Project Details	
Project Code	MRCIIAR25Ex Sanders
Title	Plasmids as AMR vectors
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Antimicrobial resistance (AMR) is rising to dangerously high levels causing a global health crisis. To develop strategies to combat AMR, we need to know how AMR genes are spreading. Plasmids as ubiquitous mobile genetic elements are key players of AMR spread. Antibiotics make carry AMR plasmids beneficial to their bacterial hosts and therefore drive plasmid prevalence and evolution. This project will investigate the evolution of highly transmissible AMR plasmids that can spread resistance within and between microbiomes. This will be done by targeted experiments and investigations of complex microbiomes using plasmid genomics and network analysis.
Description	**Background** The widespread use of antibiotics in clinical and agricultural settings has resulted in the rapid evolution and spread of antibiotic resistance causing a major health crisis (1). Bacteria can gain resistance to antibiotics through mutations or by taking up resistance genes (2). Plasmids play a key role in the spread of antimicrobial resistance (AMR) genes (3) because of their ability to transfer between different bacteria (4). The range of different bacterial hosts that plasmids interact with, i.e. plasmid generalism, is therefore crucial for the spread of AMR. There is evidence that antibiotic pressure can enhance plasmid generalism and this may not only facilitate the spread of the AMR genes under selection, but also may allow additional AMR genes to hitchhike along with the generalist plasmids (5). This could then lead to the spread of multi-drug resistant plasmids throughout microbial communities and, more worryingly, between environmental, agricultural and clinical microbiomes, a threat acknowledged in the OneHealth concept (2). AMR plasmid spread could be mitigated if plasmid generalism is a transient effect, reducing when antibiotic selection is lowered. However, it is unclear if this is the case. Plasmids can evolve incredibly quickly (6), and continued exposure to multiple hosts may lead to the evolution of plasmids that are even more successful at transmitting within microbiomes (7). Exposure to even a single antibiotic may lead to the evolution of plasmids that are highly infectious vectors of AMR genes in general. This project aims to determine how plasmids become transmissible AMR vectors. It will be experimentally tested how environmentally relevant antibiotic exposure regimes shape plasmid generalism and determine the molecular/functional changes on the plasmid. The project will further investigate the spread of AMR plasmids in complex communities (host-plasmid networks) and to pathogens combined with theoretical modelling. **Key question** Is evolved plasmid generalism the driver of AMR spread to pathogens within microbiomes? With increased plasmid generalism, we can expect significant changes to the structure of host-plasmids networks, becoming more interconnected and plasmids transmitting between

	phylogenetic distant hosts. This will have consequences for future events of novel AMR genes spread across microbial communities. **Objectives** 1. Determine if antibiotic exposure leads to evolved plasmid generalism. AMR plasmids within replicate complex experimental bacterial communities will be exposed to different levels of antibiotics for 6 weeks. Host-plasmid network structures and plasmid generalism will be measured at the end of the experiment. This objective aims to measure the (under different antibiotic pressures) evolved plasmid fitness effects across a range of hosts and to determine the genomic basis of evolved plasmid generalism. RNA sequencing on ancestral and evolved bacteria with and without the plasmid will reveal gene expression networks for generalist plasmids compared to specialist plasmids. 2. Test experimentally if plasmid generalism can predict the spread of AMR plasmids to pathogens in complex host plasmid networks. Link plasmid generalism to network structure and risk of pathogens gaining resistance. We will expose laboratory scale wastewater communities spiked with known AMR plasmids to relevant antibiotic exposure regimes and determine the resultant bacteria-plasmid networks using Hi-C metagenomics. This will allow to determine how antibiotics shape bacteria-plasmid network structure and the spread of AMR in wastewater microbiomes and to pathogens. The student will have the choice of studying a type of microbiome, e.g. soil, sewage or porcine gut (faecal) microbiome. 3. Determine the drivers of plasmid invasion of new microbiomes. We will complement the experimental work with theory to identify possible interventions to minimise AMR spread. An example for such intervention is to reduce antibiotic concentration/diversity in the environment to minimise transmission between microbiomes. As a starting point, we will use published models developed for communities containing multiple hosts and plasmids with and without antibiotics (8,9). The models describe ecological dynamics of bacteria-plasmid communities based on Lotka-Volterra competition, including horizontal transmission and plasmid loss. We will vary costs associated with plasmid generalism and the strength and specificity of selection (i.e., multiple antibiotics and resistance mechanisms). The experiments and the wider work of the supervisor team will provide crucial data about plasmid and host traits related to their importance within the networks and for AMR transmission. We will explore how plasmid survival and spread in a new environment is affected by the amount of invading host bacteria, growth rate costs of bacteria in new environments, the degree of evolved generalism, and antibiotic selection within hosts. Here the student has the freedom exploring different approaches and interventions. **References** 1. Murray et al. Lancet, 629–655 (2022). 2. Castañeda-Barba et al. Nat.Rev.Microbiol., 1–15 (2023). 3. DelaFuente et al. Nat.Ecol.Evol., 1980–1991 (2022). 4. Redondo-Salvo et al. Nat.Commun., 3602 (2020). 5. Lassalle et al. Nat.Microbiol., 1787–1798 (2023). 6. Dimitriu et al. PNAS, e2107818118 (2021). 7. De Gelder et al. Genetics, 2179–2190

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