Project Details	
Project Code	MRC22IIAREx van Houte
Title	Developing new technologies to target AMR
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Antimicrobial resistance (AMR) has been described as a slow-motion pandemic that already has a huge impact on healthcare, and will become much more problematic in the next decades if the tide is not turned. Finding new ways to eradicate AMR could be a real game changer. This project will explore CRISPR-Cas9 in combination with lytic bacteriophage as a new antimicrobial tool to target drug resistant bacteria in the gut microbiome.
Description	Antimicrobial resistance (AMR) is one of the greatest threats of our time and development of novel technologies to remove AMR genes from patients would be truly ground-breaking. CRISPR-Cas9, an adaptive immune system in bacteria that is now widely adopted for its versatile and efficient gene-editing abilities, is a highly promising next-generation tool with the potential to remove AMR genes. One of the key problems with AMR is that it transfers between different bacterial species within microbial communities, such as those in our gut. If we want to apply CRISPR-Cas9 to remove AMR genes, or stop their spread, we therefore need to find ways to deliver it to many different bacterial species in a community. In this project the student will combine CRISPR-Cas9 technology with bacteriophage-based approaches to target AMR in a microbial community context. To do so, the student will select, design and engineer suitable broad host-range vectors (e.g. conjugative plasmids) that have the ability to deliver CRISPR-Cas9 to a wide range of bacterial species. The van Houte lab has a large library of >100 lytic bacteriophages that infect various pathogens, including E. coli ST131 (a major cause of bloodstream and UT infections) and Pseudomonas aeruginosa (an opportunistic pathogen causing a.o. lung infections). The student will characterize these phages phenotypically and genotypically (e.g. phage typing and sequencing, measuring host range and evolution of phage resistance), and select the most promising phage(s) for use as a selective force to enhance the spread of the CRISPR-Cas9 delivery vector. To achieve this, the student will engineer the CRISPR-Cas9 vector to target (i) AMR gene(s) of interest and (ii) lytic bacteriophage(s). It is expected that because the CRISPR-Cas9 delivery vector now confers immunity to lytic phage, bacterial hosts will be forced to take up CRISPR-Cas9, thereby resensitizing them to antibiotics. The student will test this hypothesis through in vitro experiments using 1) clonal pathogen populations and 2) pathogen clones embedded within a microbial gut community. The abundance of the CRISPR-Cas9 delivery vector, the targeted AMR gene and the lytic phage will be monitored using fluorescence microscopy, FACS analysis, qPCR, phenotypic assays, metagenome sequencing, or a combination thereof. In vitro experiments will be complemented by mathematical modelling to generate testable predictions on (i) the optimal conditions for spread of the CRISPR-Cas9 vector, (ii) how the efficacy of AMR removal depends on different phage types, (iii) how the efficacy of AMR removal depends on the composition of the microbial community. Depending on how the student wishes to tailor the project, there will also be opportunities to determine the

	ecological and evolutionary consequences of AMR removal on microbial community composition, using long-term in vitro experiments and metagenome sequencing. Finally, to validate in vitro findings the student will measure the efficacy of the tool in vivo in a wax moth model. If relevant, a mouse gut colonization model will be used in collaboration with Dr Fernanda Paganelli, UMC Utrecht, to validate the initial in vivo findings. The student will work under the supervision of Dr van Houte, who has developed CRISPR-Cas9 based tools to target AMR and has acquired a large library of bacteriophages that form the basis of this project. Furthermore, the student will be supervised in bioinformatics analysis of genomic data on microbial community composition by Prof. Feil (University of Bath). Co-supervisors on the project also based in Exeter are experts on evolution and spread of AMR (Prof. Gaze & Dr Leonard) and mathematical modelling (Dr Recker). The student will receive training across many disciplines in synthetic biology, bioinformatics, experimental evolution, mathematical modelling, molecular microbiology and genetics.
Supervisory Team	
Lead Supervisor	
Name	Dr Stineke van Houte
Affiliation	Exeter
College/Faculty	Life and Environmental Sciences
Department/School	Biosciences
Email Address	c.van-houte@exeter.ac.uk
Co-Supervisor 1	
Name	Professor William Gaze
Affiliation	Exeter
College/Faculty	Medicine and Human Health
Department/School	Medical School
Co-Supervisor 2	
Name	Professor Edward Feil
Affiliation	Bath
College/Faculty	Department of Biology & Biochemistry
Department/School	Milner Institute for Evolution
Co-Supervisor 3	
Name	Dr Mario Recker
Affiliation	Exeter
College/Faculty	Life and Environmental Sciences
Department/School	Biosciences
Co-Supervisor 4	
Name	Dr Anne Leonard
Affiliation	Exeter
College/Faculty	Medicine and Health
Department/School	Medical School