Project Details	
Project Code	MRC23IIAREx Raymond
Title	Does resistance to antimicrobial peptides help bacteria avoid immunity and become more harmful?
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	Antimicrobial peptides are a potentially rich source of new antibacterial drugs. However, peptides also form part of the defence systems of many animals. The project will combine protein synthesis with genomics, and experiments in an insect model. It will investigate whether evolved resistance to a range of peptide structures also confers resistance to innate immunity peptides and how resistance affects the ability of bacteria to infect and harm hosts.
Description	Antimicrobial peptides (AMPs) have tremendous potential to address the rise of multi-drug resistant bacterial infections. These natural compounds are highly diverse, and they are widely used in immune systems and by other micro-organisms to suppress the growth of bacteria. The role of AMPs in basic defence mechanisms raises important questions. If bacteria evolve resistance to peptides applied clinically, will they then be better able to overcome host immunity? The importance of these compounds in the natural world suggests that it might be difficult for bacteria to evolve resistance to peptides 1,2. Nevertheless, Gram-positive bacteria can acquire resistance to peptides produced by human immune systems (defensins) after being selected for resistance to other peptides 3 a phenomenon known as cross-resistance. If bacteria can readily evolve cross-resistance to diverse peptides, without losing their ability to infect hosts, then this will create serious issues for clinical use of AMPs. This PhD will study how evolved resistance to diverse peptides affects AMP susceptibility generally; it will test whether different compounds are more or less likely to lead to cross-resistance and will evaluate how resistance affects the ability of a focal bacterium to infect a model host. The project will use Enterobacter cloacae, one of several key Gram-negative bacteria which contributes to clinically important broad-spectrum antibiotic resistance. This microbe has been developed by BR as an insect infection model, and can be used as a chronic gut infection model or as an invasive virulent parasite 4. Initially, the student will test selection for resistance in vitro using use at least five diverse compounds: magainin 2, melittin, microcin, cecropin and the clinically licensed drug colistin. Mutants with resistance to these compounds will be screened for changes in susceptibility to the other peptides used in selection and to a panel of human produced peptides (defensins) that are in clinical development. In the second part of the project the student will sequence resistant mutants and characterize resistance mechanisms. We will determine the genetic basis of novel resistance by screening for SNPs, rearrangements, new gene acquisitions or recombination that might have led to the development of resistance, through comparing evolved resistant mutants to their susceptible ancestor. We will use a range of standard bioinformatics techniques, including mapping and assembly, in a Linux environment to perform these comparative genomics tasks. Resistance to peptides is commonly based on changes in the charge of the lipopolysaccharide membrane or uptake mechanisms, so resistant mutants will also be screened for

	altered membrane potential using flow cytometry 3. The final part of the project will explore how peptide resistance affects infection in an insect model host. Resistance to peptides can affect the ability of bacteria to tolerate stress, we hypothesize that resistance could decrease bacterial persistence and transmission but may increase virulence in vivo if there is altered susceptibility to key defence molecules (e.g. cecropin or defensins). This project will be led by an inter-disciplinary team with complementary expertise in microbial evolutionary biology and the evolution of resistance (BR), peptide chemistry (LL) and bacterial genomics (LC). This project could suit students from a range of possible backgrounds (chemistry, genetics, microbiology, evolutionary biology). References (1) A Peschel, H-G Sahl (2006) Nature Publishing Group 4, 529. (2) G Yu et al. (2018) Proc R Soc B: Biol Sci 285. (3) JZ Kubicek-Sutherland et al. (2017) J Antimicrob Chemother 72, 115. (4) CJ Manktelow et al. (2020) Antimicrob Agent Chemo.
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