CFWA TOP UP SCHOLARSHIP 2021

Final Report

PROJECT TITLE: Studying How *Pseudomonas aeruginosa* Becomes Resistant to Phage Therapy to Identify How to Prevent it Occurring

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The increase in antimicrobial resistant (AMR) bacterial infections is one of the greatest threats to healthcare today. *Pseudomonas aeruginosa* is one of the deadliest AMR pathogens and there are few antibiotics being developed to continue to treat it. This will disproportionately affect those living with cystic fibrosis (CF) who require multiple antibiotic courses over their lifetime to prevent *P. aeruginosa* infection and subsequent lung damage. A promising alternative therapy uses viruses (bacteriophages or phages) that target bacteria, and work conducted has shown their effectiveness against various bacteria including *P. aeruginosa*. However, these bacteria can become resistant to this therapy and so to prevent this from occurring, phages have been combined with antibiotics. The development of phage resistance has been poorly studied in *P. aeruginosa* that have been isolated from infections in people with CF. Therefore, the aim of this study was to investigate phage resistance in CF clinical isolates of *P. aeruginosa* to inform a combination therapy that could prevent resistance occurring.

Initially, over 250 *P. aeruginosa* phages were isolated from wastewater. These were tested for activity against 94 *P. aeruginosa* isolates from both people with CF and other infection settings. The DNA of the phages with the greatest range against the panel of *P. aeruginosa* was extracted and sequenced. The genes within each of these phage's DNA were analysed and it was found that each of the 20 phages could be applicable for therapy. Four phages were chosen to represent the diversity observed within the top 20 phages' DNA isolated. These four phages (Kara-mokiny 8, 13, 16 and Boorn-mokiny 1) were further characterised to determine their stability at different temperature and pHs and to elucidate what part of the bacteria they attach to when initially infecting. The characteristics displayed by these phages made them ideal candidates for further investigation.

The four phages were then used treat three isolates of *P. aeruginosa* (M1C79, AST154 and AST234). Isolate M1C79 was isolated from a child with CF and is still susceptible to all commonly used antibiotics. AST154 and AST234 were both taken from adults with CF and were resistant to most or half the commonly used antibiotics respectively. The phages were used to treat each of the three *P. aeruginosa* isolates at different doses and bacterial growth tracked over 24 hours. In most cases bacteria were initially killed by the phages before eventually re-growing when they had become resistant. The observed killing and eventual resistance was not affected by the dose of phage used. Surviving bacteria were isolated after 24 hours of phage treatment and their DNA extracted and sequenced. Analysis of the bacterial genome revealed that genes that control the creation of surface components were altered. These surface components are recognised by the phages, allowing their attachment and subsequent infection. Therefore, by altering these components bacteria prevent phage attachment. One gene (*rfaB*) was commonly mutated in response to multiple phage treatment and in different bacterial isolates. This gene has only been identified as involved in phage resistance in one previous study and justifies detailed investigation in the future. Due to phage resistance often affecting other bacterial traits, antibiotic resistance was also investigated. The phage resistant *P. aeruginosa* were tested for resistance to four antibiotics commonly used to treat people with CF. From this, it was observed that the phage-resistant *P. aeruginosa* was more susceptible to tobramycin when compared to non-phage treated counterparts. Thus, combination of tobramycin with phages was investigated for its ability to prevent resistance.

Specifically, Kara-mokiny 16 phage and tobramycin were mixed at different concentrations and used to treat the AST154 in a preliminary experiment to determine the best combination of doses to use. There was greatest effect at 2 μg/mL of tobramycin with 10^6 or 10^7 PFU/mL of Kara-mokiny 16. These combinations were then used to measure the suppression of resistance with mixed effects. There was some prevention of resistance when these combinations were used to treat AST234 and M1C79. However, when used to treat AST154 the combination did not perform any better to either tobramycin or Kara-mokiny 16 alone. Results generated suggest that futher optimisation of the combination treatment is needed. To assess safety of the phage combination, human airway cells were grown for 28 days so they had structures and cell types resembling the human airway. Kara-mokiny 16 and tobramycin alone and in combination were then added to the cells for 24 hours before inflammatory signals, toxicity and gross changes to the cells were investigated. Overall, treatments were well tolerated as there was no increase in inflammation, toxicity or detrimental changes to the cells' appearance.

Overall, this project has added to the field of research in the area, specifically phage resistance to *P. aeruginosa* derived from people with CF. Specifically, it identified that the development of resistance differed compared to *P. aeruginosa* from other clinical settings and identified a potential combination treatment that prevented phage resistance developing. Furthermore, the combination treatment was found to be safe using preclinical human airway models.