VIRGINIA COMMONWEALTH UNIVERSITY

# Investigating the Efficacy of Phagotherapy in Combating Multi-Resistant Properties Exhibited by *Pseudomonas aeruginosa* Bacteria

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## Abstract |

*Pseudomonas aeruginosa (P. aeruginosa)* is characterized as a gram-negative, rod-shaped bacterium which has the capability to cause various infections in humans including pneumonia, UTIs, and bacteremia. In more recent years, the bacteria exhibited resistance properties to several antibiotics, causing *P. aeruginosa* infections to pose a greater risk for patients. Medical researchers have proposed using bacteria-specific phages as a way of treating multi-resistant *P. aeruginosa* infections. The reliability and effectiveness of phagotherapy must be tested and examined through extensive research prior to its use in healthcare; therefore, it is crucial to begin with an in-depth evaluation of the novel techniques involved in this form of bacterial infection treatment. This literature review will consider the effectiveness of phages on *P. aeruginosa* bacteria to investigate the efficacy of phagotherapy in combating the bacteria's antibiotic resistance. Furthermore, the review will pay special attention to any possible limitations and societal concerns associated with phagotherapy. One study used an artificial sputum-medium (ASM) model to deduce a significant decrease ($p \leq 0.01$) in *P. aeruginosa* bacteria formation 24-hours after phage treatment. Rodent studies were also conducted using a murine model, and the results showed a significant decrease ($p \leq .001$) in symptoms of *P. aeruginosa* infection compared to the control group. With both the ASM and murine model, limited host range can be a potential limitation to phage effectiveness, so it is important to have a sufficient understanding of phage diversity and dynamics to counter these issues. Bacteriophage diversity, effectiveness against their target bacteria, and their growing success in the pre-clinical field are all indicators that phagotherapy can efficiently combat *P. aeruginosa* resistance.

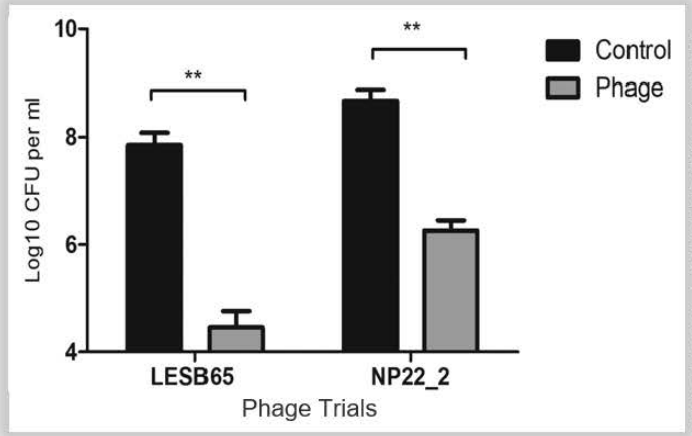


**Figure 1**
In-vitro study modeling the phage activity against *Pseudomonas aeruginosa* in an artificial sputum medium model. The specific bacterial strains used in the study were LESB65 wild type and adapted strain NP22_2. Administration of phage PELP20 (1x108 plaque-forming units) was done 72 hours after the establishment of P. aeruginosa mature biofilm in the ASM model. The figure shows colony-forming units (CFU) at 24 hours post-phage administration. The significant differences presented in the data were determined using a two-way analysis of variance (**p≤0.01). Error bars indicate the standard error of the mean (SEM) in the study, where there were 6 biological replicates and the mean values were taken from triplicate samples. The data presents no significant difference in fold reduction after phage treatment as well.

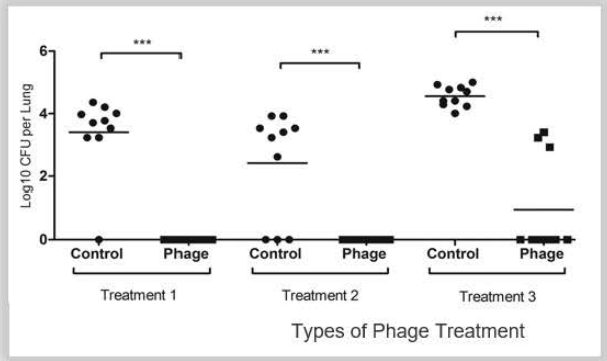


**Figure 2**
In-vivo study modeling phage activity against *Pseudomonas aeruginosa* bacterial infection in a murine lung. The specific bacterial strains used in the study were LESB65 wild type, and adapted strain NP22_2. The figure shows the colony-forming units (log CFU per lung) present per mice lung following intranasal infection with LESB65 (used in treatments 1 and 2) and NP22_2 (treatment 3). The mice were randomly assigned to a group and were then treated with phage PELP20 or phosphate-buttered saline, which was used for the control group. The mice were treated with the page using one of three treatment protocols: (1) the phage was administered at 24 and 36 hours post-infection, with bacterial CFUs calculated at 48 hours; (2) the phage was administered at 48 and 60 hours post infection, with bacterial CFUs calculated at 72 hours; and (3) phage administered at 144 and 156 hours post infection, with bacterial CFUs calculated at 168 hours. The significant differences presented in the data were determined using a two-way analysis of variance and Bonferroni's multiple comparison test. The asterisk represents the post-hoc analysis conducted (***p≤0.001). The investigators selected the time points for sampling based on a previous study they had conducted.

## Conclusions |

- In both the murine model and the ASM model, there were significant differences in trials where phages were administered
- These differences were further calculated with statistical testing and the low p-value numbers confirm the study's findings
- Both studies present limited-host range against the targeted microbials to be a possible limitation within phagotherapy, so understanding phage diversity and dynamics would be helpful in creating a more effective treatment plan
- The data presented in the figures show the efficacy of using phages as a treatment method in combating antibiotic resistance

## Further Directions |

- Phages are found to have synergistic effects when in combination with another antibiotic treatments, such as chemical antibiotics or other phages (phage cocktails). This allows phage therapy to address the potential limitation of having a narrow-host range by providing a broader spectrum of suitable microbial targets
- Bacteria is very unlikely to develop cross-resistant properties to both antibiotics and phages due to the difference in anti-resistant methods. This makes the combination of phages and antibiotics together a very viable option to combating resistant properties in P. aeruginosa and other bacteria effectively.
- The data indicates that phagotherapy has had immense success in the pre-clinical field, and can effectively combat antibiotic resistance properties in *P. aeruginosa* bacteria

## References |

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