Quantitative analysis of intrinsic antibiotic resistance in the major nosocomial pathogen Klebsiella pneumoniae

Abstract
Antibiotic resistance poses a global and severe threat to human, animal and planetary health. Typically, resistance arises through genetic mutations or via the acquisition of genes that allow bacteria to resist antibiotics. Transcription factors (TF) are part of the intrinsic response to antibiotic challenge and when upregulated control multiple genes. It has, for example, been shown that the last-line antibiotic, tigecycline, directly selects for enhanced expression of the global regulatory protein RamA. Importantly, increases in RamA levels are not limited to tigecycline exposure alone but extend to other antibiotics, thereby highlighting the relevance of RamA in the intrinsic resistome.

In this project, we set out to quantitatively characterise the gene regulatory mechanisms of RamA that allow the clinically relevant bacterium *Klebsiella pneumoniae* to adapt their gene expression machinery to antibiotic challenges. We will analyse transcriptomics data using machine learning and bioinformatics to identify promoter signatures that lead to differential expression of RamA-regulated genes.

Introduction
Bacteria can rapidly evolve under antibiotic pressure to develop resistance, which occurs when target genes mutate, or when resistance-encoding genes are transferred. Alternatively, bacteria can simply alter the levels of intrinsic proteins that allow the organism to "buy" time to resist antibiotic pressure. *Klebsiella pneumoniae* is a pathogen that causes significant blood stream or respiratory infections, but more importantly is a bacterium that is increasingly being reported as multidrug resistant.

Previous data from the Schneiders lab has demonstrated that the transcriptional regulator RamA can trigger changes on the bacterial surface that allow *Klebsiella* to survive both antibiotic challenge, degradation by host immune peptides and resist phagocytosis. The molecular basis of increased survival of *ramA* overexpressing *K. pneumoniae*, against host-derived factors is associated with RamA-driven alterations of the lipid A moiety of *Klebsiella*. This modification is likely to be linked to *Klebsiella's* ability to resist the host response so that it remains undetected by the immune system.

In this project we aim to characterise the mechanisms underpinning transcriptional regulation through RamA. We will investigate comprehensive transcriptomics and genomics data to identify sequence elements within the promoter regions of RamA-regulated genes that determine differential expression.

Research Challenge
We will train classifiers and assess the performance of different methods and further investigate the impact of different sequence encodings. Starting with simple classifiers, e.g. random forests, we aim to identify sequence features that impact differential expression, and subsequently, train regression models on the reduced feature space for sequence-to-expression prediction.

Data
The Schneiders lab has existing transcriptomics data from wildtype and RamA mutants, and it is currently collecting new data from clinical isolates.

RRI/Ethical Considerations Expected Outcome
Re RRI, lab work with Klebsiella requires heightened safety measures, however, this will only be an issue if the candidate would like to gain hands-on experience in data collection, which is a possibility. In that case, they will be able to rely on extensive experience in the Schneiders lab to ensure safe and responsible research conduct.

The project will derive new insights into resistance mechanisms in Klebsiella pneumoniae, which is a major nosocomial pathogen with high levels of antibiotic resistance.

The project will equip the student with a range of quantitative data skills and will be an opportunity for truly interdisciplinary research training.

References

- Holden, & Webber (2020). MarA, RamA, and SoxS as mediators of the stress response: survival at a cost. *Frontiers in microbiology*, *11*, 504720.
- De Majumdar, ... & Schneiders (2015). Elucidation of the RamA regulon in Klebsiella pneumoniae reveals a role in LPS regulation. PLoS pathogens, 11(1), e1004627.