Project Details	
Project Code	MRCIIAR25Ex Westra
Title	Developing phage therapy solutions for Staphylococcus aureus
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	The spread of antimicrobial resistance (AMR) is a slow-moving pandemic, identified by the WHO as a top-10 threat facing humanity. Staphylococcus aureus is an opportunistic human pathogen with high levels of antimicrobial resistance and it is a WHO priority to develop novel therapeutic solutions against this species. Phages (viruses that infect bacteria) are increasingly recognized as potential therapeutic modalities. In this project, the student will carry out large scale infection assays with diverse S. aureus isolates and phages to identify which phages are the most effective inhibitors of S. aureus growth, and tease apart why this is the case.
Description	The spread of antimicrobial resistance (AMR) is a slow-moving pandemic and has been identified by the WHO as one of the top 10 threats facing humanity. Mobile genetic elements (MGEs), such as phages and plasmids, play a key role in AMR dissemination but also offer a promising basis for non-antibiotic therapies. The activity of MGEs is fundamentally shaped by bacterial defenses, which can suppress AMR spread and limit the efficacy of phage-based therapies. Well-known bacterial defenses include Restriction-Modification (RM) and CRISPR-Cas, but it is now recognized that bacteria carry more than 100 defense systems. These defenses act at different stages of the MGE lifecycle: some cleave MGE genomes immediately following infection, others interfere with MGE transcription or replication, or induce cell death or dormancy responses. Large-scale systematic studies are necessary to develop a broader understanding of how these defenses integrate and shape bacteria-phage interactions, which is essential to outflank AMR. We predict that bacterial defenses can interact synergistically or antagonistically, and that such combinations occur more and less frequently than expected by chance, respectively. To identify interactions supporting this hypothesis, we will identify known defense genes in publicly available whole genome sequences and analyze their co-occurrence patterns using phylogenetically controlled models. Next, we will use a diverse collection of over 500 sequenced S. aureus isolates that differ in geographical, clinical origin, ecology and host. Using bioinformatics pipelines, we will build phylogenies, and identify known defense genes in these isolates. On these 500 isolates, we will perform large-scale infection assays using a panel of 15 phages that can infect S. aureus. This panel captures diverse phage families with distinct life styles, genome sizes, and replication mechanisms, allowing us to test whether certain defenses are specific to particular phage types and whether combinations of defenses provide complementary or overlapping resistance ranges. Our team has recently developed high-throughput analyses of phage resistance and infectivity based on OD600 plate reader assays, which will be adopted in this project. We will analyze the resulting

	infectivity/resistance data using phylogenetically controlled statistical models to examine how defense types and combinations correlate with phage resistance phenotypes, and to what extent this depends on the phage type. The correlational analysis will highlight certain defense combinations that provide synergistic levels or broader ranges of resistance. To test for causality, we will express defense genes individually or in combination in S. aureus lab strains. The bioinformatics analysis may also point towards combinations observed less frequently than expected, suggesting potential antagonistic interactions. Strains with such combinations can be engineered by combining the respective constructs. Using the same approaches, we will test how individual defenses and their combinations impact phage infectivity. Finally, we will select individual defenses and synergistic combinations for follow-up bulk infection experiments to gain deeper insights into the consequences for bacterial and phage population dynamics. These experiments will also reveal whether phage can evolve to overcome defenses, and how this depends on the combinations of defenses in the host bacteria. We will identify phage mutants that evolved to overcome host defenses by comparing their infectivity against that of the ancestral phage. Where increases in infectivity are observed, we will perform Illumina sequencing to identify the genetic basis. We expect that in some cases, phage resistance or infectivity patterns cannot be explained by known defense genes, suggesting the presence of unknown defenses. To identify these, we will apply our recently developed Tn mutagenesis assays (Maestri et al Cell Host Microbe 2024) where a Tn library is infected with selective or fluorescent marker-labelled temperate phage that poorly infect the ancestral WT genotype of the isolate. Selective plating or FACS sorting of lysogens (i.e. bacteria infected with the temperate phage, which integrate into the genome) followed by Tn-seq will enable the identification of defense genes. The applicant will benefit from being embedded in a dynamics and prolific team of researchers who study bacteria-phage interactions, as well as being embedded in the BBSRC sLoLa Multidefence network (https://sites.exeter.ac.uk/multidefence/) and the BBSRC Mission Award "Safephage" network. Key publications from supervisory team related to the project: Bacteriostatic antibiotics promote CRISPR-Cas adaptive immunity by enabling increased spacer acquisition. Dimitriu T et al Cell Host Microbe. 2022 Exploitation of the Cooperative Behaviors of Anti-CRISPR Phages. Chevallereau A et Cell Host Microbe. 2020 Targeting of temperate phages drives loss of type I CRISPR-Cas systems. Rollie C, et al Nature. 2020 Bacterial biodiversity drives the evolution of CRISPR-based phage resistance. Alseth EO et al Nature. 2019 Ultrafast search of all deposited bacterial and viral genomic data. Bradley P, et al Nature Biotech 2019 Anti-CRISPR Phages Cooperate to Overcome CRISPR-Cas Immunity. Landsberger M et Cell. 2018

	The diversity-generating benefits of a prokaryotic adaptive immune system. van Houte S, et al Nature. 2016 Rapid antibiotic-resistance predictions from genome sequence data for Staphylococcus aureus andMycobacterium tuberculosis. Bradley P Nat Commun. 2015
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