Project Details	
Project Code	MRCIIAR26Ba Jones
Title	Co-evolution of bacteriophage within bacterial microbiomes and development of phage therapies
Research Theme	IIAR
Project Type	Wet lab
Summary	Viruses that infect and kill bacteria (bacteriophage) are promising alternatives to antibiotics and could contribute to controlling antibiotic resistant pathogens. Working with the United Kingdom Health Security Agency, you will employ molecular, genomic, bioinformatic, and directed evolution techniques, in conjunction with models of polymicrobial infection, to answer fundamental questions about the co-evolution of bacteriophage within bacterial microbiomes. These studies are essential to understand how we could apply bacteriophage to treat problematic infections, including those which are antibiotic resistant. They also open up opportunities to use phage for microbiome modulation as a new way of preventing infections.
Description	**Background** Antimicrobial resistance (AMR) constitutes a major global threat to public health and without intervention, this crisis is predicted to claim ~10 million lives and cost $1 trillion USD a year by 2050. This has renewed interest in the development of alternatives to antibiotics that could limit the spread of AMR. Viruses that specifically infect and kill bacteria (bacteriophage or phage) are a promising approach to control a range of infections, particularly those associated with indwelling medical devices where biofilm formation is a major problem. Biofilms are surface-associated bacterial communities encased in an extracellular polymeric matrix which affords protection against immune clearance and antibiotic treatment. Phage have evolved mechanisms to penetrate the extracellular matrix and infect biofilm associated cells which could be exploited to develop new treatments for these infections. Our previous work has already highlighted the potential for phage to control catheter associated urinary tract infection (CAUTI) and inhibit biofilm formation on these devices. However, the development and application of effective phage therapies requires a deeper fundamental understanding of factors that modulate the interaction of phage and bacterial hosts. Bacteria predominantly exist within polymicrobial communities (or microbiomes) in many clinically relevant habitats, including infections such as CAUTI. This raises important questions regarding the co-evolution of phage and target species within these communities, how effective phage are at killing target species within a polymicrobial community, and the effect of phage treatment on mobilisation of antibiotic resistance genes within microbiomes. **Aims & Objectives** Focusing on phage infecting Klebsiella pneumoniae and using a clinically relevant in vitro model of polymicrobial CAUTI, this project will address key questions regarding the co-evolution of bacteriophage and hosts within bacterial communities, and the application of phage to resolve biofilm associated infections. Klebsiella pneumoniae is a prominent opportunistic pathogen, a particular concern in terms of AMR, and forms

	extensive biofilms on catheter surfaces which incorporate and protect other pathogens. The CAUTI model provides a tractable microbiome system replicating both planktonic and biofilm components, and facilitates the use of directed evolution, genomic, metagenomic, and biochemical approaches to understand community response and validate new therapeutic approaches. Objective 1 - Phage isolation and characterisation. Bacteriophage will be isolated against a panel of K. pneumoniae clinical isolates and characterised in terms of genome composition, capsid structure, host-range, and ability to infect and disrupt K. pneumoniae biofilms. Factors influencing phage host range and infection will initially be explored using directed evolution approaches, comparative genomics, and targeted mutagenesis approaches. The synergistic activity of phage combinations to increase host range and mitigate bacterial resistance will also be explored. This will allow us to address questions regarding the emergence of phage resistance, the impact on fitness of host bacteria, and identify effective phage combinations. Objective 2 - Phage therapy and host-phage co-evolution in polymicrobial communities. Models of CAUTI will be used to evaluate the capacity of individual phage, or phage combinations, to target and eliminate K. pneumoniae alone or when embedded in a bacterial community. This will include analysis of both planktonic and biofilm associated components of these microbiomes, through phenotypic and genomic characterisation of bacterial and viral populations recovered pre and post treatment. This will allow us to address questions regarding the impact of community membership on the efficacy of phage therapy, the co-evolution of phage and host species within a clinically relevant microbiome, and the effect of eliminating the target species on wider community dynamics. Objective 3 - Plasmid transfer and phage defence systems. The transfer of plasmids between bacterial species is a key mechanism in the dissemination of antibiotic resistance, but these mobile genetic elements have also been found to encode defence systems that confer protection against bacteriophage. Therefore, there is potential for target species to rapidly acquire mechanisms that undermine phage therapy and for the application of phage to inadvertently promote the spread of plasmids encoding antibiotic resistance genes. Using our polymicrobial CAUTI model we will investigate the impact of phage exposure on transfer of plasmids between community members. This will include plasmids encoding phage defence systems and antibiotic resistance genes, either alone or in combination. **Student Ownership** The student will be encouraged and supported to take ownership of the project from the outset. The supervisory team will enable the student to take the lead on experimental design and the specific focus of work in each objective. Initial "prep-period" activities and training will enable the student to more specifically define the research questions and lead implementation of experiments to test hypotheses they develop.

Supervisory Team	
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