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In Vitro Activity of Bacteriophage Cocktail Tested Against *Pseudomonas aeruginosa* Cystic Fibrosis Isolates

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Introduction

- *P. aeruginosa* can persist within cystic fibrosis (CF) infections due to a host of antibiotic resistance mechanisms including efflux pumps, reduced permeability and over-expression of intrinsically encoded β-lactamase genes.
- The emergence of multidrug resistance in *P. aeruginosa* highlights the need for additional therapeutic options.
- Bacteriophages are bacterial viruses that invade bacterial cells via specific receptors, regardless of antimicrobial susceptibility, and might cause bacterial lysis.
- We evaluated the *in vitro* activity of a bacteriophage cocktail against a collection of *P. aeruginosa* isolates collected from CF and non-cystic fibrosis bronchiectasis (NCFB).

Materials and Methods

- A total of 80 *P. aeruginosa* isolates were collected from patients with CF or NCFB as part of SENTRY Antimicrobial Surveillance Program were used in this study.
- Isolates were tested by CLSI broth microdilution method and separated into multidrug resistant (MDR; resistant to ≥3 antimicrobial classes) and non-MDR groups.
- Bacteriophage cocktail was prepared fresh on the day of testing by combining each individual lysate in an equal ratio.
- Spot assays were performed for each isolate using a double-layer soft agar method and spotting a dilution series of the bacteriophage cocktail as 5 µL spots.
  - Activity in the spot assay was determined by presence of countable plaques or zones of lysis.
- Each isolate was evaluated in a kinetic culture lysis assay at three multiplicities of infection (MOIs) with growth monitored using OD_{600}.
  - Activity in the lysis assay was determined if ≥50% inhibition was observed at 10 hours.

Results

- Overall, the phage cocktail was active against 31/80 (38.8%) of the isolates in the spot assay and 65/80 (81.3%) of the isolates in the culture lysis assay.
- There was an observable difference in the percentage of isolates sensitive to the phage cocktail in the spot assay between the various groups, but not in the lysis assay:
  - Six of 31 (19.4%) CF MDR isolates (19.4%) were sensitive compared to 14/34 (41.2%) of CF non-MDR isolates.
  - Six of 9 (66.7%) NCFB MDR isolates (66.7%) were sensitive compared to 5/6 (83.3%) of NCFB non-MDR isolates.
  - Twenty-five of 31 (80.6%) CF MDR isolates (80.6%) were sensitive compared to 25/34 (73.5%) of CF non-MDR isolates at any MOI.
  - All 9 (100%) NCFB MDR isolates (100%) were sensitive along with 6/6 (100%) of NCFB non-MDR isolates at any MOI.
- Activity of the phage cocktail in the culture lysis assay increased with MOI:
  - 39/80 (48.8%) at MOI 10, 54/80 (67.5%) at MOI 100 and 59/80 (73.8%) at MOI 1000.
- Figure 1 shows a heatmap of the bacteriophage cocktail activity against the entire collection of isolates.

Conclusions

- Phage therapy is an attractive alternative to treat bacterial infections.
- The activity of a bacteriophage cocktail is independent of antimicrobial susceptibility, with similar activity against MDR and non-MDR *P. aeruginosa* isolates collected from CF and NCFB patients.
- Sensitivity in the spot assay demonstrated a high correlation with the culture lysis assay; 30/31 isolates sensitive by spot assay also had activity in the culture lysis assay.
- The culture-based kinetic assay may be a more sensitive assay in evaluating the activity of phage compared to the single end-point spot assay.

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Figure 1. Heatmap of bacteriophage cocktail activity against collection of *P. aeruginosa* clinical isolates