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Making Antibiotics Great Again: Phage Resistance In Vivo Correlates to Resensitvity to Antibiotics in Pan-Resistant *Pseudomonas aeruginosa*

Introduction

The emergence of antibiotic resistance is undermining modern medicine. The problem is compounded by the inherent adaptability of bacteria in the face of intense selective pressures. Phages are viruses that infect bacteria. They are diverse, evolvable, and have been proposed as a possible solution for this crisis. Phage therapy or the use of phages for treatment of infections, has a long-standing history in Eastern Europe. In the West, since 2007 the group at the Queen Astrid Military Hospital (QAMH) in Brussels, Belgium has been treating patients with phage therapy utilizing protocols based on the experiences of the George Eliava Institute of Bacteriophages. A retrospective analysis was performed on a 100 of these cases which included an assessment of phage-resistant bacteria isolated from some of these patient cases.

Methods

Our lab received 21 bacterial isolates from 5 patient cases with varied infections, but all caused by the pathogen *Pseudomonas aeruginosa*. Whole genome sequencing was performed on these isolates combining long-read (Illumina) and short read technology (Nanopore) in order to determine genetic changes (e.g. single-nucleotide polymorphism (SNP) and insertions or deletions) related to phage resistance. In order to assess the phenotype of these resistant isolates an analysis was also performed that included virulence determination using the *Galleria mellonella* model system and MIC (minimal inhibitory concentration) assays.

Results

Analysis of sequencing results show changes in phage-resistant isolates recovered from patients correlates with changes in known phage receptors. These included receptors of virulence factors in *P. aeruginosa* such as type IV pili (T4P) and lipopolysaccharide (LPS). Other important changes we noted include a phage resistor with a SNP in a gene that encodes a critical component of the MexAB-OprM efflux pump. For this case in particular we see changes associated with resensitvity of pan-resistant bacteria to antibiotics which was demonstrated in patient antibiogram results as well as in the MIC assays we performed. Moreover, further fitness costs were observed when resistors were isolated from patients that received phages that target multiple receptor types.

Discussion

Phage resistance can occur in patient cases of phage therapy as observed in these 5 patient cases. Nevertheless, effective strategies can be devised to ensure successful treatment despite this challenge. One such approach involves the use of phage and antibiotics synergistically, which has shown promise in addressing resistant and difficult-to-treat infections. In order to implement this strategy effectively, it is crucial to evaluate the phage receptors and assess fitness costs related to phage resistance.