Bacteriophage Treatment of Chronic Multidrug-Resistant *Pseudomonas aeruginosa* Periprosthetic Joint Infection

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INTRODUCTION:

Periprosthetic Joint Infection (PJI) is the most common cause of revision in total knee arthroplasty. PJI and other biofilm implant-associated infections pose a significant challenge in Orthopedic Surgery and medicine. Multidrug-resistant (MDR) *Pseudomonas aeruginosa* is a challenging pathogen in PJI as conventional antibiotic treatments are often ineffective and biofilm formation is prevalent.

Lytic bacteriophages have the potential to be an effective therapy in these difficult-to-treat infections. Case reports have shown phage treatment for conditions such as cystic fibrosis, mycobacterial infections, urinary tract infections, and more. Currently, there are no known randomized control trials of phage therapy, which limits the available knowledge on the effectiveness of this therapy. However, exploring the potential of phage therapy in such cases could be a promising avenue for future research and the development of effective treatment options.

This study assesses bacteriophage therapy to establish clinical control of a chronic multidrug-resistant *P. aeruginosa* PJI.

METHODS:

This is a prospective study of a patient who presented with chronic multidrug-resistant *pseudomonas* infection of an ipsilateral hip, femur, and knee PJI. Initially sensitive to oral antibiotics, the organism developed antibiotic resistance over prolonged treatment with antibiotics and multiple failed surgical interventions. The infection was non-operable given the patient's complex implants and comorbidities, and he had been placed on palliative care after beginning to fail intravenous antibiotics.

Using FDA-expanded access, two separate bacteriophages were used, along with a debridement, antibiotics, and implant retention (DAIR) procedure. The two bacteriophages are PASA16, with a titer of 4.7×10^{10} particle forming units (PFU)/mL, and Φ83, with a titer of 7.5×10^{9} PFU/mL. A total of 9 sets of bacteriophage injections were provided into both the knee and joint space over the course of 26 months.

These bacteriophages were further studied using a *C. elegans* model. *C. elegans* were infected with clinical isolates of *P. aeruginosa* followed by phage treatment. Multiple different strains of *P. aeruginosa* were given with subsequent phage administration, and survival rates were compared to an untreated control group.

To investigate potential phenotypic changes before and after phage therapy, we further characterized *P. aeruginosa* clinical isolates for biofilm formation. Inhibition and eradication assays were performed and concurrently incubated with Φ83 and PASA16 bacteriophages. These incubations were compared to untreated *P. aeruginosa* controls and examined for biofilm formation.

RESULTS:

In the FDA-expanded-access patient, clinical control of PJI was obtained and maintained on ~3-month injections of Φ83 and PASA16 into the joint spaces. The patient continued to culture multidrug-resistant (MDR) *P. aeruginosa* on both hip and knee aspiration cultures but displayed no clinical symptoms (the patient is asymptomatic, ESR/CRP within normal limits). This is a significant improvement to pre-phage therapy.

Significant survival improvement was seen in all *C. elegans* infected with patient-derived *P. aeruginosa* and subsequently treated with PASA16 and Φ83. S1 and S2 strains were cultures obtained from the patient before initial phage treatment. Strain 1 (S1) had 7-day survival rates of 66% and 86% with PASA16 and Φ83, respectively, compared to a 13% survival rate in untreated *C. elegans* ($p < 0.0001$). This trend continues with S2 (73% PASA16 and 80% Φ83 compared to 6.6% untreated) ($p < 0.0001$). The same is true with *C. elegans* inoculated with patient strains obtained after patient had received phage therapy. S8 treated with combined PASA16 and Φ83 had 86% survival compared to 6.2% untreated ($p = 0.0001$).

Biofilm analysis presented significantly reduced biofilm production with phage therapy compared to untreated assays. Significant biofilm reduction was exhibited with patient-derived pre-phage-treated *P. aeruginosa* ($p = 0.0001$), mid-treatment samples ($p = 0.0001$), and post-treatment samples ($p = 0.0001$). Additionally, after allowing biofilm to form uninhibited for 48 hours, treatment with phage therapy exhibited significant biofilm eradication with every stage of patient-

derived *P. aeruginosa* ($p < 0.005$). This quantitative analysis was verified with qualitative analysis through photographic review, further supporting the apparent decrease in biofilm formation in the phage-treated strains.

DISCUSSION AND CONCLUSION: Our study shows that bacteriophage therapy is a possible effective alternative to antibiotics in treating PJI caused by MDR *P. aeruginosa*, with the potential to significantly improve treatment outcomes. After treatment with PASA16 and Φ83, the study patient exhibited clinical recovery from a chronic PJI unresponsive to prior interventions. Significant survival increases were shown in the *C. elegans* Kaplan-Meier survival analysis. Additionally, a significant reduction in biofilm production, a natural hurdle to PJI infections, was seen in *P. aeruginosa* strains treated with PASA16 and Φ83. This study emphasizes the potential of bacteriophage therapy in combating PJI and provides valuable insights into genetic and biofilm-related mechanisms. Further investigation is needed to establish a role for phage therapy in everyday clinical care.