Genetic regulation of antibiotic resistance in the major pathogen *Klebsiella pneumoniae*

Supervisors

Dr Andrea Weisse, School of Informatics & School of Biological Sciences
Dr Thamarai Dorai-Schneiders, School of Medicine

Abstract

Antibiotic resistance poses a severe threat to human, animal and planetary health. It means that common infections are becoming harder, and at times, impossible to treat. Already, resistance is associated with almost 5 million annual deaths globally, with numbers expected to rise rapidly as resistance spreads.

Resistance typically arises through genetic mutations or through gene acquisition that enable bacteria to resist antibiotics. In contrast to acquired resistance, transcription factors form part of the intrinsic response to antibiotic challenge and when upregulated control multiple genes that can impact bacterial susceptibility to antibiotics.

The global regulatory transcription factor RamA controls a multitude of drug and immune responses in the pathogen *Klebsiella pneumoniae*, which causes severe infections, particularly, in vulnerable hospital patients. In previous work, we showed that RamA contributes to the systemic dissemination of *Klebsiella pneumoniae* in mouse models of infection – thus highlighting its role as a key factor in pathogenesis and antibiotic resistance.

Here, we set out to quantitatively dissect mechanisms that promote RamA-mediated resistance. We will integrate public, clinical and our own lab data to quantitatively study how regulation via RamA differentially adapts the gene expression machinery to antibiotic challenge. With RamA upregulated in strains resistant to last-line antibiotics, it presents as a promising target for the development of novel treatments, and so this work will establish a mechanistic base to investigate viable strategies.

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 alter levels of intrinsic proteins that allow the pathogen to "buy" time to resist antibiotic pressure. *Klebsiella pneumoniae* is a pathogen that causes severe blood stream and respiratory infections, and importantly, it is a bacterium that is increasingly reported as multidrug resistant.

We previously demonstrated that the transcriptional regulator RamA can trigger changes on the bacterial surface that allow *Klebsiella* to survive antibiotic challenge, evade degradation by host immune peptides and resist phagocytosis. The molecular basis of increased survival of *ramA*-overexpressing *K. pneumoniae* results from changes in permeability reducing entry of antibiotics and host-derived factors – potentially through RamA-driven alterations of the lipid A moiety in *Klebsiella* – likely enabling

overexpressing *Klebsiella* to remain undetected by the immune system and thus resist the host response.

Current data suggest that multiple novel genes, beyond those already characterised, are relevant to the phenotypes linked to reduced antibiotic susceptibility and immune evasion. Given the ubiquity of clinical *Klebsiella* strains that overexpress RamA, we hypothesise that overexpression exploits a targeted regulatory regime, affecting a select group of downstream genes, as biological costs associated with global changes in the expression of all RamA-regulated genes would otherwise impose substantial selective pressure.

To identify relevant genes, we set out to quantitatively characterise the mechanisms underpinning transcriptional regulation by RamA. We will integrate transcriptomics, genomics and physiological data from clinical and lab strains to untangle regulatory mechanisms of RamA and their physiological consequences *in vivo*.

Research Challenge
We will integrate genomic and transcriptomic data to predict differential expression from variations in the promoter sequences of RamA-regulated genes. Data will comprise well-characterised lab-strains along with clinical isolates and environmental samples, posing challenges in integrating datasets of heterogeneous qualities.

A further challenge will be the high ratio of predictors to samples, and thus to reduce the feature space. We will start by training classifiers to identify predictive sequence signatures, and based on the reduced feature space, we then aim to train regressors for quantitative prediction.

Finally, we aim to integrate these insights into mechanistic models to investigate their dynamic impact on physiological responses of *Klebsiella* to antibiotic challenges.

Data & Methodology
The Schneiders lab has extensive genomic and transcriptomic data from wildtype and RamA mutants, and it is currently collecting new data from clinical isolates, all of which will inform the ML analysis. Inhouse data will further be supplemented with publicly available data from various environmental and clinical samples. Additionally, the lab will collect physiological data from growth assays, which we will use to train mechanistic models, developed in the Weisse group, to predict dynamic responses of *Klebsiella* growth and physiology.

We will employ bioinformatics, classic ML and dynamic systems modelling, and if desired, the project will also be an opportunity to gain hands-on lab experience. The project will thus be an opportunity to develop extensive interdisciplinary, data and ML skills.

RRI/Ethical Considerations
Lab work with *Klebsiella* requires heightened safety measures, however, this will only be an issue if the student 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, safety protocols (both HSE and local Biological risk assessments are already in place) in the Schneiders lab to ensure safe and responsible research conduct.

Expected Outcome

The project will derive new insights into resistance mechanisms in *Klebsiella pneumoniae*, a major nosocomial pathogen with high levels of antibiotic resistance, with the potential to highlight mechanisms that can be targeted for novel therapy.

The student will be embedded in a highly interdisciplinary research environment as part of the Biomedical Computation lab led by Dr Weisse (Schools of Informatics and Biological Sciences) and the Clinical Bacteriology lab led by Dr Schneiders (Institute of Regeneration and Repair). The project will be an exquisite opportunity to develop invaluable interdisciplinary research skills as well as data and ML skills, and an opportunity to contribute to concerted research efforts to combat a major global health challenge.

References

1. Holden, & Webber (2020). MarA, RamA, and SoxS as mediators of the stress response: survival at a cost. *Frontiers in microbiology*, *11*, 504720.
2. De Majumdar, ... & Schneiders (2015). Elucidation of the RamA regulon in Klebsiella pneumoniae reveals a role in LPS regulation. *PLoS pathogens*, 11(1), e1004627.
3. Weisse et al (2015), Mechanistic links between cellular trade-offs, gene expression, and growth, *PNAS*, 112 (9) E1038-E1047.