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Performance of a Lytic Bacteriophage Cocktail against Pseudomonas aeruginosa in Conditions That Simulate the Cystic Fibrosis Lung Environment

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Abstract : Objectives: The cystic fibrosis (CF) lung is a unique microbiological niche, wherein harmful bacteria persist for many years despite antibiotic therapy. Pseudomonas aeruginosa (Pa), the major culprit leading to lung decline and increased mortality, thrives in the lungs of patients with CF due to several factors that have been linked with poor antibiotic performance. Our group is investigating alternative therapies including bacteriophage cocktails with which we have previously demonstrated efficacy against planktonic organisms. In this study, we explored the effects of a 4-phage cocktail on Pa grown in two different conditions, intended to mirror the CF lung: a) alongside standard antibiotic treatment in pre-formed biofilms (structures formed by Pa-secreted exopolysaccharides which provide both physical and cell division barriers to antimicrobials and host defenses and b) in an acidic environment postulated to be present in the CF airway due both to the primary defect in bicarbonate secretion and secondary effects of inflammation. Methods: 16 Pa strains from CF patients at the Royal Brompton Hospital were selected based on sensitivity to a) ceftazidime/ tobramycin and b) the phage cocktail in a conventional plaque assay. To assess efficacy of phage in biofilms, 96 well plates with Pa ($5x10^7$ CFU/ ml) were incubated in static conditions, allowing adherent bacterial colonies to form for 24 hr. Ceftazidime and tobramycin (both at 2 × MIC) were added, +/- bacteriophage ($4x10^8$ PFU/mL) for a further 24 hr. Cell viability and biomass were estimated using fluorescent resazurin and crystal violet assays, respectively. To evaluate the effect of pH, strains were grown planktonically in shaking 96 well plates at pH 6.0, 6.6, 7.0 and 7.5 with tobramycin or phage, at varying concentrations. Cell viability was quantified by fluorescent resazurin assay. Results: For the biofilm assay, treatment groups were compared with untreated controls and expressed as percent reduction in cell viability and biomass. Addition of the 4-phage cocktail resulted in a 1.3-fold reduction in cell viability and 1.7-fold reduction in biomass ($p < 0.001$) when compared to standard antibiotic treatment alone. Notably, there was a 50 ± 15% reduction in cell viability and 60 ± 12% reduction in biomass (95% CI) for the 4 biofilms demonstrating the most resistance to antibiotic treatment. 83% of strains tested (n=6) showed decreased bacterial killing by tobramycin at acidic pHs ($p < 0.01$). However, 25% of strains (n=12) showed improved phage killing at acidic pHs ($p < 0.05$), with none showing the pattern of reduced efficacy at acidic pH demonstrated by tobramycin. Conclusion: The 4-phage anti-Pa cocktail tested against Pa performs well in pre-formed biofilms and in acidic environments; two conditions intended to mimic the CF lung. To our knowledge, these are the first data looking at the effects of subtle pH changes on phage-mediated bacterial killing in the context of Pa infection. These findings contribute to a growing body of evidence supporting the use of nebulised lytic bacteriophage as a treatment in the context of lung infection.

Keywords : biofilm, cystic fibrosis, pH, Pseudomonas aeruginosa, lytic bacteriophage
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