Project Details	
Project Code	MRCIIAR26Ex van Houte
Title	Developing CRISPR-Cas antimicrobials to tackle antibiotic resistance spread in Klebsiella pneumoniae.
Research Theme	IIAR
Project Type	Wet lab
Summary	Antimicrobial resistance (AMR) poses a major threat to human health, which is for a large part driven by plasmids (mobile pieces of DNA) that facilitate AMR gene spread between bacteria. In this project you will develop new tools based on CRISPR-Cas to stop the spread of AMR-carrying plasmids in the important pathogen Klebsiella pneumoniae. Proof-of-concept work shows that this is theoretically possible, but challenges remain in discerning drivers of plasmid competition. This interdisciplinary project integrates microbiology, genomics, and synthetic biology to tackle one of the most urgent challenges in infectious disease, providing training in leading-edge approaches with real-world impact.
Description	Widespread antibiotic use has made modern healthcare possible, and treatment of bacterial infections accessible and cheap. However, recent decades have seen a rise of antimicrobial resistant (AMR) bacterial infections which cannot be treated by conventional means, and finding a solution to this AMR crisis is urgently needed. Bacteria frequently take up AMR genes through a process called horizontal gene transfer – the transfer of genetic material between bacterial strains and species. Mobile genetic elements such as plasmids are an important vector of AMR genes, and these are particularly relevant to the opportunistic human pathogen Klebsiella pneumoniae and close relatives. K. pneumoniae has a particular propensity to cause hospital- or community-acquired AMR infections including pneumonia, urinary tract, and bloodstream infections. Extended-spectrum-beta-lactamase-producing and carbapenem-resistant K. pneumoniae infections account for nearly 100,000 infections and >7000 deaths annually within Europe. Mobile genetic elements are abundant in environmental and clinical strains of K. pneumoniae and often carry clinically relevant genes, including virulence factors and AMR. Beyond being an opportunistic pathogen of humans, K. pneumoniae is also associated with plants, animals, and the environment. K. pneumoniae is known to capture AMR genes and plasmids from environmental bacteria and act as a conduit to pass these elements on into other human pathogens, thus building a link between environmental reservoirs of AMR and the clinic through plasmid transfer. The fact that many AMR genes are carried by plasmids provides an opportunity to control their spread: bacteria have evolved a wide range of defence systems to selectively associate with mobile genetic elements. A well-known example of this is the CRISPR-Cas defence system. Many defences are themselves carried by mobile genetic elements, including plasmids – and it is hypothesized such mobile defences are important in competition between plasmids. Recently, we have shown that the effectivity of CRISPR-Cas carried by a plasmid is determined by the mobility of the plasmid, and that the strength of CRISPR-Cas can influence the outcome of competition between plasmids

	(Sünderhauf et al. 2025 bioRxiv). Furthermore, we have been able to harness the ability of CRISPR-Cas to specifically cleave a sequence in order to target AMR genes carried by plasmids, which allows us to resensitise pathogens to make them susceptible to antibiotics (Sünderhauf et al. 2023 Microbiology). The successful candidate will use a collection of human-associated K. pneumoniae isolates collected in clinical settings and from the wider community in Thailand and Italy to identify the role that plasmid-encoded microbial defences such as CRISPR-Cas play in plasmid competition, and how other competitive plasmid genes can modulate this interaction. By first describing which plasmids, resistance genes, and virulence genes are most commonly found amongst diverse K. pneumoniae isolates, the project will aim to develop a CRISPR-Cas9-based technology to stop their transfer. Plasmid transfer rates from human-associated K. pneumoniae isolates into laboratory strains, and between isogenic K. pneumoniae and E. coli strain backgrounds will be measured (objective 1). These data will be considered in the context of the presence of relevant plasmid-borne genes, including AMR, virulence, microbial defences, counter-defences, and other genes which may influence the plasmids' competitive ability. Having identified targets for a CRISPR-Cas AMR resensitisation treatment, molecular cloning techniques will be used to generate CRISPR-Cas plasmids which can target and remove K. pneumoniae AMR plasmids (objective 2). Finally, plasmid competition experiments will be carried out with natural and fluorescently labelled plasmids to identify whether plasmid genes involved in competition are more effective when static or mobile, and to discover under which conditions a CRISPR-Cas plasmid can outcompete K. pneumoniae AMR and virulence plasmids and thus prevent their transfer (objective 3). The successful applicant will take an active role in developing new bioengineering approaches to remove AMR genes from clinically relevant bacterial strains, and provide helpful insights to discern which plasmid genes influence the outcome of plasmid competition. We are excited to work with a student who is willing and able to take ownership of the project, and steer the direction based on data generated within earlier objectives. K. pneumoniae isolates carry many diverse plasmids and other mobile genetic elements with many types of AMR, virulence, and defence genes, and the student will be encouraged to choose their own focus within the second part of their PhD. The student will be based at the Cornwall campus of the University of Exeter, within the Environment and Sustainability Institute under the supervision of Prof Stineke van Houte and Dr David Sünderhauf. They will work closely with members of the van Houte group of all career stages and will be part of the Penryn Microbiology Early Career Researcher network at the University of Exeter (~ 100 members). Bioinformatic aspects of the PhD will be supervised by Prof Ed Feil at the Milner centre at the University of Bath and can be carried out remotely or during research visits to Bath.

Supervisory Team	
Lead Supervisor	
Name	Professor Stineke van Houte
Affiliation	Exeter
College/Faculty	Environment, Science and Economy
Department/School	Environment and Sustainability Institute
Email Address	C.Van-Houte@exeter.ac.uk
Co-Supervisor 1	
Name	Dr David Sünderhauf
Affiliation	Exeter
College/Faculty	Environment, Science and Economy
Department/School	Environment and Sustainability Institute
Co-Supervisor 2	
Name	Professor Ed Feil
Affiliation	Bath
College/Faculty	Department of Life Sciences
Department/School	Milner Centre for Evolution
Co-Supervisor 3	
Name	
Affiliation	
College/Faculty	
Department/School	