Project Details	
Project Code	MRC22IIARBa Blagbrough
Title	Polyamine-based antimicrobials for treatment of antibiotic resistant bacterial pathogens
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Antimicrobial resistance (AMR) is one of the top 10 global health issues. Lack of novel drugs in the clinical pipeline are severely hampering treatment options and driving AMR. We have identified novel polyamine-based compounds displaying activity against major human bacterial pathogens. This project will further define the molecular activity of these compounds using a suite of chemical and molecular microbial methods together with invertebrate infection models.
Description	Preliminary Studies Biofilms are a notoriously difficult to treat therapeutic impediment. S. aureus is classified as a priority pathogen for the development of new antimicrobials. Polyamine chemistry and targeted molecular microbiology afford a rational use in targeting bacterial pathogens to overcome AMR as a framework for novel antibiotic discovery or facilitating biofilm disruption. We have recently identified a suite of polyamines that show antimicrobial activity against a panel of genetically distinct, highly-drug resistant S. aureus clinical isolates with a minimum inhibitory concentration (MIC) = 2-4 µg/mL. Importantly, experiments evaluating nephrotoxicity have indicated these compounds have an IC50 of 64 µg/mL, with no erythrocyte haemolysis observed in concentrations as high as 128 µg/mL. The aim of this PhD studentship is to characterise the molecular mechanisms of action of these polyamines to direct future structure-activity relationship (SAR) studies in the search for more potent and clinically relevant antimicrobials. The efficiency of these compounds to treat and clear infection will be assessed using the fifth instar Manduca sexta invertebrate model and biological activity against single- and mixed-biofilm communities. Phenotypic responses and the probability of AMR arising over time will also be assayed. #1: Characterise molecular mechanism of action Polyamines bind to regions of negative charge, e.g. nucleic acids and cellular phospholipids. In vitro assessment of drug induced DNA oxidative damage using pure DNA and DNA isolated from treated S. aureus (in vivo) will be analysed using alkaline electrophoresis gel assay and DAPI-treated bacteria and confocal microscopy. Membrane permeabilization assays will be conducted using flow cytometry and propidium iodide staining and evaluation of intracellular esterase activity. The student will monitor the effects on oxidation-reduction potential monitoring glutathione levels in cells treated with polyamines and antibiotics. ROS generation will be investigated, as a common mechanism of bacterial death is caused by the generation of free radicals derived from oxygen following oxidative damage. The student will follow localisation to see if FITC-labelled polyamines enter the bacterial cytosol, using the fluorescent membrane marker (FM4-64) and confocal microscopy. #2: In vivo model for AMR The student will develop an in vivo invertebrate model using fifth instar M. sexta caterpillars. It is important to note that these experiments align with the UKRI National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs): Pioneering better science. Both ML (Bath)

	and RCM (Bristol) laboratories have experience in designing infection experiments in using the M. sexta disease model, quantifying both toxicity and the ability to cure S. aureus infection. Our previous research shows that this model can reliably report on bacterial and disease burden through analysis of hemolymph and weight respectively. #3: Biofilm disruption We have unpublished results on polyamines inhibiting and disrupting single and mixed species biofilms. Both static and flow biofilm models will therefore be established where analysis will inform on biomass (crystal violet staining) and metabolic activity (TTC staining). Using a microfluidic platform (with TB, Exeter), how polyamines inhibit biofilm attachment and maturation under flow will be assayed in real time using microscopic imaging of biofilm development. Does this lead to synergy with current licensed antibiotics, and moving towards translational research, is activity retained in human serum? #4: Resistance studies Emergence of resistance to our novel polyamines will be measured by serial passages, microfluidic single cell experiments, and real time diffusion mutations assays e.g. MEGA plate assays to investigate mutational and adaptive pathways towards high-level AMR while evaluating mutant fitness.
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