

Bacteriophage therapy for the treatment of *P. aeruginosa* infections in cystic fibrosis patients

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INTRODUCTION

Chronic lung infections caused by *Pseudomonas aeruginosa* (PA) are a major cause of morbidity and mortality in cystic fibrosis (CF) patients. In some cases effective antibiotic therapy is no longer available, with multi-drug resistant (MDR) forms of these bacteria becoming increasingly challenging to treat. Thus, new alternative means of controlling MDR PA infections are urgently needed. Bacteriophage (phage) therapy is a potential therapeutic tool for the treatment of bacterial infections. However, due to the specific nature of phages, questions have been raised about the clinical practicality of bacteriophage based products and their ability to be effective against a range of clinical isolates.

We have previously reported in the development of three prototype phage mixes and shown that phages are efficacious in reducing both bacterial load and inflammation in a murine lung infection model [1]. In this study, we have expanded the in vitro testing and developed a bacteriophage mix (AB-PA01) active against relevant clinical PA isolates collected from around the world. In addition, we demonstrated the efficacy of AB-PA01 in vivo in a murine lung infection model.

METHODS

In vitro testing: Lytic bacteriophages were isolated from environmental sources in Australia and England and their activity screened against a reference collection of 67 *P. aeruginosa* from CF patients. A prototype combination of four phages was then developed and tested for its activity against 429 global CF and non-CF clinical isolates collected between 2007 to 2015. Isolates included were both antibiotic susceptible/resistant and mucoid/non-mucoid.

In vivo testing: Immunocompetent CD-1 female mice were inoculated intranasally (IN) with $6.26 \log_{10}$ CFU in 50 μ L of TSB. At 2 hrs post-infection (PI), 50 μ L 4-phage mix was administered IN to three dosage groups (n=5) of mice consisting of 7.5×10^9 , 7.5×10^8 , and 7.5×10^7 PFU/mL per phage (for a total of 1.5×10^9 , 1.5×10^8 , or 1.5×10^7 PFU administered). A second identical dose was administered IN at 6 hrs PI. Meropenem (25 mg/kg) was administered subcutaneously at 2 hrs and 6 hrs PI to a fourth group. A fifth group was infected, but treated with the phage diluent. All mice were euthanized at 24 hrs and CFU/lung pair determined. Statistical analysis was performed using Tukey's multiple comparisons test (Graphpad Prism 6, $p < 0.05$).

RESULTS

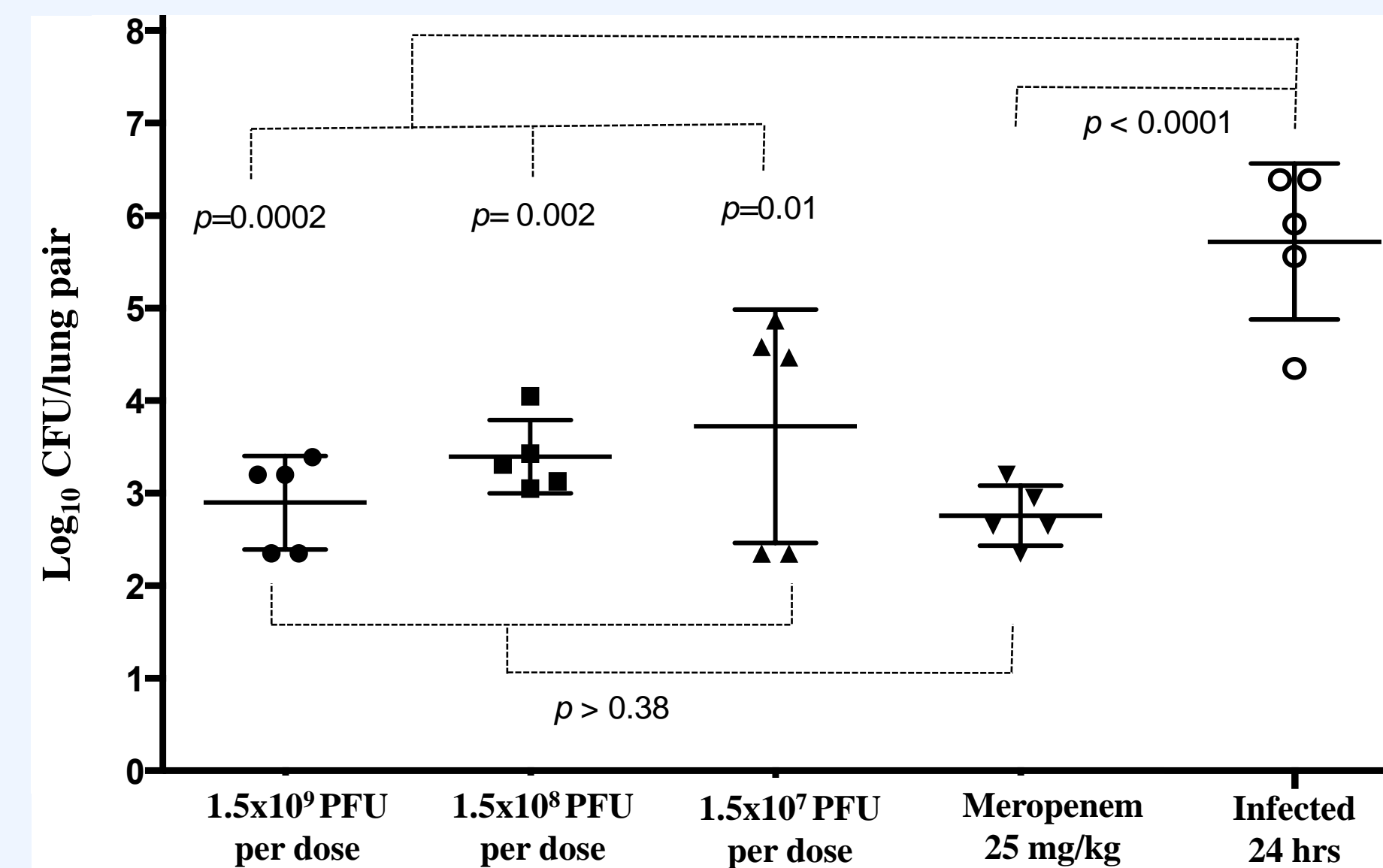
AB-PA01 Phage Mix Host Range

Phages were isolated from a variety of environmental sources in Australia and the UK, using different protocols as previously described [2]. Four phages were selected based on the spectrum of activity against a reference panel of 67 distinct CF isolates, with the number of isolates targeted by ≥ 2 phages considered an important selection criteria. The overall activity of the selected 4-phage mix **AB-PA01** is summarised in Table 1.

Number of Isolates	Area (Year of Isolation)	Type	AB-PA01 % of Activity	% of isolates sensitive to ≥ 2 phages in mix
67*	UK/AU/USA (2012-13)	CF	95.5%	87.5%
120	UK (2012-13)	CF	85.0%	95.0%
60	AU (2007-2013)	CF	93.3%	98.2%
40	USA (2014)	CF	87.5%	91.4%
82	UK (2015)	CF	81.7%	92.5%
60	USA/EU/AU (2013)	Non-CF	83.3%	80.0%
369	Total CF Isolates		87.8%	93.2%
429	Total isolates (CF + Non-CF)		87.2%	91.4%

Table 1 Activity of AB-PA01 against global CF and non-CF *P. aeruginosa* isolates. Phage mix (10^8 PFU/ml) was used to spot onto *P. aeruginosa* lawns. Each strain was considered sensitive if more than 20 plaques were observed. *Reference panel used for phage selection composed of 67 distinct CF strains that included well characterized CF epidemic clones. UK: United Kingdom; AU: Australia; USA: The United States of America. % of activity=[no. sensitive isolates/total no. of isolates tested] x 100

Murine Lung Infection Model



CONCLUSIONS

- Four phages were isolated and combined into an effective prototype phage mix capable of infecting *P. aeruginosa* clinical isolates collected around the world. The developed 4-phage mix was shown to infect both antibiotic susceptible/resistant and mucoid/non-mucoid CF strains.
- This study has shown that AB-PA01 has a broad range of activity addressing concerns that the specificity of phages could make this therapy impractical in the clinical environment. However, it is likely that, like the flu vaccines, these broad spectrum preparations will need to be reformulated overtime as the bacterial populations evolve.
- AB-PA01 administered at the three dosage levels demonstrated efficacy similar to meropenem in a *P. aeruginosa* murine lung model of infection. However, there seems to be a non-significant trend suggesting a possible dose-dependent effect.
- In addition, we have confirmed the usability of AB-PA01 for:
 - ✓ Clinical use (exclusively lytic, efficacious in vivo)
 - ✓ Nebulisation (no significant decreases in titre were observed)
 - ✓ GMP Manufacturing (long-term stability, current process optimisation)

The use of phages as therapeutic tools continues to be a viable option for the treatment of PA infections in CF patients. AmpliPhi Biosciences, in collaboration with the Brompton Hospital, plan to evaluate the safety and efficacy of AB-PA01 in CF patients.

REFERENCES

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