Rational Mining of the Human Microbiome for Novel Antibiotic Candidates that Target Antimicrobial Resistant Pathogens

Widespread antimicrobial resistance (AMR) is now rendering some bacterial infections untreatable with current antibiotics. The problem is dire, especially considering there are very few new or novel classes of antibiotics in the discovery pipeline. Many believe we are headed for a post-antibiotic era where common bacterial infections and routine clinical procedures become life or death scenarios. For example, colistin is a last resort antibiotic and there are now reports of the emergence of colistin resistant *Klebsiella pneumoniae* meaning that lives are now at risk of infections once thought to be easily and routinely treatable. Novel approaches and technologies are required to find candidate antibiotics to replenish the discovery pipeline with products that spare the co-resident microbiota.

Over the past few years, extensive research into the human microbiota has led to insights on interactions between health-associated bacteria and enteric pathogens that could be exploited to discover novel metabolites or other bacterial by-products that could be novel classes of antibiotic candidates. We believe that during intestinal health, some commensal bacteria produce molecules that can interfere with or kill pathogens or hold a pathogen colonization in stasis, thereby preventing overgrowth. How do we accurately identify these pathogen restraining commensals?

Using metagenomic analysis of the human microbiome we find that about 5-10% of healthy individuals carry low levels of known pathogens (*Klebsiella, E. coli, Enterococci, Camplyobacter, Salmonella, C. perfringens* - unpublished data). However, nobody has yet systematically and thoroughly data mined the human microbiome for beneficial bacteria with anti-pathogen activity. The Finn group has recently completed a meta-analysis of >13,000 human microbiome WGS datasets, identifying thousands of novel genomes. Meanwhile, the Lawley group has largely overturned the viewed that the microbiota is “unculturable”, developing novel methods to culture the majority of bacteria from the human intestinal tract and identifying many novel bacterial taxa (family, genera and species) that are ubiquitous to humans (Browne et al. 2016. Nature). Combining the two group’s resources (700 genomes from isolates and >2000 metagenome assembled genomes, representing the most comprehensive human gut microbiome genome reference catalogue) paves the way for performing a systematic analysis to identify and validate bacteria harbouring anti-pathogenic activity.

The project will leverage the informatics expertise in the Finn group to initially perform large-scale reference-based mapping using the aforementioned catalogue, to produce a fine grain taxonomic and functional analysis. This approach will identify bacterial species and subspecies and associated abundances with a high degree of confidence (compared to amplicon or raw read analysis), and will be used to develop a co-occurrence network. Commensal bacteria that could exclude pathogens from the community will be identified using network analysis and machine learning. Detailed phylogenies for pathogens carried at low level within the healthy human population will be analysed in the context of metadata including known pathogenic outcomes to differentiate the genetic vs environmental factors underpinning the pathogen inhibition factors of interest. Based on the co-occurrence analysis...
we will identify species and/or specific metabolic pathways/gene clusters that could lead to the identification of novel commensal or antibiotic candidates. Thereafter, these anti-pathogenic traits will be validated within the Lawley Laboratory.

SPECIFIC AIMS
One key strength of this project is the complement of a high-end bioinformatics group with a well-supported, state-of-the-art CL2 experimental laboratory that will allow the post-doctoral fellow to achieve the following specific aims:

1. To develop pipelines and perform comprehensive genomic and phylogenetic analysis of extensive pathogen genome collections to identify key marker genes capable of detecting and determining the lineage of AMR pathogens within metagenomics datasets.

2. To use commensal and pathogenic genome sequences and associated phylogenies for reference based analysis of WGS metagenomes to establish positive and negative co-occurrence correlations for AMR pathogens. Unmapped/unassembled sequences will undergo de novo co-assembly and binning to establish lower abundant, potential commensal bacteria absent from the reference genome collections. Metagenomes containing commensal organisms that have the strongest co-occurrence correlations (both positive and negative), hence likely to be harbouring antimicrobial resistance genes or antibiotic synthesis operons, will have metadata verified and enriched from associated publications. The Finn group will help use the reference isolate genomes, metagenome assembled genomes and sample metadata to mine for potential pathways (e.g. antibiotics, immunity proteins, metabolic pathways) to provide a rationale for the observed relationships.

3. To assemble a collection of pathogens and commensal organisms (based on the findings from Aims 1 and 2) to initially test commensals for anti-pathogen activity. The Lawley Lab maintains a large diverse culture collection of over 500 species with whole genomes and the capacity to target culture desired bacteria from microbiota samples. The lab has a variety of experimental platforms to investigate the molecular and genetic basis of host-microbiota interactions (Figure 1). Candidate bacteria will be taken forward for validation and mechanistic studies in the Lawley Lab with the options to use a variety of in vitro (e.g. microbiology, intestinal organoids) and in vivo methods (e.g. germ free mouse colonization) in combination with metatranscriptomic and metabolic analysis. The lab maintains a fermentation system to grow pure cultures or model communities, if warranted. Thus, the fellow will have a variety of experimental platforms to interrogate and validate hypotheses generated from Aims 1 and 2 – fostering a cycle of experimental validation follow by refinement of informatics analysis. The overarching goal is to determine the unpinning molecular mechanisms of candidate commensals/natural products, both in terms of the target in the pathogen and the spectrum of action against other pathogens and the microbiota.

EXPECTED OUTCOMES
This project offers a unique opportunity for a post-doctoral fellow in the burgeoning field of human microbiome research and asks deep questions about the co-evolution of human commensals and pathogens. The resulting human microbiome data generated will have broad applications for deciphering the mutualistic co-evolution of the human microbiome and understanding health and diseases of the intestine that are associated with the microbiota. Once developed, the pipelines and experimental work could be readily applied to other biomes (e.g. oral cavity or cow rumen) to deepen our understanding of the interplay of micro-organisms in different biomes. Finally, this collaboration between the EBI and WTSI will form the basis of a unique, world-class research programme, integrating state-of-the-art bioinformatic and experimental approaches.