This project aims to investigate the interactions mediating toxin activation by host chaperones. This work involves production of the recombinant target proteins, binding and interaction studies, and structural characterization of the protein complexes. We plan to use a combination of structural biology techniques including cryo-EM, X-ray crystallography, SAXS/SANS and molecular dynamics simulations. We expect that this work provides structural details about a key molecular mechanism of pathogenesis in bacteria, which can serve as a starting point for developing new treatment strategies against diarrheal diseases.

AN INVESTIGATION OF THE COMPETENCE REGULON IN *STREPTOCOCCUS DYSGALACTIAE* BY RNA-seq

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Naturally competent bacteria can take up naked DNA from the environment and incorporate it into their genomes. This ability may contribute to antigenic variation and spread of antibiotic resistance. The proteins required for DNA uptake and homologous recombination during this process are conserved across species. These genes are expressed during a specific physiological state known as competence in response to an inducing peptide. Despite the presence of genes required for natural transformation in most streptococci, not all have been shown to be naturally competent, including the human and animal pathogen *Streptococcus dysgalactiae*. The aim of this study is two-fold; (1) to understand the role of competence in this species and (2) to make it amenable for genetic modification via natural transformation. *S dysgalactiae* genomes of human and animal origin were analyzed, revealing that only 49 % (n=180) of the genomes encode the full set of intact genes required for natural transformation, the rest being truncated or inactivated by phages. Three strains harboring the full repertoire of genes were selected and by using a luciferase reporter, we show that expression of SigX, the master regulator of competence, as well as late competence genes are induced upon addition of the competence inducer ComS. Notably, we were also able to observe transformation of DNA into these strains *in vitro*, however, the transformation efficiencies were extremely low. To further understand this phenomenon and identify possible barriers to the low transformation efficiencies, a time-resolved RNA-seq analysis was performed to define the *S. dysgalactiae* competence regulon. This revealed a surprising variation in competence response within and between strains, and we also observed regulation of genes not associated with competence. Our results suggest that *S. dysgalactiae* can take up DNA by natural transformation, but also that the competence system may have other roles in this species.

DISCLOSING THE BIOFILMOME OF *ESCHERICHIA COLI*

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The relationship between biofilm formation in clinical infections and the genetic background of *Escherichia coli* is unknown. *E coli* biofilms are associated with osteomyelitis, urinary tract- and device-associated infections, all of which may cause systemic disease (sepsis). Consequentially, such clinical biofilms can result in difficult-to-treat recurring infections and increased mortality.

We aim to disclose the biofilmome of *E. coli*, resolving its biofilm forming potential by analysing 3254 isolates from human blood cultures. First, we will identify all genes coding for products excreted from the cytosol by genomic analyses and a structured literature search. Second, the presence/absence of known and putative biofilm-associated genes will be correlated with the biofilm formation (determined through a panel of different phenotypic methods).

Initial results suggest that the ability to produce curli and cellulose, previously described matrix-components of *E. coli* biofilms, is widespread across the collection, but two central genes responsible for cellulose synthesis are absent in parts of the study population. PGA is also highly prevalent, but completely missing from ST69. Fimbria are important for initial adhesion, and certain fimbria-associated genes such as *sfa* and *fimH* are highly prevalent whereas others vary, being a potential foundation for biofilm-associated genetic profiles.

This research can reveal determinants of biofilm formation, providing a deeper understanding the role of this important mode of growth in shaping the virulence of clinical *E. coli*. Additionally, it may reveal potential novel targets for antimicrobial treatments focused on the non-essential process of biofilm formation, thus imposing less selective pressure for resistance development compared to traditional drugs.

BACTERIAL ANTIMICROBIAL RESISTANCE MECHANISMS - THE NOVEL DUAL CARBAPENEMASE INHIBITOR APC247

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Background: The constant ability of bacteria to adapt, evolve and acquire new mechanisms of resistance to avoid elimination, has led to an acute demand for improved treatment regimes. The presence of several resistance mechanisms has left numerous of the previous powerful antimicrobial components inefficient. This emergence of multi-resistant bacteria is a devastating global health problem, caused directly by use and over-use of antibiotics. Although the need for new antimicrobial agents is a necessity, the efficacy of several antimicrobials has recently been restored by targeting the resistance mechanisms by inhibition.
Carbapenemases are enzymes which destroy carbapenems (broad-spectrum β-lactam antibiotics) and can roughly be divided into two categories: serine β-lactamases (SBLs) and metallo-β-lactamases (MBLs). Carbapenem-resistant strains (*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* etc.) are all on the Priority 1 (Critical) part of the WHO priority pathogens list for R&D of new antibiotics. Although there are a few compounds on the market that inhibit the SBLs, no compounds are currently available that targets the MBLs, or both.

Project outlook: AdjuTec Pharma addresses this problem by developing an adjuvant technology with the aim of inhibiting both MBLs and SBLs. The discovery of APC247 and its dual carbapenemase inhibitory effect will be an important contribution to the fight against antimicrobial resistance. Initial testing has shown that the combination of APC247 and meropenem, lowers the minimum inhibitory concentration (MIC) of several WHO priority 1 bacterial strains.
The aim of developing APC247 towards clinical trials, and with the long-term goal to provide a life-saving new drug reversing resistance in critical human infections, creates an urgency of further broadening the knowledge about this putative dual inhibitor. Investigating the mechanism of action (MoA), frequency of resistance (FoR) and mechanism of resistance (MoR), will provide the insight needed to ensure appropriate usage, later in the clinic.

THE ROLE OF SCAVENGER ENDOTHELIAL CELLS IN BACTERIOPHAGE PHARMACOKINETICS

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Bakcground: Intravenous administration of phages is primarily used when treating critically ill patients with widespread infections/bacteremia. Studies of phage pharmacokinetics/dynamics in individuals receiving phage therapy are limited, and few reports exist about the cells and mechanisms involved in phage blood clearance. Endocytosis of phages is postulated as a major process determining pharmacokinetics, but it has been studied mostly in cell lines, which are often very different from normal cells in the body. According to the currently available literature, after systemic administration phages accumulate in liver and spleen. In the liver, Kupffer cells and liver sinusoidal endothelial cells (LSECs) are important scavenger cells actively engaged in the clearance of foreign antigens, including viruses. Our leading hypothesis is that many phages reaching the liver will be endocytosed by LSECS, which have a very high endocytic capacity, and degraded. This may constitute a vital role in elimination of phages from blood during phage treatment. We further hypothesize that rapid uptake and efficient degradation of phages in LSECs is a silent process, and that this may explain why phages are well tolerated by the human body.

Methods: Our research group recently reported that T4 phages are efficiently endocytosed and degraded by primary rat LSECs *in vitro*. We have therefore started a project to investigate how various i.v. administered phages interact with scavenger cells in a complete physiological system, by studying the blood clearance and organ distribution in a mouse model.

Results: Preliminary results from our current work with high doses of bacteriophage K1F-GFP (T7-like lytic phage against *E.coli*) show the elimination of 80% of circulating particles as quickly as 20 minutes after inoculation with a concurrent high uptake of phage in liver.

Conclusion: The liver is a vital organ in blood clearance of K1F-GFP and phage interaction with LSEC and other resident liver cells clearly warrants further investigation.

Machine Learning for Antimicrobial Resistance

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The rising prevalence of antimicrobial resistant (AMR) bacteria has caused alarm in medical fields and has been listed as major threat to global health by the World Health Organization. These resistances can spread rapidly through both vertical and horizontal gene transfer. Consequently, the overuse of an- timicrobials can and has driven a rapid increase of AMR but it is not the only cause for it. Certain microbes with highly plastic genomes and higher rates of horizontal gene transfer can acquire resistances quite often and efficiently.

Enterococcus faecium is a commensal microbe native to the gut of many animals and humans. While this makes it a vital part of a human's internal microbiome, some E. faecium strains are infectious and resistant to glycopeptide antibiotics. These virulent strains are often found in clinical settings, resulting in nosocomial infections.

Understanding the genetic differences between the commensal and virulent clinical strains can help inform the development of safe and effective treatment plans. The first step in this process is to be capable of isolating the genetic vari- ations that are overrepresented in clinical strains of E. faecium. Genome-wide association studies (GWAS) attempt to use statistical tests to isolate gene vari- ations responsible for observed phenotypes. Recent developments in sequencing technology have made larger genome datasets available, opening the door to develop and apply a multitude of machine learning techniques to enhance the power of GWAS.

SIGNALLOME ANALYSIS OF A BETA-CLAMP INHIBITOR AND PUTATIVE THYMIDYLATE MONOPHOSPHATE KINASE INHIBITOR

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Introduction: World-wide increase in antimicrobial resistance far outpaces the development of new antimicrobial drugs. A putative thymidylate monophosphate kinase (TMPK) inhibitor (JK274) combined with a beta-clamp inhibitor (MDR26) exhibited a four-fold reduction in the MIC of *Staphylococcus aureus*. To better understand the synergistic effect between the two antimicrobials and further elucidate the MoA of the two antimicrobials separately, we conducted a proteomic study using *S. aureus*.

Method: Logarithmic phase *S. aureus* was treated with either JK274, MDR26, or a combination. Two doses of each antimicrobial were included – a high and low dose. The high dose mimicked the growth curve of the combination treatment. The combination treatment consisted of low dose JK274 and MDR26. Pellets were sampled at 10, 25, 50 and 180 minutes after treatment and processed for signallome analysis using a multiplexed inhibitor bead assay. A control with no treatment was also sampled.

Results: The effects of MDR26 were seen in the 10-minute samples while the effects of JK274 treatments were not significantly detected until the 50-minute samples. After 10 min, proteins involved in translation and modification of proteins and RNAs showed reduced enrichment compared to the control. Additionally, the essential cell division protein FtsA was strongly reduced in all timepoints. At 25 min, the protein FtsZ also exhibited reduced pull-down with high dose MDR26. On the other hand, JK274 strongly reduced enrichment of TCA cycle proteins. JK274 did not reduce levels of TMK as expected, but instead yielded reduced pull-down of nucleoside diphosphate kinase (NDK).

Conclusion: Synergism between MDR26 and JK274 might be due to a combined assault on both translation and respiration that overwhelms the bacterial cell. As a general NDK inhibitor, JK274 will most likely not be suitable as an antimicrobial as it may be toxic to human cells. However, MDR26 shows promise as novel antimicrobial drug.

A COHORT STUDY OF COVID-19 NESTED WITHIN AN RCT OF PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA (COVID-19-CAPNOR STUDY). SUB-STUDY ON CAP BIOMARKERS: TRANSCRIPTIONAL AND PROTEIN BIOMARKER PROFILING IN PATIENTS WITH COVID-19 OR ACUTE LOWER RESPIRATORY TRACT INFECTIONS WITH ALTERNATE MICROBIAL ETIOLOGY.

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COVID-19-CAPNOR study: We recruited patients (initiated on 25th September 2020) hospitalized for community-acquired pneumonia (CAP) at the Emergency Department (ED), Haukeland University Hospital (HUS), Bergen, into a pragmatic randomized controlled trial (RCT) [NCT04660084]. The proposed patient-centered, opportunity-driven nested cohort study will contribute to optimising sampling strategies, patient management, microbial detection, mapping of protein and transcriptional responses, and supporting capacity building and infection control measures. To date, SARS-CoV-2 was identified in 17.4% of patients recruited in the RCT (74/425). A bacterial co-infection was detected in 42% of COVID-19 patients, with *H. influenzae* and *S. aureus* is the most common bacteria detected in mixed infections. Despite a bacterial co-infection, 52.9% of COVID-19 patients with a bacterial co-infection were clinically deemed as not requiring treatment with an antibiotic.

COVID-19-CAP biomarker nested sub-study

Background: To enable optimal care, prevent unnecessary antibiotic prescriptions, and maximize the use of hospital resources, a precise differential diagnosis between acute viral and bacterial illnesses is essential.

Methods: Whole blood RNA samples from patients with CAP due to pure bacterial etiology (n=11), pure viral etiology, other than COVID-19 (n=11) and COVID-19 (n=10), and plasma samples from the pure bacterial etiology (n=49), pure viral etiology (n=35), and pure COVID-19 (n=40) were selected and analysed using high throughput methods for transcriptional (Clariom™ S assay) and protein profiling (Bio-plex multiplex assay), respectively.

Results: The transcriptional analysis shows that 177 differentially expressed genes overlapped between the following comparisons: pure bacterial vs. pure viral and pure bacterial vs. pure COVID-19. Of these, 103 genes were down-regulated, and 74 were up-regulated in the pure bacterial group compared to pure viral and COVID-19 groups. Protein biomarker profiling of COVID-19 patients compared with those with acute respiratory tract infections with an alternate microbial etiology (bacterial/viral) showed that the concentration of GCF, IL6, IL8, MCP1, bFGF, IL1ra, IL4, MIP1a, and MIP1b was increased while, the concentration of IP10 and TNF was decreased in pure bacterial etiology as compared with those with viral etiology (including COVID-19). Further, compared to the pure bacterial and other viral etiology, the concentration of IL1b and IP10 was elevated in COVID-19 patients, whereas the concentration of IL7 was decreased in COVID-19 patients.

THE ROLE OF TRKA AND C-DI-AMP IN *STREPTOCOCCUS MITIS*

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Introduction and objectives: The opportunistic pathogen and commensal bacterium *Streptococcus mitis* can cause serious bacteremia in neutropenic patients and infective endocarditis. We have shown that the second messenger cyclic di-adenosine monophosphate (c-di-AMP) influences phenotypical characteristics such as growth, biofilm formation, susceptibility to antibiotics, exposure to UV radiation and osmotic stress tolerance. The mechanisms and cellular processes behind these phenotypical characteristics are yet to be found. When looking into the literature of the effector proteins that bind to c-di-AMP, we found TrkA.
C-di-AMP is an important regulator of the K+ homeostasis in many bacteria. It controls the K+ transporter activity by binding to an effector protein such as TrkA. When TrkA is bound to c-di-AMP, TrkA reduces the K+ transporter activity. In this study, we investigate if c-di-AMP, through TrkA influences phenotypical characteristics of *S. mitis*.

Methods: We utilized our set of KO-mutants of the genes encoding c-di-AMP turnover proteins and the knockout of the *TrkA* gene in these KO-mutants, creating double mutant. The KO-mutants are adenylate cyclase (CdaA), which synthesizes c-di-AMP, and mutants of the phosphodiesterases Pde1 and Pde2, which degrade c-di-AMP to AMP via phosphadenylyl-adenosine (pApA).

Biofilm, MIC, growth, UV-radiation and osmotic stress tolerance assays were performed to study the influence of TrkA through c-di-AMP on these phenotypical characteristics.

Results: TrkA does not influence susceptibility to ampicillin, biofilm formation, or growth. Preliminary data indicate that TrkA may influence susceptibility to UV stress, ciprofloxacin, and osmotic stress, but it is not yet established if this is via c-di-AMP signaling.

Conclusion: TrkA does not influence biofilm formation, growth, or susceptibility to ampicillin in *S. mitis*.

IDENTIFICATION OF VIRULENCE FACTORS IN METHICILLIN-RESISTANT - *STAPHYLOCOCCUS AUREUS* BY USING ACTIVITY-BASED PROTEIN PROFILING (ABPP)

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Staphylococcus aureus is a major human pathogen in hospital and community-acquired infections. Prevention and treatment of *S. aureus* infections by antibiotics are difficult for the development of resistance and the ability of *S. aureus* to persist in biofilms. Thus, developing new diagnostic strategies and options for treating life-threatening bacterial infections is an urgent task. Chemical probes have become invaluable in studying the physiology of bacteria, their virulence, and their resistance to antibiotics and using this knowledge to develop point-of-care diagnostic tools. Of note, activity-based protein profiling (ABPP) is a powerful method for deciphering specific functional enzymes in bacterial systems. The study aimed to identify novel (functional) enzymatic activities in S. *aureus* under biofilm-promoting growth conditions.

Competitive ABPP was used to identify functional enzymes and inhibitors activity in live *S. aureus* by treating intact cells with inhibitors, carmofur/5-fluorouracil, and fluorescent ABP (compound **1**) followed by analysis of labeled proteins by SDS–PAGE analysis. A mass spectrometer was used to identify target enzymes using biotin-tagged ABP (**1**) & mutant strains for target validation. MIC & time-kill assay of inhibitors, carmofur and 5-fluorouracil was done by the broth microdilution method.

The ABP (compound **1**) showed broad activity in targeting functional enzymes on biofilm-promoting growth conditions. Compound **1** labeled fluorophosphate-binding serine hydrolase virulence factors (fphB, fphE, and fphF) and other teichoic acids D-Ala esterase (FmtA) and IMP cyclohydrolase (PurH). Most bands labeled by compound **1** can be completed out by pretreatment with the unlabeled parent inhibitors carmofur and active drug, 5-fluorouracil of compound **1**, and both drugs showed the same antibacterial activity (MIC-5µM) & time-dependent killing against *S. aureus* USA 300.

The ABP (compound **1**) could be used for targeting & inhibiting a group of hydrolytic enzymes whose functions are likely to be important for various aspects of cellular physiology and host- pathogen interactions.

COMBATING TUBERCULOSIS: CHEMICAL TAILORING OF ISONIAZID

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Introduction and Objectives: Tuberculosis (TB) is one of the leading infectious diseases caused by *Mycobacterium tuberculosis* (*Mtb*). According to the WHO, 10 million new cases and 1.5 million deaths were reported in 2021. Isoniazid (INH) is one of the key molecules employed in the treatment of TB, inhibiting Mtb cell wall biosynthesis. However, the efficacy of INH has seriously decreased due to emerging INH-resistant and INH/rifamipicin-resistant (MDR) *Mtb* strains. Therefore, novel candidate anti-TB drug inventions target mycobacterial growth rate and aim to minimize drug resistance.

Methods: INH was chemically tailored by applying the molecular hybridization approach to link INH with various azole rings, alkyl carboxylate, and sulfonate esters through hydrazone functionality. New compounds were screened for their antitubercular activity against *Mtb* H37Rv and INH-resistant clinical *Mtb* isolates using the Resazurin Microtiter Assay (REMA). Additionally, the cytotoxic effects of active compounds were assessed on different healthy host cell lines using tetrazolium bromide (MTT) assay.

Results: The stability of the INH hydrazone moiety in the chemical structure of the final compounds was confirmed in both aqueous medium and DMSO. Compounds carrying azoles exhibited moderate antitubercular activity in REMA, similar to or slightly better than generic INH, whereas compounds with alkyl carboxylates were inactive. Some of the compounds with sulfonate esters were equipotent to isoniazid also against INH-resistant *Mtb* clinical isolates, with IC_{50} of 1.5 µM against *inhA* promoter mutants and 6.2 µM agaist *katG* mutants. All the compounds were found to be safe against HEK293, IMR-90, and BEAS-2B with improved therapeutic indexes as compared to INH.

Conclusion*:* Taken together, the results suggest that the INH-hydrazone-sulfonate esters should be further optimized for candidate antitubercular drug development.

UNDERSTANDING THE ROLE OF THE SIGNAL PEPTIDE IN OPTIMIZING PRODUCTION OF LPXTG-ANCHORED HETEROLOGOUS PROTEINS IN *LACTIPLANTIBACILLUS PLANTARUM*

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Introduction: The lactic acid bacteria *Lactiplantibacillus plantarum* is a part of the human microbiome, and strategies for exploiting the commensal bacterium as a vaccine delivery vehicle of antigens are being explored. *L. plantarum* carrying surface displayed tuberculosis hybrid antigen Ag85B-ESAT-6 are shown to induce specific immune responses in mice. However, a drawback of using strains with cell wall anchored antigens as a delivery vehicle is growth inhibition. This study aims to construct a strain producing LPxTG-anchored antigen with optimized growth and simultaneously understand factors influencing growth retardation.

Methods: The construction of 6 strains with different LPxTG-anchors and 11 strains with various signal peptides were made to optimize production and improve the growth. In addition, to gain a deeper understanding of what causes the growth retardation, the antigen was exchanged with a HaloTag protein in the 11 strains with different signal peptides. By labelling the HaloTag protein with membrane impermeable and permeable ligands a measure of translocation efficiency depending on the signal peptide were analyzed using western blot analysis and fluorescent microscopy.

Results: We found that varying the anchor sequences did not improve the growth, but that the growth rate could be increased by 50% by changing the signal peptide without compromising the amount of antigen on the bacterial surface. However, fluorescence microscopy of strains that produce and surface display the HaloTag protein showed that the translocation efficiency greatly varied among the different signal peptides.

Conclusion: The choice of signal peptide greatly influences the translocation efficiency and growth rate of strains producing heterologous genes fused to a LPxTG cell wall anchor. Further experiments will be conducted to elicit how interactions between the signal peptide and components of the secretion machinery may increase or decrease the translocation efficiency. These findings will be beneficial for future strain design.

NOVEL BACTERIOCIN ROMSACIN FROM STAPHYLOCOCCUS HAEMOLYTICUS INHIBITS GRAM-POSITIVE ESKAPE PATHOGENS

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New antimicrobial compounds are important to combat antibiotic resistant bacteria. Bacteriocins are ribosomally synthesized peptides that bacteria produce to inhibit growth of competing bacteria occupying the same niche. Due to differences in the modes of action between bacteriocins and antibiotics, bacteriocins can be potent against antibiotic-resistant strains. To find new antimicrobials such as bacteriocins, we investigated if *Staphylococcus haemolyticus* produce them. *S. haemolyticus* is a skin bacterium causing hospital infections, and it is often multidrug-resistant.

A collection of clinical and commensal *S. haemolyticus* isolates (n= 174) were screened for antimicrobial activity against three indicator strains: *Lactococcus lactis*, *S. haemolyticus*, and *Staphylococcus aureus*. One *S. haemolyticus* commensal produced a distinct inhibition zone against all three indicators, and was the only isolate with antimicrobial activity after being heated to 100 °C. We chose this isolate for further characterization due to its strong antimicrobial activity. The antimicrobial was stable to pH (2.1-11.9, 30 min) and temperature (4-121 °C, 15 min), but sensitive to trypsin protease, which are typical characteristics of bacteriocins. The antimicrobial was purified using cation exchange and reversed-phase chromatography. We confirmed that the antimicrobial was a novel two-peptide lantibiotic bacteriocin by performing matrix assisted laser desorption/ionization time-of-flight mass spectrometry, whole-genome sequencing, and structure prediction. The bacteriocin was active against a broad range of gram-positive bacteria, including various coagulase-negative staphylococcal species, and the ESKAPE pathogens *S. aureus*, and *Enterococcus faecium.* The bacteriocin did also eradicate *S. haemolyticus* and *S. epidermidis* biofilms. Electron microscopy and a pore formation assay indicated that the bacteriocin mode of action involve inhibition of cell wall biosynthesis without pore formation.

We have described a new bacteriocin, romsacin, with broad antimicrobial activity, which is promising to combat antimicrobial resistant pathogens.

PRECLINICAL DEVELOPMENT OF A NOVEL METALLO-β-LACTAMASE INHIBITOR APC148

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Antimicrobial resistance (AMR) is an emerging and devastating global health problem caused directly by use and over-use of antibiotics. AMR jeopardizes our ability to perform many life-saving procedures such as surgery and cancer chemotherapy. β-lactamases are enzymes which break down β-lactam antibiotics, conferring drug resistance in pathogenic bacteria. In contrast to serine β-lactamases (SBL) for which inhibitors exist, metallo-β-lactamases (MBL) are zinc-containing enzymes for which there are currently no approved inhibitor products available on the market.

APC148 is a novel zinc-chelating MBL inhibitor which has demonstrated promising ability to restore the efficacy of carbapenem antibiotics in clinical strains of Gram-negative bacterial pathogens *in vitro* and *in vivo*. Here we present preliminary results from a preclinical development program of APC148. The compound has previously been found to reduce the minimum inhibitory concentration of meropenem to susceptible levels in MBL producing clinical strains. Here we have expanded this analysis to map the frequency and mechanism of resistance toward an APC148/meropenem combination in a wider set of carbapenem resistant clinical isolates of *Escherichia coli* and *Klebsiella pneumonia*, indicating that the frequency of resistance development to the combination in general is low. Furthermore, animal studies have previously shown that APC148 exhibits low *in vivo* toxicity. Here, Caco-2 assays in the presence of extrusion pump inhibitor cocktails showed low biological membrane permeability, in line with the low toxicity profile of the compound. Future studies on the mechanism of resistance toward the APC148/meropenem combination in the expanded set of Gram-negative pathogens will involve selection of genetically stable mutants and systematic whole genome sequence comparisons to high quality reference genomes.