Identification and characterisation of barriers to antimicrobial resistance gene transfer

Background

Antimicrobial resistance (AMR) is a severe threat within public health, limiting bacterial infection treatment options and increasing patient risk of mortality. The overuse of frontline antibiotics is primarily responsible for the rapid spread of AMR worldwide. The constant presence of the selective pressure causes the dissemination of resistance genes amongst susceptible, exposed bacteria, pathogenic or otherwise. Whilst AMR can be obtained through gene mutation, the primary mechanism via which worldwide AMR gene dissemination is occurring is horizontal gene transfer (HGT), whereby genetic components are transferred between bacteria. HGT occurs via three possible methods. The first is transformation, whereby bacteria take up genetic material from the surrounding environment. The second, most well studied method is conjugation, where bacteria directly obtain mobile genetic elements (MGE) from other, permissible bacteria through direct contact. The final technique is transduction, which uses bacteriophages to transfer genetic material from a host bacterium to a new recipient.

HGT can occur within the host microbiome. Commensal bacteria harbouring AMR genes endemically exist within the human microbiome due frequent exposure to antibiotic selection pressure. They are generally inert. In the event of pathogenic infection, however, they act as an AMR gene reservoir. Their abundance increases during antibiotic treatment, and subsequently so does the risk of HGT of AMR genes to the invading pathogen. This is a major health concern, as this can facilitate the rise and spread of pathogenic strains resistant to new or multiple antibiotics and reduces the effectiveness of antibiotic treatment on the patient. Not only this, but it can result in chronic infection. AMR pathogenic bacteria that survive the course of antibiotics may then lose the resistance genes in order to increase their fitness and ability to outcompete the microbiota, leading to secondary infection. Understanding exactly how HGT of AMR genes occurs is therefore imperative. Understanding the gene pathways via which transformation, conjugation, transduction and gene loss are controlled could allow us to identify potential treatment targets to decrease the incidence of AMR within patients and increase the effectiveness of antibiotic treatment.

Staphylococcus aureus is a gram-positive bacteria and common commensal of the human nasal tract and skin. Methicillin-resistant *S. aureus* (MRSA) is currently one of the greatest problems facing healthcare professionals, endemic to hospital settings and more, recently, the community. Certain strains of MRSA have been showing mounting resistance to other antibiotics through HGT acquisition; identifying the mechanism via which it controls HGT could help prevent its AMR progression. Genes associated with *S. aureus* HGT have already been identified within *S. aureus*. The Sau1 locus has been shown to encode genes for a type I restriction-modification system that restricts gene transfer from *Escherichia coli* (Waldron and Lindsay, 2006). It has been shown, however, that ablation of this system is not sufficient to permit *S. aureus* to accept foreign DNA (Veiga and Pinho, 2009). This suggests that other genes exert control over HGT, the identification of which could shed light on the mechanism via which it is controlled.

Furthermore, the primary mechanism of HGT in *S. aureus* is transduction (Lindsay and Holden, 2006). Recent results indicate that the type of antibiotic as well as the phage responsible for the generalized transduction may influence the rate of HGT, and implied that antibiotics may act as a signal for the initiation of transduction (Stanczak-Mrozek, Laing and Lindsay, 2017). Once again, a study into the genes responsible for the HGT mechanism in *S. aureus* may allow us to inhibit antibiotic-mediated transduction.

The analysis of *S. aureus* genes implicated in the transfer of AMR genes may identify new tools that allow us to insert transgenes via previously unavailable methods, allowing more in-depth research. Furthermore, it may identify potential targets for preventing the

acquisition of AMR genes within an MRSA-infected patient, ultimately slowing the spread of AMR and maintaining patient treatment options.

Question

Are there *S. aureus* genes implicated in the mechanism of horizontal gene transfer that can be identified, functionally analysed and manipulated in order to generate new tools for genome manipulation, or treatments for preventing antimicrobial resistance incidence in a patient?

Methodology

1. Identification

I plan to first analyse for genes associated with HGT in *S. aureus* using an unpublished assay developed by [removed] at [removed]. Using two clinically isolated strains of *S. aureus* harbouring different antimicrobial resistance genes (such as *eryR* and *tetR*), I will grow them in co-culture and analyse the rate at which HGT occurs by identifying the number of double resistant progeny that are produced.

Using the Nebraska Transposon Mutant Library (NTML), I will perform a reverse genetic screen (Bose, Fey and Bayles, 2013). The NTML contains well microtiter plates containing *S. aureus* mutant strains, each with a transposon inserted into one of 1,952 nonessential genes. The transposon contains a restriction enzyme binding site that allows for the insertion of a marker gene, and an erythromycin resistance gene (*eryR*). For each well, I will use the aforementioned assay procedure using the bacteria carrying the *eryR* gene, and another parent harbouring *tetR* and *kanR* as marker genes, that was generated by the [removed] team. These two strains will harbour different AMR genes, and both lack the function of one of the 1,952 nonessential genes. I will co-culture them and analyse the rate of HGT. I then will be able to identify whether loss of a particular gene's function has no effect on HGT, or whether it enhances or reduces it.

2. Functional analysis

Upon identification of associated genes, I will then perform bioinformatic ontology and structural analysis to identify associated pathways and potential ligands to give me initial ideas on the functions of the genes (e.g. QuickGO, PBD and BLAST). Experimental analysis of gene/gene pathway transcriptional changes through RNA-sequencing will also provide more information as to how loss of gene function contributes to HGT rate alteration. Using the knockout bacteria, we can perform phenotypic analysis looking at the rates of growth and metabolic changes in the bacteria. Considering the associated genes will be implicated in controlling HGT, they could act as transcription factors. Identifying whether they contain a helix-turn-helix domain would identify whether they could be transcription factors, in which case performing a ChIP-seq assay may identify downstream effectors. It is possible they be stress response genes, which are inducing increased prophage excision. Looking specifically at the transcription levels of sigma factors such as *sigB* using q-PCR would help clarify. Finally, the associated genes may have a similar function to the Sau1 system, and may degrade foreign DNA, decreasing HGT. Once again, identifying protein domains could help us to identify protein regions with hydrolytic function.

Applicant background

Throughout my studies at [removed], I specialised in cell biology, with a particular focus on infectious disease and molecular biology. This has provided me with a wealth of understanding regarding molecular techniques and bacterial infection. I have experience

working in a molecular laboratory, having undertaken an internship with [removed] at [removed], researching the development and function of innate lymphoid cells with particular focus on ILC2s. I have also been working more recently with [removed], analysing the impact of the microbiota and innate immune system on the progression of the neurodegenerative disease, Gaucher disease, in *Drosophila*. This has involved a great degree of wet lab and molecular work, and has familiarised me with molecular techniques, most applicably q-PCR. This research has been fascinating, but I would much rather turn my focus back to cell and infection research. Considering the failing of antibiotics, and the burden infectious disease has on the developing world, I would like to direct my research towards improving existing infection treatments. The opportunity to study genes associated with AMR would be just that. The opportunity to potentially identify bacterial genes that could one day aid in the fight against the spread of AMR, as well as improving patient prognosis. Furthermore, identifying the genes and gene function is step one. Further research would be required to utilise them efficiently. If I were to be accepted onto this studentship, I would relish the chance to help develop the findings into a treatment mechanism or genetic tool.

References

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