

Targeting *Klebsiella pneumoniae* Biofilms With Depolymerase-Encoding Bacteriophages: Functional and Genomic Characterization

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Biofilm formation is a major driver of antimicrobial resistance and persistent infections, particularly in medical device-associated settings. These structures protect bacteria from antibiotics and host immune responses, underscoring the urgent need for alternative strategies against high-priority pathogens such as *Klebsiella pneumoniae*. This study investigated the antibiofilm potential of bacteriophages isolated against *K. pneumoniae*. Antibiofilm activity was evaluated in vitro in both early-stage formation and mature biofilms, including clinically relevant assays on hospital catheter surfaces. Phages K23, K75, and K79 effectively inhibited biofilm formation, while K23 and K79 also disrupted established biofilms. Notably, phage pre-treatment of catheter surfaces reduced bacterial adhesion, demonstrating potential for preventing device-associated infections. Phage stability under varying environmental conditions was assessed, revealing sustained lytic activity across a broad range of temperature and pH, indicating high robustness for potential clinical application. Genetic analysis confirmed the DNA nature of the phages. Given its superior antibiofilm performance, phage K23 was selected for genome sequencing. Bioinformatic analysis identified genes encoding proteins with glycosyl hydrolase domains consistent with depolymerase activity, suggesting a mechanistic role in extracellular matrix degradation and biofilm disruption. These findings position bacteriophages as promising therapeutic agents and a source of antibiofilm enzymes, supporting their development as innovative strategies to combat biofilm-associated infections. Future studies will focus on the heterologous expression and purification of K23-derived depolymerases as adjunctive therapies.

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