Antimicrobial Resistance:

Summary of Abstracts for the Poster Session

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P82	Identification of Novel Biosynthetic Enzymes in Peptide Natural Products	Mary Bond	School of Biological Sciences, The University of Auckland, Auckland, New Zealand
P83	New Method for Nanopore Metagenomic Sequencing of Pathogens from Retail Poultry	Alice Bradbury	Molecular Epidemiology and Public Health Laboratory, School of Veterinary Science, Massey University, Palmerston North, New Zealand
P84	Unravelling the complexity of Staphylococcus aureus in Chronic Rhinosinusitis	David Broderick	Department of Surgery, The University of Auckland, Auckland, NZ
P85	The Role of Efflux Pumps in Potentiating the Evolution of Untreatable Multidrug-Resistant Strains of Mycobacterium tuberculosis	Caitlin E Cunniffe	Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand
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P87	The International Literature and Policy on the Prevalence and Management of Antimicrobial Resistance in Waterways	Dixit, D	School of Medical Sciences, Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ
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P95	Unravelling the oligomeric forms of the membrane-interacting EsxE-EsxF complex	Hoang, A.V	Biomolecular Interaction Centre, Christchurch, NZ, School of Physical and Chemical Sciences, University of Canterbury, NZ.

P96	Altered physiology of drug-resistant M. tuberculosis restricts growth and metabolic adaptability	Jowsey, W.J	Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand, Mātai Hāora – Centre for Redox Biology and Medicine, Department of Pathology and Biomedical Science, University of Otago Christchurch, Christchurch, New Zealand
P97	Targeting Pseudomonas aeruginosa biofilms using peptide-gold nanoparticles conjugates	Kumarage, P.M.	Department of Microbiology & Immunology, University of Otago, Dunedin, NZ
P98	Synthetic studies towards novel oligomers to address antibacterial resistance	Mullin, R.	University of Auckland
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P100	Extracellular DNA joining in the multi-drug resistant pathogen Neisseria gonorrhoeae	Pan, J.	School of Science, University of Waikato, Private Bag 3105, Hamilton, 3240, New Zealand
P101	Investigating phage infection dynamics during lesser studied physiological states to improve anti-biofilm therapeutics	Smith, L.M	Department of Microbiology and Immunology, University of Otago, Dunedin, NZ Maurice Wilkins Centre for Molecular Biodiscovery, University of Otago, Dunedin, NZ Genetics Otago, University of Otago, Dunedin, NZ
P102	Feedback regulation of iron-sulfur cluster biogenesis	Stephanie M. Stuteley	School of Biological Sciences, The University of Auckland; Auckland, Private Bag 92019, New Zealand
P103	Allosteric regulation of isocitrate lyase 2 as a potential target against Mycobacterium tuberculosis	Jamie Taka	

P104	PBT2-mediated metal ion disruption: uncovering new metabolic vulnerabilities and overcoming resistance in MRSA	Todd Rose, F.O.	Department of Microbiology and Immunology, University of Otago, Dunedin, NZ,
P105	Sulfur assimilation and metabolism in Neisseria gonorrhoeae.	van Niekerk, S.	Te Huataki Waiora, School of Health, University of Waikato, Hamilton New Zealand
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P107	Antimicrobial susceptibility of Kingella kingae in Australasia: Implications for empirical treatment of paediatric osteoarticular infections	Wolf, K	Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ
P108	Validation of genomic antimicrobial resistance markers (AMR) for national AMR surveillance for Salmonella spp. Identifies discrepancies in azithromycin resistance for S. Paratyphi A	Wright, J	Institute of Environmental Science and Research, Porirua, NZ, 2Health Security, NZ Institute for Public Health and Forensic Science, Porirua, NZ,
P109	Structural and Enzymatic approaches to 3-methyl-L-tryptophan production for antimicrobial peptide development	Yosaatmadja, Yuliana	School of Biological Sciences, The University of Auckland, Auckland 1142, Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Auckland 1142.
P110	Identifying genes involved in resistance against combinatory treatment on K. pneumoniae infection	Islam, R.	Department of Microbiology and Immunology, University of Otago, Dunedin, NZ

P81 Surveillance of vancomycin-resistant enterococci in New Zealand.

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Vancomycin-resistant enterococci (VRE) are under passive surveillance in New Zealand (NZ), with low numbers of VRE found historically. The epidemiology of VRE in NZ in 2023 is described, including information on a large outbreak, caused by ST17 *Enterococcus faecium* with *vanB*.
Diagnostic medical laboratories refer VRE to the Antimicrobial Resistance (AMR) Laboratory at PHF Science for confirmation and further characterisation, including susceptibility testing and Illumina-based whole genome sequencing.
A total of 391 vancomycin-resistant *E. faecium* and *Enterococcus faecalis* from 387 patients were confirmed. *E. faecium* remains the dominant vancomycin-resistant enterococcal species in NZ (388/391, 99.2%). The total number of isolates found in 2023 was nearly three times more VRE than the highest number of cases recorded previously in a single year.
Clinical infections were uncommon, with the majority (95.4%, 373/391) of isolates in 2023 from screening samples. The VRE showed a mix of resistance genes; 11.5% *vanA* (45/391), 88.2% *vanB* (345/391) and 0.3% *vanN* (1/391). The high number of isolates was primarily due to the spread of one ST17 strain of *E. faecium*, which were predominantly referred from Waikato and Tauranga Hospitals (290/391, 74.2%). This strain was first identified at the end of 2022. The utilisation of WGS-based methods to type VRE in 2023 improved VRE surveillance in NZ and enhanced our understanding of this outbreak.

VRE continues to be considered non-endemic in NZ and hospitals continue to implement a ‘stamp it out’ approach to VRE. This requires ongoing and highly resource intensive infection prevention action, with particular attention to cleaning, transmission-based precautions and patient screening in an attempt to contain and eliminate VRE from NZ healthcare facilities.

P82 Identification of Novel Biosynthetic Enzymes in Peptide Natural Products

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Antimicrobial resistance poses a significant challenge, urging the scientific community to explore novel compounds with antimicrobial properties. Ribosomally Synthesized and Post-Translationally Modified Peptides (RiPPs) are naturally occurring molecules produced by various microorganisms, including bacteria and fungi. These peptides are structurally diverse and carry out diverse functions, including bacterial defense, making them promising candidates for antibiotic discovery. We have used genome mining approaches to identify novel RiPP Biosynthetic Gene Clusters (BGCs), encoding enzymes that modify and produce RiPP natural products. These enzymes install features such as azoline and macroamide rings, which are crucial for RiPP bioactivity.

This research aims to identify novel bioactive RiPP natural products and to investigate the enzymes responsible for their production. Two modifying enzymes from each of the YcaO and radical *S*-Adenosylmethionine (rSAM) enzyme families and their corresponding peptides have been produced recombinantly in *E. coli*. Peptide modification experiments with YcaO enzymes have been carried out *in vitro*, while an *in vivo* modification strategy has been pursued for the rSAM enzyme-peptide pairs. The resulting RiPP products will be characterized using mass spectrometry, while enzyme function will be further explored using biochemical and structural techniques. Studying RiPPs and the enzymes that are involved in their maturation will lead to the discovery of novel bioactive compounds and enzymatic tools, supporting efforts to overcome the challenges presented by antimicrobial resistance.

P83 New Method for Nanopore Metagenomic Sequencing of Pathogens from Retail Poultry

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Poultry is well-established as a common source of foodborne pathogens, including *Salmonella* and *Campylobacter*. Illness is typically self-limiting, but long-term complications and work disruption mean these pathogens have a significant economic impact. The rising antimicrobial resistance in *Campylobacter*, such as ST-6964, emphasises the need for ongoing pathogen surveillance. Current detection methods are culture-based, taking a week to confirm pathogen presence alone. A broader approach to surveillance of pathogens from retail poultry is necessary.

A method of metagenomic Nanopore sequencing for exploring retail poultry microbiomes and pathogen presence has been developed. Short enrichments for *Campylobacter* and *Salmonella* were tested to increase pathogen DNA, followed by testing of depletion of chicken DNA, based on Bloomfield *et al'*s method, with consumables accessible in NZ [1]. For each chicken sample, non-enriched, *Campylobacter*-enriched, and *Salmonella*-enriched samples were sequenced. A non-enriched chicken rinse was sequenced to investigate how chicken carcass microbiomes may impact pathogen presence. DNA extraction kits were compared. Library preparation protocols native barcoding 24 (NB24) and native barcoding 96 (NB96) were compared.

We found that a 24-hour enrichment in blood-free CAT broth enriched *Campylobacter* sufficiently, while an 8-hour pre-enrichment in BPW, followed by a 16-hour enrichment in RVS, was able to enrich *Salmonella* DNA for Nanopore sequencing. Host-DNA depletion significantly reduced chicken DNA (t-test, p-value = 0.0077). The Qiagen HMW MagAttract Kit was used, as this produced DNA of a HMW compared to other kits we tested. Library preparation using the NB24 protocol yielded library preps with higher DNA concentrations compared to the NB96 protocol. For each sequencing run, six samples were run on the MinION using an R10.4.1 flow cell.

A faster and more insightful method for detecting retail poultry pathogens has been developed, providing deeper and more comprehensive surveillance.

[1] SJ Bloomfield *et al.* 2023, doi: 10.1016/j.fm.2022.104162.

P84 Unravelling the complexity of Staphylococcus aureus in Chronic Rhinosinusitis

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Staphylococcus aureus is frequently cultured from patients with chronic rhinosinusitis (CRS), a persistent inflammatory condition that impacts quality of life as much as chronic lower back pain or mild-to-moderate congestive heart failure. Despite frequent antibiotic use in this population, treatment is often ineffective, likely due to high prevalence of bacterial biofilms. As *S. aureus* is also commonly found in healthy individuals, this study aimed to identify differences between *S. aureus* isolates from CRS patients and healthy controls and investigate potential drivers of pathogenicity.

We conducted phenotypic, genomic, and evolutionary analyses on clinical isolates. Longitudinal samples were collected monthly over three months from CRS patients with cystic fibrosis (n=37) and healthy controls (n=12), and combined with cross-sectional isolates from idiopathic CRS (n=14) and healthy controls (n=18).

Phenotypic assays assessed biofilm-forming capacity (BFC), minimum inhibitory concentration (MIC), and minimum biofilm eradication concentration (MBEC) for doxycycline. Whole-genome sequencing using Oxford Nanopore technology identified virulence and resistance genes. Evolutionary assays subjected isolates to repeated biofilm growth and low-dose antibiotic exposure, mimicking conditions seen in CRS.

Cross-sectional and longitudinal comparisons revealed no significant differences in MIC, MBEC, or BFC between isolates from healthy individuals and CRS patients. However, evolutionary assays demonstrated that adaptation in biofilms can lead to increased antimicrobial resistance, which is preserved in planktonic growth states. Notably, *S. aureus* isolates from CRS patients were more likely to evolve into strong biofilm formers over time.

These findings suggest that while baseline traits may not differ significantly, the potential for *S. aureus* to evolve increased virulence under CRS-like conditions may be higher. Understanding these adaptive processes offers new diagnostic and therapeutic opportunities for managing this burdensome and recalcitrant disease. It also highlights the importance of biofilm-specific antibiotics to prevent the formation of virulent reservoirs in this population.

P85 The Role of Efflux Pumps in Potentiating the Evolution of Untreatable Multidrug-Resistant Strains of *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis, the etiological agent of tuberculosis (TB) in humans, is the leading cause of mortality attributable to a sole pathogen. Importantly, drug-susceptible infections are highly treatable; however, the four-drug regimen is strict and lengthy, often resulting in patient non-compliance.

Due to this, *M. tuberculosis* has evolved resistance to almost all clinically available antibiotics, including the cornerstone drug bedaquiline (BDQ) used to treat multidrug-resistant infections. Of particular concern to this growing resistance and threat to treatment outcomes is the contribution of efflux pumps due to their ability to act as evolutionary steppingstones towards high-level drug resistance. The MmpS5L5 efflux pump, physiologically used for siderophore recycling, is known to be overexpressed as a result of transcriptional repressor mutations, causing widespread low-level BDQ resistance clinically. Indeed, subsequent mutations, commonly in the BDQ target AtpE, have the ability to confer high-level resistance. Critically, mutations in the Rv0678 repressor have been reported in BDQ-naïve patients, having important implications in TB therapy. Despite this, the role of MmpS5L5 in potentiating the evolution of high-level BDQ resistance and untreatable multidrug-resistant *M. tuberculosis* strains remains to be fully elucidated.

To understand this, we aim to recognize the chemical properties that determine whether an antibiotic has the ability to be an efflux substrate of MmpS5L5 while unravelling the molecular mechanism by which high-level and multidrug resistance is potentiated. This knowledge aims to allow the future development of novel therapeutics and adjuvants with the ability to synergize with current drugs to overcome resistance and improve patient outcomes.

P86 Enzymatic production of modified amino acids for synthesis of antimicrobial peptides

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Antimicrobial resistance (AMR) is a major global health challenge, predicted to cause about 10 million deaths annually by 2050, without urgent intervention. Antimicrobial peptides (AMPs) are a promising class of therapeutics to combat AMR, showing broad-spectrum antimicrobial activity, high target specificity and binding affinity, and less likelihood of resistance development. Ambocidin, a recently discovered AMP, exhibits potent activity against multi-drug-resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococcus faecium* (VRE).[1] A key structural feature of ambocidin is the presence of non-canonical amino acids, including β-hydroxyaspartic acid.

In this study, we report a one-step enzymatic synthesis of L-*threo*-3-hydroxyaspartic acid using the enzyme TobO—an alpha-ketoglutarate-dependent hydroxylase. The reaction has been extensively optimised to achieve near complete (>95%) conversion of the substrate, L-aspartic acid, significantly simplifying downstream processing. Product purification was accomplished using cation exchange chromatography. Additionally, we determined the crystal structure of TobO, using X-ray crystallography, revealing the molecular details of its substrate binding site.

This study establishes a practical enzymatic route for producing L-*threo*-3-hydroxyaspartic acid, facilitating chemical synthesis of ambocidin and analogues for structure-activity relationship studies. The structural insights into TobO open new avenues for engineering alpha-ketoglutarate-dependent hydroxylases for synthesis of hydroxy amino acids, supporting novel AMP development.

1. Lai, H.-E. *et al. Calcium-dependent lipopeptide antibiotics against drug-resistant pathogens discovered via host-dependent heterologous expression of a cloned biosynthetic gene cluster*. Angewandte Chemie International Edition 63, e202410286 (2024).

P87 The International Literature and Policy on the Prevalence and Management of Antimicrobial Resistance in Waterways

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Rising antimicrobial resistance (AMR) has greatly reduced the efficacy of antimicrobials. Waterways represent a potentially significant reservoir of AMR and may be clinically relevant as a source of AMR infections. It is important to develop strategies to detect and manage AMR in waterways. This investigation aimed to evaluate the current international literature and policies on the detection, prevention and management of AMR in waterways and analysis of the advantages and disadvantages of different policy options for managing AMR in New Zealand's waterways. A scoping review was undertaken to identify studies and policy documents published in the last 20 years, that investigated or discussed AMR in waterways. The following databases were searched: SCOPUS, Ovid MEDLINE(R), Epub Ahead of Print, Web of Science/Web of Knowledge & the World Health Organisation.

The analysis of the international literature showed that rivers, lakes, groundwater, wells and aquifers are the most significant waterway reservoirs of AMR. Rates of prevalence are high in many waterways and tend to be highest in waterways located in or running through urban areas, or near agricultural estates and wastewater treatment facilities. This investigation showed that the potential impacts of AMR in waterways include downstream AMR infections and a reduction in water quality. There remains a lack of comprehensive management strategies across most of the investigated AMR action plans, with initiatives to test AMR in waterways either not discussed or currently in progress. The United Kingdom and Singapore emerged as cases of best practice for managing AMR in waterways. Urgent action is needed to control the spread of AMR in New Zealand and management strategies from the international literature and policies may be integrated into a plan for managing AMR in New Zealand's waterways.

P88 Unravelling the effect of cellular homeostasis regulators on the quinone pool

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Microorganisms can adapt to changing environments by modulating the composition and redox potential of what is called the quinone pool. This pool is composed of different quinones, quinols, and their derivatives with different redox potentials, and is crucial for energy generation and respiration. Hence, regulation of the quinone pool is important for microorganism survival, and inhibition of key regulatory proteins that control pool composition could lead to growth termination.

The menaquinone (MK) biosynthesis pathway is an important source of quinone pool constituents. This pathway presents in bacteria but not humans, and its products function as electron carriers in the electron transport chain and energy generation in microorganisms. The final step is catalysed by MenG, a membrane-associated *S*-adenosyl-methionine (SAM)-dependent methyltransferase. The balance of methylated and demethylated forms of menaquinone are well-documented to be significant in adapting electron transport to different redox environments. In *Mycobacterium tuberculosis*, *menG* (*rv0558*) forms a cluster with two other protein-coding genes, *rv0559c* and *rv0560c*, that can be transcribed convergently, and may contribute to adaption of energy generation during infection. *rv0560c* is another predicted SAM-methyltransferase, while *rv0559c* is a predicted secreted protein. We are interested in determining the structure-function relationships of these proteins, and how they may interplay as homeostasis regulators to alter the composition and redox potential of the quinone pool.

We have investigated the recombinant expression and purification of MenG from three human pathogens: *M. tuberculosis*, *Staphylococcus aureus*, and *Listeria monocytogenes*. The cell lines, media, and purification buffer systems that can provide modest yields of *L. monocytogenes* MenG in a soluble form have been determined. We have also constructed different plasmids for recombinantly expressing MenGs, Rv0559c and Rv0560c and completed expression trials. These results are an important step towards the structural characterisation of MenG and a strong foundation for structure, function, and relationship studies of the gene cluster.

P89 Covalent targeting of bacterial enzymes for specific detection of antibiotic-resistant *Staphylococcus aureus* infections

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Around a third of healthy humans are carriers of *Staphylococcus aureus*, such that they have the bacteria on their skin or nose without any active infection or disease. Despite being harmless in most individuals, *S. aureus* can cause severe pathogenic infections. The increased occurrence of community-acquired antibiotic-resistant *S. aureus* strains, is a major health threat, requiring urgent development of new diagnostic and therapy options.

Serine hydrolases are a large family of enzymes that play key roles in bacterial homeostasis and survival at the host-pathogen interface during infection. This makes serine hydrolases promising new anti-virulence and anti-infectivity targets.

Here we report our ongoing efforts to develop new molecular imaging and anti-virulence tools for antibiotic-resistant *S. aureus* by targeting serine hydrolases. We report recent accomplishments from the Fellner, Lentz and Bogyo laboratories to covalently target the active site serine residue in these proteins in *S. aureus* bacteria. The targeting is accomplished via a range of new functional groups like a cyclic peptide fused to a covalent binding diphenylphosphate group, oxadiazole group (by themselves or linked to mRNA), as well as a boron-based group. These covalent inhibitors are then developed further into diagnostic probes for the specific detection of *S. aureus* infections, with additional anti-virulence benefits. The developed molecular imaging agents have the potential to tell us where exactly the infection is located, how significant it is and if it is responding to antibiotic treatments; important information that is not available with current methods used in the clinic.

P90 Characterization of amino acid-modifying enzymes in natural product biosynthesis pathways

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Antimicrobial peptides (AMPs) are a promising class of therapeutics that show broad-spectrum antimicrobial activity, high target specificity and less likelihood of resistance development. AMPs consist of modified amino acids that play a key role in exerting their antimicrobial effects. Ambocidin, a recently discovered AMP, exhibits calcium-dependent antimicrobial activity against gram positive bacteria and contains two modified amino acids, β-hydroxy-aspartic acid and L-N^{ϵ}-hydroxy-arginine.[1] This project is focused on the characterization of amino-acid modifying enzymes in the ambocidin biosynthetic cluster.

AmbT is a heme-dependent oxygenase that catalyses the production of L-N^{ϵ}-hydroxy-arginine in ambocidin.[1] We produced heme-incorporated AmbT in *E.coli* by co-expression of AmbT and hemin receptor (ChuA). Supplementing the growth media with hemin allowed incorporation of exogenous heme into AmbT. Acidified butanone extraction showed that heme is non-covalently bound to AmbT. Preliminary experiments using AmbT and L-arginine or linear ambocidin as substrates did not show hydroxylation activity.

AmbQ is an alpha-ketoglutarate-dependent hydroxylase, however its substrate specificity is unknown. We hypothesize that AmbQ catalyses the production of β-hydroxy-aspartic acid in ambocidin. Structural characterization of AmbQ by X-ray crystallography showed presence of the 'jelly roll' fold (characteristic of alpha-ketoglutarate- dependent oxygenase) and revealed alpha-ketoglutarate bound in the active site. However, preliminary experiments using AmbQ and L/D-aspartic acid or linear ambocidin as substrates did not show hydroxylation activity.

We have conducted preliminary biochemical and structural characterization of amino acid-modifying enzymes, AmbT and AmbQ, from the ambocidin biosynthetic cluster. Further structural and biochemical studies are required to investigate their substrate specificity. This project will give insights into the function of novel amino-acid modifying enzymes, and may have implications in biocatalytic synthesis of synthetic AMPs.

1. Lai, H.-E. *et al.* Calcium-Dependent Lipopeptide Antibiotics against Drug-Resistant Pathogens Discovered via Host-Dependent Heterologous Expression of a Cloned Biosynthetic Gene Cluster. *Angewandte Chemie International Edition* **63**, e202410286 (2024).

P91 Evaluating the effect of feeding calves waste milk on the taxonomic profile and resistance genes in faecal and milk samples in dairy farms in New Zealand: a cross-sectional study

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Exposure to antimicrobials may increase the risk of emergence of antimicrobial resistance in bacteria found in livestock. On dairy farms, an indirect pathway of exposure to antimicrobials is feeding young calves non-saleable milk containing antimicrobials from treated lactating cows, known as waste milk (WM). Overseas studies suggest feeding WM to calves can result in a higher prevalence of antimicrobial resistant bacteria in their faeces than in those not fed WM. However, this has not been confirmed in New Zealand, which has one of the lowest rates of antimicrobial use on dairy farms worldwide.

Six farms that feed WM to their replacement calves were recruited. Faecal samples from calves fed WM (replacement calves), and those calves fed milk replacer (non-replacement calves). Samples of the WM and milk replacer were also collected. DNA extraction, sequencing, and short-read metagenomic studies were conducted to compare the bacterial taxa and antibiotic resistance genes in all samples.

There were differences in the taxonomic profiles of faeces between groups, with non-replacement calves having a more diverse taxonomic faecal composition than replacement calves. Within the top 10 families found in faecal samples, the mean abundance of the *Enterobacteriaceae* family was higher in non-replacement than replacement calves ($p<0.01$), whereas the mean abundance of the *Oscillospiraceae* family was higher in replacement than in non- replacement calves ($p<0.01$). The highest prevalence of antimicrobial resistance genes were those which confer resistance against tetracyclines, beta-lactams and aminoglycosides.

These data indicate that WM feeding impacts bacterial taxonomic distribution of the faecal samples from calves with further analysis underway to understand whether exposure to antimicrobials via WM feeding impacts the faecal resistome of calves

P92 Loss of aminoarabinose lipid modification leads to cephalosporin antibiotic resistance in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic, multidrug-resistant pathogen frequently causing respiratory tract infections in cystic fibrosis patients, as well as urinary tract and burn wound infections. A particularly hypervirulent and multidrug-resistant strain of *P. aeruginosa* is the Liverpool Epidemic Strain (LESB58). This pathogen utilises resistance mechanisms including enzymes such as β-lactamases, to hydrolyse β-lactam antibiotics. The polymyxin and cephalosporin classes of antibiotics are commonly used as last-resort treatments against complex multidrug-resistant *P. aeruginosa* infections. Polymyxins target and disrupt the bacterial cell membrane, while cephalosporins inhibit cell-wall synthesising enzymes. A polymyxin resistance mechanism in many *P. aeruginosa* strains is the aminoarabinosylation (Arn) system, that incorporates a positively charged aminoarabinose sugar into the lipid A of the lipopolysaccharide (LPS) to repel positively charged polymyxin antibiotics. This lipid modification system is encoded by the *arnBCADTEF-ugd* operon (*arn*; 9,380-bp) and regulated by five different two-component systems, including PhoPQ and PmrAB. We hypothesised that deletion of the *arn* operon could result in resistance to other antibiotics targeting the bacterial cell wall. Antimicrobial susceptibility assays demonstrated that deletion of *arn* (Δ*arn*) caused increased resistance to the cephalosporin antibiotics ceftazidime (32-fold), cefepime (8-fold) and cefotaxime (8-fold). RNA-sequencing of Δ*arn* and wild-type strains following exposure to sub-inhibitory ceftazidime concentrations revealed significant upregulation of the β-lactamase enzyme *ampC* within Δ*arn*. The double mutant Δ*arn*/Δ*ampC* rescued cephalosporin resistance almost to WT levels (2-fold MIC difference) and an *ampC* promoter fluorescence study using green fluorescent protein (msfGFP) showed continued activity of *ampC* in Δ*arn* following ceftazidime treatment. Deletion of an *arn* regulatory two-component system *pmrAB* (Δ*pmrAB*) caused 10-fold increased resistance to ceftazidime. Our findings reveal a novel link between the lipid modification system and cephalosporin resistance. As polymyxin and cephalosporin antibiotics are heavily relied upon treatments, these findings have a potential to implicate therapeutic strategies against the clinically relevant *P. aeruginosa* LESB58.

P93 Novel Broad-Host-Range Listeria Phages from Aotearoa: Discovery, Characterisation, and Industrial Potential

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Listeria monocytogenes is a serious foodborne pathogen with major implications for food safety in the food industry[1]. While commercial phage solutions targeting *Listeria* are available, local isolates may offer superior efficacy or regulatory advantages. In this study, we isolated and characterised *Listeria*-infecting bacteriophages from environmental samples collected in Twizel, Mackenzie District, New Zealand.

Phages were initially identified using a high-throughput screening approach[2]. Plaques with distinct morphologies were selected, purified through multiple rounds, and amplified to high titre. DNA from 13 purified isolates was extracted and submitted for Illumina whole-genome sequencing. Genome analysis revealed 5 distinct isolates belonging to the same species within the *Pecentumvirus* genus. Those isolates are closely related to the well-characterised broad-host-range phages A511 and CKA15.

Consistent with their genus, our phages exhibited broad host range activity. *In vitro* lytic assays demonstrated effective infection of *L. monocytogenes* isolates (19 isolates covering serogroups IIa, IIb, IVa, and IVb), as well as *L. seeligeri* (2 isolates) and *L. innocua* (2 isolates). Comparative assays against a commercial phage cocktail revealed equivalent efficacy against *L. monocytogenes* and *L. seeligeri*. However, our phages outperformed the commercial product against *L. innocua*, forming distinct plaques, whereas only lysis-from-without was observed with the commercial product.

These findings support the potential industrial application of our newly discovered New Zealand phages. Their strong lytic activity, broad host range, and ability to replicate efficiently in non-pathogenic *Listeria* species highlight their promise as effective, locally sourced biocontrol agents for New Zealand's food industry.

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2. Olsen, N. S., Hendriksen, N. B., Hansen, L. H., & Kot, W. (2020). *A New High-Throughput Screening Method for Phages: Enabling Crude Isolation and Fast Identification of Diverse Phages with Therapeutic Potential.* PHAGE (New Rochelle, N.Y.), 1(3), 137–148.

P94 Mass spectrometry techniques for structural and functional biology

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Mass spectrometry (MS) coupled with electrospray ionisation (ESI) is a powerful technique for analysing macromolecules such as proteins. This gentle ionisation method, in combination with the right MS instrumentation, allows the determination of protein molecular weights with high accuracy in both denatured and native conditions, advancing research on the structure and function of proteins.

At the NZ Centre for Native MS at the University of Canterbury, we have been establishing and expanding MS capabilities to empower protein research in Canterbury and across New Zealand. Our specialised time-of-flight (TOF) MS instrument, equipped with a nano-flow ESI source for improved sensitivity, is tuned for gentle ionisation of proteins while retaining their native conformations in gas-phase. The approach, called native MS, is valuable for studying protein structure and interactions (e.g. self-association, multi-subunit complex formation) with opportunities to analyse protein-small molecule interactions (e.g. evidence of binding, interaction stoichiometries).

We have also implemented bottom-up proteomics approaches to validate protein identity and amino acid sequence. Here we can determine the mass of proteolysis-derived peptides from target proteins. Gas-phase fragmentation of these peptides provides information on the amino acid sequence, and this technique is beneficial to protein research in a range of applications, from confirming the identity and modification of lab-purified proteins (e.g. post-translational modification, disulfide bond formation) to screening proteins in biological samples.

This presentation will demonstrate applications of these techniques, which are being developed and open for collaboration at the NZ Centre for Native MS at the University of Canterbury.

P95 Unravelling the oligomeric forms of the membrane-interacting EsxE-EsxF complex

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Mycobacterium tuberculosis, the causative agent of tuberculosis in humans, produces an extracellular toxin that induces necrosis of host macrophages. The toxin-harbouring protein (CpnT) is encoded in an operon containing the genes for two WXG100 proteins, EsxE and EsxF. This protein pair is critical for mediating the translocation of CpnT and thus toxin secretion across the cell envelope and thereby is essential to the survival and virulence of *M. tuberculosis*.[1] Like other proteins of the WXG100 family, EsxE and EsxF can form a canonical heterodimer, which has been suggested to further oligomerize into membrane-interacting pores and filaments.[1] We are investigating EsxEF oligomerisation to understand how the complex assembles and interacts with lipid membranes to perform its crucial role.

EsxE and EsxF were individually and co-expressed using *Mycobacterium smegmatis*, a non-pathogenic cousin of *M. tuberculosis*. We have found that EsxEF forms predominantly a hetero-tetramer, EsxE by itself is preferentially a monomer, while EsxF has a significant tendency for self-oligomerisation into a variety of higher-order structures. Analysis of the interactions between individually expressed EsxE and EsxF wild types was extended to variants of conserved residues, which showed detrimental effects on the stability of the oligomeric forms. The unexpectedly diverse structural behaviour of these proteins indicates a remarkable structural adaptability, which calls into doubt their assumed 1:1 heterodimeric arrangement, and other reported higher oligomeric forms.[1] Further decoding these behaviours may hold the key to revealing the mechanism of their role(s) during toxin secretion.

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P96 Altered physiology of drug-resistant *M. tuberculosis* restricts growth and metabolic adaptability

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Mycobacterium tuberculosis is an obligate human pathogen and the causative agent of tuberculosis, the world's leading cause of death due to an infectious disease. Resistance to all clinically available antibiotics have been reported in *M. tuberculosis*, significantly undermining the effectiveness of antitubercular treatments. Antibiotic resistance mutations often disrupt essential cellular processes, yet the physiological consequences of these mutations remain poorly understood. Here we have used a combination of phenotypic growth assays and mass spectrometry to address how drug resistance mutations affect the physiology of *M. tuberculosis*.

We uncover that drug resistance constrains metabolic flexibility of *M. tuberculosis*, with host-relevant conditions inhibiting the growth of bedaquiline- and rifampicin-resistant mutants. We also show that central carbon metabolism is altered across various drug-resistant strains. Collectively, this study highlights how drug resistance impacts the physiology of *M. tuberculosis* and how resistance places metabolic constraints on the ability of *M. tuberculosis* to adapt to host-like environments.

P97 Targeting *Pseudomonas aeruginosa* biofilms using peptide-gold nanoparticles conjugates

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Pseudomonas aeruginosa is a Gram-negative bacterium classified by the World Health Organization as a critical priority pathogen due to its high virulence and multidrug resistance (MDR). It is a leading cause of severe infections in the lungs, urinary tract, chronic wounds and bloodstream, posing a substantial burden on global public health systems. A key factor contributing to its persistence and tolerance to conventional antibiotics is its ability to form biofilms, highlighting the urgent need for new treatment strategies. In this study, we investigated a novel combinatorial treatment approach using the enantiomeric peptide D-3006 in combination with citrate-capped gold nanoparticles (Cit-AuNPs) or glutathione-capped gold nanoparticles (GSH-AuNPs) against MDR *P. aeruginosa* biofilms. The antimicrobial activity was assessed using the broth microdilution method, while biofilm eradication was evaluated on preformed biofilms. Both assays were conducted under standard laboratory conditions using Mueller-Hinton Broth. D-3006, Cit-AuNPs, and GSH-AuNPs exhibited individual minimum inhibitory concentrations of 12.5 µg/mL, 0.625 µg/mL and 1.25 µg/mL, respectively. When preformed biofilms were individually treated with D-3006, Cit-AuNPs, and GSH-AuNPs at a concentration of 50 µg/mL, none of the tested compounds exhibited a significant reduction in viable biofilm-associated cells compared to the untreated. However, when combined, D-3006 with either Cit-AuNPs or GSH-AuNPs resulted in a synergistic ~100-fold reduction in viable biofilm-associated cells at a similar concentration of 50 µg/mL compared to the untreated control. At 50 µg/mL, both AuNPs showed no cytotoxicity, while a minimum toxicity (~5%) was observed with D-3006 towards human keratinocytes, as determined by the MTS viability assay. In summary, the D-3006-AuNP combination demonstrates synergistic antibiofilm efficacy with low toxicity against *P. aeruginosa* biofilms, warranting further studies focusing on the antibiofilm mechanism of action of the combination therapy in a mouse model.

P98 Synthetic studies towards novel oligomers to address antibacterial resistance.

Mullin, R.

University of Auckland

We are rapidly approaching a post-antibiotic era. Initially effective at preventing loss of life in the early 20th century, resistance to mainstay antibiotics has developed rapidly.

With their short generations, the fight against emerging multi drug-resistant strains is an uphill battle. Compounding this is the fact that fewer antibacterial drugs are being approved, as they are considered unprofitable to develop.

The Gausemycins are a family of cyclic, glycosylated lipopeptides discovered in 2016. As yet, no reported chemical synthesis for the scaffold exists. They show activity against Gram-positive bacteria and are thought to cause membrane permeabilization and cell death. My research is thus focused on performing chemical synthetic studies towards the Gausemycin family to provide access to a new class of antibiotic that could be used as a therapeutic. Further Structure Activity Relationship (SAR) studies would determine which amino acids are important for activity and/or toxicity, and which are amenable to substitution to produce optimized analogues.

My research will also focus on the synthesis of peptoid mimics of known antimicrobial peptides. Peptoids differ from peptides in that their side chain is attached to the amide nitrogen instead of the α carbon. This means that they are stable to proteases which bacteria use to cleave antimicrobial peptides. They disrupt bacterial membranes, biofilms, and, in some studies, bacteria do not gain resistance to them, even after >800 passages at a sublethal dose. They have been shown to be active against MDR *P. aeruginosa* and MRSA. Their synthesis is analogous to traditional SPPS. It has also been shown that peptoids often retain the activity of their parent peptide. I am also interested in collateral sensitization, polypharmacology and virulence factor attenuation as strategies to address the resistance problem, and I plan to investigate peptides and peptoids that are designed with these principles in mind.

P99 From Genomes to Function: Utilizing Genome Mining Tools to Uncover Novel Enzymes in Secondary Metabolite Biosynthesis

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Secondary metabolites are structurally diverse natural products that play critical roles in biological interactions, defence, and communication. Among them, ribosomally synthesized and post-translationally modified peptides (RiPPs) represent a particularly valuable class due to their therapeutic and industrial applications. The bioactivity of these largely results from complex post-translational modifications (PTMs) catalyzed by unique enzymes such as YcaO-domain proteins and radical *S*-adenosylmethionine (rSAM) enzymes. However, despite advances in genomic sequencing and bioinformatics, our current understanding of the catalytic diversity and capabilities of these modifying enzymes remain limited.

This project aims to uncover novel RiPP-specific modifying enzymes through genome mining and protein biochemistry. Functional genome mining of YcaO and rSAM superfamilies uncovered six candidate RiPP biosynthetic gene clusters, each encoding a single putative modifying enzyme. These modifying enzymes and their corresponding precursor peptides have been overexpressed in *E. coli*. Production of the selected YcaO enzymes was hampered by toxicity issues that are currently being addressed. In contrast, optimization of expression conditions enabled the FPLC purification of two rSAM enzymes under anaerobic conditions with intact iron-sulfur clusters, as confirmed by their characteristic colour and UV spectra. These purified rSAM enzymes will be incubated with their specific precursor peptides under defined *in vitro* conditions. Reaction products will be analysed by mass spectrometry to identify any modifications introduced by the enzymes. Concurrently, expression and purification protocols will be refined to attempt crystallization trials, facilitating structure determination by X-ray crystallography, and offering mechanistic insight.

Through this approach, the project aims to expand the known repertoire of RiPP-modifying enzymes and uncover new biosynthetic pathways, ultimately leading to the development of novel bioactive compounds for pharmaceutical and industrial applications.

P100 Extracellular DNA joining in the multi-drug resistant pathogen *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae, the causative agent of the sexually-transmitted infection gonorrhea, is an expert at acquiring novel DNA by horizontal gene transfer. Its natural competence at all stages of growth is the major route by which *N. gonorrhoeae* gains antibiotic resistance. Our group have identified a DNA-joining enzyme 'Ligase E' (Lig E) which has the potential to accelerate antibiotic resistance as well as bacterial persistence in *N. gonorrhoeae*. Lig E is an unusual 'minimal' form of DNA ligase which is predicted to localise to the periplasm or be transported further outside the cell. Previously we demonstrated that Lig E contributes to biofilm formation, likely by increasing the molecular weight of extracellular DNA which comprises a significant component of the gonococcal extracellular matrix. Here we show that Lig E also significantly enhances transformation of *N. gonorrhoeae* by damaged extracellular DNA, providing a route for novel gene acquisition from environmental DNA in a DNA-degrading environment. The recent emergence of *N. gonorrhoeae* strains exhibiting resistance to all front-line therapeutics raises the imminent possibility of untreatable multi-drug-resistant gonorrhea. A greater understanding of the processes behind competence-driven horizontal gene transfer in this bacterium may provide potential routes for limiting resistance transmission and preserving last-resort treatments in the future.

P101 Investigating phage infection dynamics during lesser studied physiological states to improve anti-biofilm therapeutics

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Phages – viruses that infect bacteria – represent a promising solution towards rising global health concerns like antibiotic resistance[1,2]. However, phage-based therapies are limited by gaps in our understanding of fundamental phage biology. For example, most phage characterization has been performed under conditions where planktonic bacterial hosts are in a state of exponential growth. Since bacterial growth in nature is heavily influenced by nutrient availability, phages have likely evolved genetic adaptations to bacteria in various metabolic states. We propose that phages with enhanced infection properties can be experimentally evolved using stationary phase planktonic cultures. Additionally, evolved phages may have increased efficacy against bacterial biofilms – cellular aggregations that pose problems for humans in clinical settings. Here, we established that phage replication and killing of the opportunistic pathogen *Pseudomonas aeruginosa* was reduced during stationary phase when compared to exponential growth. Jumbo phages (genome >200kbp) showed the greatest decline in killing during stationary phase, indicating that phages with complex lifecycles may be greater impacted by host metabolic state. Deep sequencing identified that phages readily acquire single nucleotide polymorphisms which become fixed in populations, highlighting the need to establish mutational baselines. These findings provide the foundation for phage evolution experiments against which improvements in phage killing and replication capacity can be assessed.

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P102 Feedback regulation of iron-sulfur cluster biogenesis

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Iron-sulfur (Fe-S) clusters are ubiquitous and chemically versatile protein cofactors utilised in a variety of important cellular processes, such as energy production and transcriptional regulation. In *Mycobacterium tuberculosis* (*Mtb*) over fifty such Fe-S cluster proteins are present, suggesting a particular reliance on these cofactors[1]. Understanding how these clusters are assembled could lead to the identification of inhibition strategies, which would impart a pleiotropic effect, disrupting multiple pathways simultaneously. The process of Fe-S cluster biogenesis in *Mtb* is primarily carried out by proteins of the sulfur mobilisation factor (Suf) pathway. Suf comprises a network of proteins that mobilise sulfur and iron, assemble nascent clusters, and then transfer them onto Fe-S proteins. As both free iron and sulfur are cytotoxic, this process in *Mtb* is tightly controlled at both transcriptional and post-translational levels.

Here, we present a novel feedback inhibition of the Suf pathway through modulating the activity of cysteine desulfurase SufS. SufS obtains sulfur from cysteine and transfers it to the core Suf machinery *via* sulfur transferase SufU. Our results demonstrate that *Mtb*-SufU binds [2Fe-2S] clusters and blocks the zinc-mediated interaction between SufS and SufU, preventing SufS activation and thereby restricting the supply of sulfur for Fe-S cluster assembly. In addition, our crystal structure of the [2Fe-2S] cluster-bound SufU and zinc-bound SufS/SufU complex shows that the [2Fe-2S] cluster occupies the zinc binding site. These findings reveal a third level of regulation in the assembly of Fe-S clusters, underscoring the importance of regulating this process in *Mtb*. Given that the Suf system is not present in humans, it represents a particularly attractive target in the search for novel, potent antimicrobials.

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P103 Allosteric regulation of isocitrate lyase 2 as a potential target against Mycobacterium tuberculosis

Jamie Taka, James Jung, Evelyn Huang, Brooke Kwai, Ivanhoe Leung, Ghader Bashiri

Isocitrate lyase (ICL) isoforms 1 and 2 enable *Mycobacterium tuberculosis* to preferentially utilise lipids as a carbon source, through their roles in the glyoxylate and methylcitrate cycles. This capability is a key metabolic feature that supports chronic infection and persistence. Our X-ray crystal and cryo-electron microscopy structures of *Mtb*-ICL2 reveal that the protein forms a homo-tetramer, with the N-terminal domains forming a central catalytic core, while the C-terminal domains create dimers at either end of the molecule in the apo form. Upon binding to the products of fatty acid metabolism, acetyl-CoA or propionyl-CoA, the C-terminal domains of *Mtb*-ICL2 undergo a dramatic conformational change, resulting in a 100-fold increase in *Mtb*-ICL2 enzymatic activity. Our time-resolved cryo-electron microscopy analyses reveal the trajectory of conformational changes with the addition of acetyl-CoA from a time point as early as 150 ms through to a complete transition, illustrating the population shift of protein conformations over time. Our findings provide unprecedented insights into the mechanisms underpinning *Mtb*-ICL2 allosteric activation. Together, these results indicate that ICL2 allostery plays a pivotal role in regulating carbon flux between the TCA and glyoxylate cycles under high lipid concentrations, highlighting a promising target for therapeutic intervention against *Mycobacterium tuberculosis*.

P104 PBT2-mediated metal ion disruption: uncovering new metabolic vulnerabilities and overcoming resistance in MRSA

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Amid rising AMR and a shortage of effective antibiotics, there is renewed interest in using metal ions either as novel antimicrobials or to potentiate the activity of ineffective antibiotics. Transition metals, particularly zinc (Zn), manganese (Mn), and iron (Fe), are crucial for bacterial survival, acting as cofactors in cellular processes, including oxidative stress management and central carbon metabolism. Our research has shown that the Zn ionophore PBT2, combined with Zn at low levels, can resensitise methicillin-resistant *Staphylococcus aureus* (MRSA) to the antibiotic oxacillin. Using transposon mutagenesis and transcriptomics, we identified the genetic and metabolic pathways disrupted by PBT2+Zn (PZ). Importantly, we have identified that PZ targets central carbon metabolism in MRSA, particularly glycolysis, a pathway essential for pathogenesis and virulence. Notably, our transposon mutagenesis screen identified the Δ*gpmA* isolate, lacking the manganese (Mn)-independent enzyme phosphoglycerate mutase involved in glycolysis, greatly increases sensitivity to PBT2 + Zn. Notably, pyruvate supplementation alleviates PZ-mediated killing of MRSA, though the underlying mechanism is unknown. We show that Mn depletion and Zn toxicity expose a unique glycolysis-related metabolic vulnerability, offering potential for novel therapeutic targets while enhancing our understanding of pathogen adaptation during host infection.

P105 Sulfur assimilation and metabolism in *Neisseria gonorrhoeae*.

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Bacterial pathogens acquire essential sulfur in the form of organic or inorganic sulfur readily available at the host-pathogen interface. Once acquired, all sulfur metabolism pathways convene at cysteine, either by processing organic sulfur compounds into cysteine or reducing inorganic sulfur for *de novo* cysteine biosynthesis. Among a plethora of other roles, cysteine acts as a sulfur revisor, and is an essential intermediate in making environmental sulfur available to microbial cells. Due to this central role of cysteine in sulfur metabolism, involved pathways, such as the *de novo* cysteine biosynthesis pathway and the sulfur assimilation pathways, have gained considerable interest as promising candidates for novel therapeutics in the face of antimicrobial resistant human pathogens.

Niesseria gonorrhoeae displays unique features of inorganic sulfur assimilation for cysteine biosynthesis. For *N. gonorrhoeae,* the final step of cysteine synthesis in requires sulfide, which is traditionally obtained via the successive reduction of sulfate. The gonococcus is incapable of sulfate reduction due to large genomic deletions, and therefore cannot grow when sulfate is the only available sulfur source. *N. gonorrhoeae* can, however, grow in the presence of thiosulfate and glutathione, yet lacks glutathione transporters and the conventional enzyme required to utilize thiosulfate for cysteine production.

The aim of this research is to characterise the unconventional inorganic sulfur assimilation pathway within *N. gonorrhoeae*. We have identified two single domain sulfurtransferases that reduce thiosulfate to sulfite and sulfide. We propose these enzymes to be the missing link in how *N. gonorrhoeae* obtains sulfur for the synthesis of cysteine.

A multidisciplinary approach including kinetic characterisation of the recombinant proteins, phenotypic characterisation of deletion strains, metabolomics, and transcriptomics has been undertaken to elucidate the involvement of Str and PspE in cysteine biosynthesis within *N. gonorrhoeae*. Additionally, this work provides insights into the sulfur requirements of *N. gonorrhoeae* during growth, survival, and infection.

P106 The FitAB type II toxin-antitoxin system from *Neisseria gonorrhoeae*: a novel function for toxin-antitoxin systems?

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Toxin-antitoxin systems are crucial elements of most bacterial genomes, particularly the most abundant type II systems. These proteinaceous systems have diverse functions, playing important roles in plasmid maintenance, virulence, biofilm formation, growth, and stress responses, including antibiotic tolerance. Under standard growth conditions, these systems, which are encoded on a bicistronic operon, are expressed, where the labile antitoxin binds and inactivates the toxin. However, under stress, the antitoxin is degraded, leaving the toxin to accumulate and carry out its function.

Neisseria gonorrhoeae contains only three type II toxin-antitoxin systems, with only a single VapBC system. In comparison, the genome of the pathogen *Mycobacterium tuberculosis* can encode up to 90 type II systems, with around 50 of these constituting VapBC systems. The VapBC system from *N. gonorrhoeae* was discovered by Hopper et al. (2000) during a transposon mutagenesis study, where a fast intracellular trafficking phenotype was observed when the system was inactivated; as such, the cassette was termed *fitAB*.

Using the structure of FitAB and methods previously established in our laboratory, we have determined that the toxin FitB exhibits ribonuclease activity. To further investigate the role of FitAB in intracellular trafficking, we have generated an insertion inactivation mutant of *fitAB* in *N. gonorrhoeae*. The mutant demonstrates a higher association with cervical epithelial cells, reflected in increased adhesion and internalisation, compared to the wild-type strain. Additionally, the mutant exhibits a distinct colony morphology in liquid culture and his unable to undergo antigenic variation of a key virulence determinant.

Our data suggests that FitAB is not only important for the intracellular lifecycle of *N. gonorrhoeae* but also for regulating population dynamics of the bacterial colonies. This proposed function of FitAB represents a novel function for toxin-antitoxin systems and may not only shed light on the infection dynamics during gonococcal infection, but also for other bacterial pathogens.

P107 Antimicrobial susceptibility of *Kingella kingae* in Australasia: Implications for empirical treatment of paediatric osteoarticular infections

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Kingella kingae is an emerging cause of osteoarticular infections (OAI) in young children. While generally susceptible to a broad range of antibiotics, there is limited regional data on the antimicrobial susceptibility patterns of K. kingae strains circulating in Australia and New Zealand. In this study, 61 local K. kingae isolates were tested to inform empirical treatment guidelines, with particular focus on first-generation cephalosporins.

Antimicrobial susceptibility testing was performed using broth microdilution, disk diffusion, or both, following ISO 20776-1:2019 and EUCAST guidelines. All isolates were beta-lactamase negative and exhibited high susceptibility to penicillin, ampicillin, amoxicillin, and second- and third-generation cephalosporins. First-generation cephalosporins—particularly cefalexin and cefazolin—demonstrated potent in vitro activity ($MIC_{90} \leq 2$ mg/L). In contrast, flucloxacillin and cloxacillin exhibited significantly higher MIC_{90} values (16 mg/L and 4 mg/L, respectively), indicating limited efficacy against K. kingae. All isolates were fully susceptible to azithromycin and erythromycin. Notably, clavulanic acid substantially reduced amoxicillin MICs even in beta-lactamase-negative strains, suggesting a possible intrinsic target effect.

This study provides the first regional antimicrobial susceptibility data for K. kingae in Australasia, supporting the emerging clinical preference for first-generation cephalosporins in empirical treatment of paediatric OAI. These findings also highlight the urgent need for EUCAST interpretive criteria specific to K. kingae, particularly for commonly used oral beta-lactams. Additional susceptibility data from other geographic regions are essential to guide clinical decision-making and inform the development of evidence-based breakpoints—especially for first-generation cephalosporins.

P108 Validation of genomic antimicrobial resistance markers (AMR) for national AMR surveillance for Salmonella spp. Identifies discrepancies in azithromycin resistance for S. Paratyphi A

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The Institute for Public Health and Forensic Science (PHF Science) undertakes national surveillance of clinical salmonellosis on behalf of the NZ Ministry of Health and is routinely utilising real-time whole genome sequencing analysis (WGS) for epidemiological typing.

As antimicrobial resistance (AMR) inference was not included in the initial PHF Science WGS validation for *Salmonella* spp, this study was undertaken to compare phenotypic susceptibility and genotypic resistome data[1,2] for 257 strains of *Salmonella* spp. isolated from clinical cases in NZ between 2019 and 2022. The purpose was to evaluate the utility of ongoing WGS analysis for national *Salmonella* AMR surveillance in replacing retrospective national phenotypic prevalence surveys.

Correlation between phenotypic resistance and genomically inferred resistance genetic markers demonstrated a high level of concordance for most antimicrobial agents with 100% concordance for sulfamethoxazole, and meropenem; and greater than 98.5% concordance for ampicillin, ceftazidime/cefotaxime, chloramphenicol, ciprofloxacin, tetracycline, and cotrimoxazole. However, no genotypic azithromycin resistance mechanisms were identified in any of the 23 phenotypically azithromycin-resistant strains.

WGS analysis of NZ *Salmonella* isolates reliably identified phenotypically resistant strains for most antimicrobial agents when compared to disc diffusion susceptibility testing and may be used for ongoing national AMR surveillance purposes. However, WGS results should not be relied upon for predicting resistance to azithromycin as discrepancies require further investigation.

The use of WGS analysis to infer AMR information for national surveillance purposes in no way supplants diagnostic laboratory susceptibility testing for clinical purposes.

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P109 Structural and Enzymatic approaches to 3-methyl-L-tryptophan production for antimicrobial peptide development

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Antimicrobial resistance (AMR) is one of the most pressing global health challenges, driving the need for new antibiotics with novel modes of action. Naturally occurring antimicrobial peptides (AMPs), such as telomycin, offer promising scaffolds for new generations of antibiotics due to their potent activity. However, these peptides often contain non-proteinogenic amino acids that hinder synthetic access and structure-activity relationship (SAR) studies. One such residue, 3-methyl-L-tryptophan (3-MeTrp), is crucial for telomycin's bioactivity yet is challenging to synthesise efficiently.

Here, we present an enzymatic strategy to generate enantiopure 3-MeTrp from inexpensive L-tryptophan using enzymes from the maremycin biosynthetic gene clusters. We have expressed and purified three candidate enzymes; MarG (amino transferase), MarI (methyl transferase) and MarH (epimerase)—which act in tandem to methylate L-tryptophan. We used mass spectrometry and nuclear magnetic resonance to confirm the formation of 3-MeTrp, and initial optimisation of the reaction conditions is underway to improve yield and ensure the correct stereochemistry.

Further, we have determined the crystal structures of MarG, providing atomic-level detail of the active site architectures and catalytic mechanisms. Structural analysis has identified active site residues suitable for mutagenesis, enabling future enzyme engineering to enhance catalytic efficiency, regioselectivity, or broaden substrate scope.

This work establishes a flexible and scalable platform for accessing non-canonical amino acids that have been previously inaccessible through synthetic routes. Together, our work integrates structural biology and enzymology, to overcome key bottlenecks in natural product AMP production. This platform lays the foundation for systematic SAR studies and next-generation antibiotic design targeting AMR.

P110 Identifying genes involved in resistance against combinatory treatment on *K. pneumoniae* infection

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Abstract

Klebsiella pneumoniae is a multidrug-resistant (MDR) pathogen of critical health concern, especially in the context of hospital-acquired infections and pneumonia. This study outlines a comprehensive screening strategy using a defined transposon mutant library of *K. pneumoniae* KPNIH1 to identify genetic determinants involved in resistance or hypersensitivity to the combinatory treatment of meropenem and polymyxin B (PMB). This screening strategy enables the identification of key resistance-associated genes and provides a foundational blueprint for understanding synergistic drug interactions in *K. pneumoniae*. We screened 21 antibiotic combinations against *K. pneumoniae* using checkerboard synergy assay and ZIP synergy model that quantifies the drug interaction relationships by comparing the change in the potency. We found a strong synergistic effect was observed between polymyxin B and meropenem (FICI = 0.375; ZIP score = 18.64). A total of 12,000 mutants were screened with meropenem, PMB, and a combination of both. The primary screen identified 153 PMB-resistant, 105 PMB-hypersensitive, 77 meropenem-resistant, and 37 meropenem-hypersensitive mutants. Screening with the combination treatment revealed 53 mutants resistant to both antibiotics, and 10 mutants showed their hypersensitivity to both drugs. Subsequent hit (High throughput screening) validation narrowed down 19 mutants resistant to the combined treatment and 1 common hypersensitive mutant. We identified the disrupted genes *mtr, cysE, rfaB, mutY* that are responsible for tryptophan permease, serine acetyltransferase, glycosyl transferase, and adenine glycosylase respectively. The screening also revealed that gene disruptions in transmembrane transporters, porins, and regulatory elements could contribute to altered susceptibility. This study uncovers new resistance genes under clinically relevant dual-drug conditions for treating MDR *K. pneumoniae* infections in the lung.