Project Details	
Project Code	IIAR27Br Spencer
Title	Understanding beta-lactam antibiotic targets to improve melioidosis treatment
Research Theme	Infection, Immunity & Antimicrobial Resistance
Project Type	Wet lab
Summary	Melioidosis, caused by the bacterium Burkholderia pseudomallei, is a severe, frequently fatal, tropical disease. Beta-lactam antibiotics are cornerstones of melioidosis treatment, but are variably effective in resolving B. pseudomallei infections. New, more effective treatments are needed for this under-studied disease. Beta-lactams target transpeptidase enzymes, primarily penicillin-binding proteins (PBPs), that catalyse cross-linking of the bacterial cell wall; B. pseudomallei transpeptidases are however little studied. This project combines structural, biochemical, computational and microbiological approaches to investigate B. pseudomallei transpeptidases, aiming to establish their reactivity towards different beta-lactams in vitro, and subsequently whether combining beta-lactams represents a route to more effective melioidosis treatments.
Description	Melioidosis, caused by the soil-borne Gram-negative bacterium Burkholderia pseudomallei, is an under-recognised tropical disease, with mortality rates that can exceed 40 % for respiratory infections, that causes c. 100 000 deaths worldwide. It is widespread in tropical regions, with the global epicentre Northeast Thailand, where agricultural workers constitute a high-risk group. Treatment is complicated by long latency and diverse symptoms which can hamper infection diagnosis. Beta-lactam antibiotics (penicillins and related agents such as cephalosporins (e.g. ceftazidime) and carbapenems (e.g. meropenem)) are key components of melioidosis treatment. Typical regimes involve 2-8 weeks intensive therapy with intravenous ceftazidime or meropenem, followed by several months of eradication therapy with trimethoprim-sulphamethoxazole or the beta-lactam-based amoxicillin-clavulanate combination. Failure to complete extended therapy can result in disease recurrence; shorter treatments that are more effective in eliminating infection are thus highly desirable. This project investigates interactions of beta-lactams with their cellular targets- enzymes involved in cross-linking the bacterial cell wall- aiming to establish the reactivity of different beta-lactams towards their various targets, and apply this information to identify new combinations of beta-lactams that collectively inhibit multiple targets and manifest increased anti-B. pseudomallei activity. Beta-lactams inhibit transpeptidase enzymes- penicillin-binding proteins (PBPs) and the related L,D transpeptidases (LDTs)- that catalyse cross-linking of peptide side chains of bacterial peptidoglycan during synthesis and maintenance of the bacterial cell wall. B. pseudomallei possesses multiple PBPs, with our preliminary in silico analysis of the well-studied K96243 strain identifying 11 candidate PBPs (including three distinct allelles of PBP3, the primary target of ceftazidime) alongside a single LDT. In this project we will use recombinant Escherichia coli to express the complete panel of B. pseudomallei PBPs and LDTs, identified from sequence data from multiple B. pseudomallei strains, and investigate

	their interactions with clinically relevant beta-lactams using biochemical and biophysical assays. Inhibition assays will be based on (commercially available) chromo- and fluori-genic beta-lactam reporters, enabling calculation of dissociation constants; while biophysical assays will utilise differential scanning fluorimetry (DSF) and circular dichroism (CD) spectroscopy to identify interacting beta-lactams based on increases in the mid-point temperature of thermal unfolding (Tm) in the presence of ligand. We envisage using DSF as an initial, medium-throughput screen to screen individual PBPs/LDTs against a panel of beta-lactams, in particular aiming to identify the cellular targets of the most clinically relevant agents. CD and inhibition assays will then enable more detailed, quantitative characterisation of observed interactions. Based on these findings we will determine structures of selected beta-lactam/PBP or LDT complexes using X-ray crystallography and/or cryo-electron microscopy, and investigate their stability and dynamic behaviour using molecular simulations. This information will rationalise the structure-activity relationships that we uncover and, potentially, enable exploration of potential non-beta-lactam inhibitors using computational approaches. Beta-lactams, in particular combinations of multiple beta-lactams that collectively inhibit multiple PBP/LDT targets, identified by this approach will be evaluated for activity against B. pseudomallei and its close relative B. thailandensis in antimicrobial susceptibility assays. As B. pseudomallei is a Biological Safety Level (BSL) 3 organism we will first establish susceptibility of B. thailandensis in disc diffusion and broth dilution assays. Subsequent testing of B. pseudomallei will be done through Thai collaborators (Prof. N. Chantratita, Mahidol University, Bangkok, who has agreed to be a co-supervisor), who has access to an extensive panel (>1000 strains) of whole-genome sequenced B. pseudomallei from both human clinical and environmental samples. We will evaluate the most promising agents/combinations against a subset of these strains, including those resistant to existing treatments, and use sequence information to rationalise variations in susceptibility with respect to sequence polymorphisms of PBPs/LDTs and other potential contributors such as beta-lactamases, porins and efflux pumps. Overall the project provides a workflow for rational development of improved melioidosis therapies based on multitargeting of B. pseudomallei transpeptidases using the established beta-lactam class of antibiotics. Multiple opportunities exist for the student to take ownership, and shape direction of, the project. Beyond our core goals of establishing the spectrum of activity of beta-lactams towards B. pseudomallei transpeptidases, and based on this information evaluating agents with complementary specificities as potential new approaches to melioidosis treatment, the project provides opportunities for students to specialise in approaches and methodologies that most interest them. These are enabled by the multidisciplinary nature of the project, and underpinned by the specific and complementary expertise within the supervisory team. Potential areas of focus include, but are not limited to, structural characterisation of beta-lactam:target complexes using X-ray crystallography and/or cryo-electron microscopy; computational investigations of such interactions using molecular dynamics (MD)- and/or quantum mechanics (QM)- based approaches; identification of

	new (non-beta-lactam) ligands for B. pseudomallei PBPs/LDTs using computational methodologies; and investigating the effects of strain-specific variations in PBP/LDT (and, potentially additional cellular components such as porins or efflux pumps) sequence/expression on beta-lactam susceptibility.
Can be completed part time?	Yes
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