Title: Potential application of bacteriophage therapy as an alternative treatment against *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen responsible for severe healthcare-associated infections and is increasingly associated with multidrug resistance, particularly against carbapenem antibiotics. Biofilm formation by this bacterium further complicates treatment by enhancing bacterial persistence and tolerance to antimicrobial agents. Therefore, alternative therapeutic strategies are urgently required.

Two novel lytic bacteriophages, Phage_Pae01 and phiPA1-3 targeting *P. aeruginosa* was characterized and evaluated their antibacterial and antibiofilm efficacy against multidrug-resistant strains. A novel virulent *P. aeruginosa* phage, Phage_Pae01 has broad-spectrum activity against 83.6% of clinical isolates, including multidrug-resistant and carbapenem-resistant strains. It belongs to *Myoviridae* family. Phage_Pae01 remained stable at 4–60°C and pH 4–10, with optimal lytic activity at an MOI of 0.01. The 93,182 bp genome contains 176 ORFs and lacks virulence or antibiotic resistance genes. Phage_Pae01 effectively prevented biofilm formation, eliminated established biofilms, and when combined with gentamicin, significantly enhanced biofilm disruption. Bacteriophage phiPA1-3 is a moderate host-range lytic phage capable of infecting 20% of clinical *P. aeruginosa* isolates, including carbapenem-resistant strains (CRPA). The phage is stable between pH 7.0 and 9.0. At 37°C, phiPA1-3 has a burst size of 619 PFU/cell and exhibits lytic activity dependent on the multiplicity of infection (MOI). Its genome is 73,402 bp. (54.7% G+C content) and encodes 93 open reading frames (ORFs), 31 of which have known functions. Phage phiPA1-3 possesses hallmarks of N4-like phages, including three RNA polymerases, leading to its classification within the N4-like *Schitoviridae* family. Based on these characteristics, phiPA1-3 shows potential for controlling CRPA infections by eradicating *P. aeruginosa* biofilms.

In conclusion, the results demonstrate that the lytic bacteriophage treatment effectively controls both planktonic growth and biofilm-associated populations of multidrug-resistant *P. aeruginosa*. These results support the potential application of phages as powerful alternative or adjunct antimicrobial agents in the clinical management of chronic and drug-resistant infections.

Reference:

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