Title: A Three-Phage Combination Effectively Controls a Clinical High-Risk *Klebsiella pneumoniae* ST307 Isolate Through Complementary Killing and Evolutionary Trapping

Guanwen HUANG*, Subhankar MUKHOPADHYAY, and Hein Min TUN
System Microbiology and Antimicrobial Resistance (SMART) Lab, Li Ka Shing Institute of Health Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
The Jockey Club School of Public Health and Primary Care, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

Keywords: Bacteriophage therapy; Antimicrobial resistance (AMR); *Klebsiella pneumoniae*; Phage cocktail; Evolutionary trapping; Carbapenem-resistant Enterobacterales (CRE)

Background: The 2024 WHO priority pathogens list ranks carbapenem-resistant *Klebsiella pneumoniae* (CRKP) as a critical superbug. The CRKP sequence type 307 (ST307) requires alternative therapies. Bacteriophage therapy is promising but often limited by resistance evolution. This study developed a resistance-suppressing phage cocktail using a rational strategy.

Methods: Three novel lytic phages (ΦK-8 [χ-like], ΦDΔ [T5-like], ΦEΔ [T4-like]) were isolated from wastewater against a clinical CRKP ST307 strain (CKP3). Killing efficacy was assessed via time-kill assays (MOI=0.1, 48h). Resistance emergence was monitored with spot tests, and fitness costs were quantified by comparing mutant to wild-type growth.

Results: Monotherapy with ΦK-8, ΦDΔ, or ΦEΔ against CKP3 resulted in bacterial regrowth after 12, 4, and 4 hours, respectively. In contrast, the triple combination achieved near-complete killing within 4 hours and suppressed regrowth for the entire 48-hour period. Resistance profiling showed that ΦK-8 selected for fit, genotypically resistant mutants (50% of isolates by 12h). Besides, ΦDΔ/ΦEΔ selected for phenotypic adaptations with fitness costs, reducing stationary phase density by 30% and 50%, respectively. While the cocktail selected for some single-phage resistant strains (~20% by 36h), no strains were observed to be resistant to all three phages. With these evidence, the killing efficacy could be hypothesized as an evolutionary trap where ΦK-8 kills mutants adapted to ΦDΔ/ΦEΔ, and ΦDΔ/ΦEΔ eliminates ΦK-8-resistant mutants.

Conclusion: We demonstrated that a rationally designed phage cocktail can achieve sustained killing of a high-priority CRKP ST307 strain by exploiting complementary resistance trade-offs. Future work will elucidate the molecular synergy and validate this approach to direct phage therapy development.

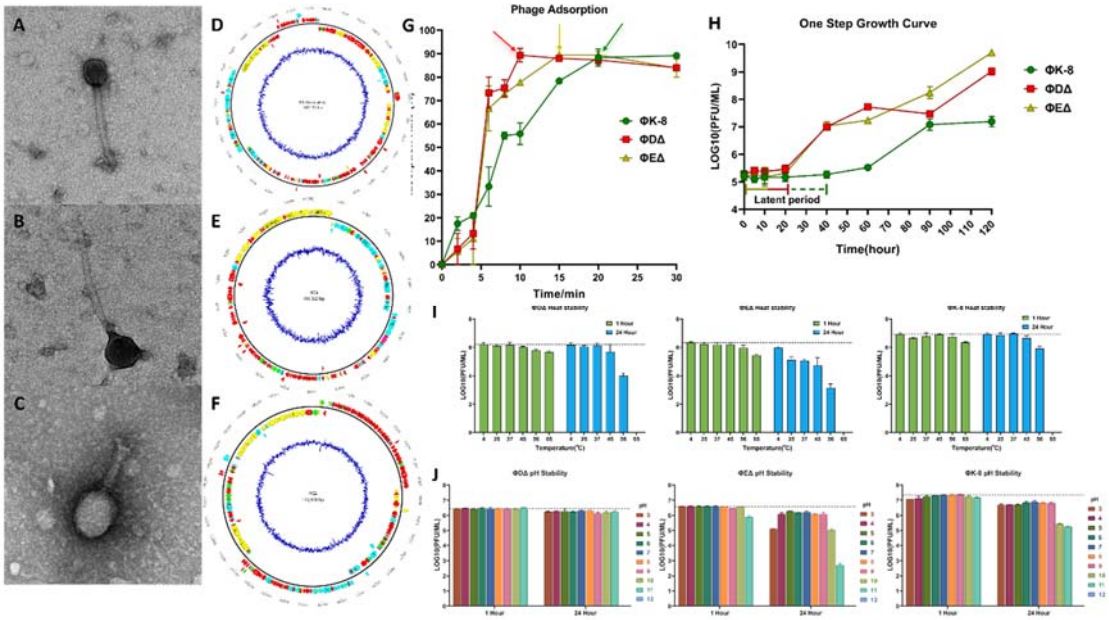

Fig 1. Isolation and characterization of three novel bacteriophages against *Klebsiella pneumoniae* ST307.

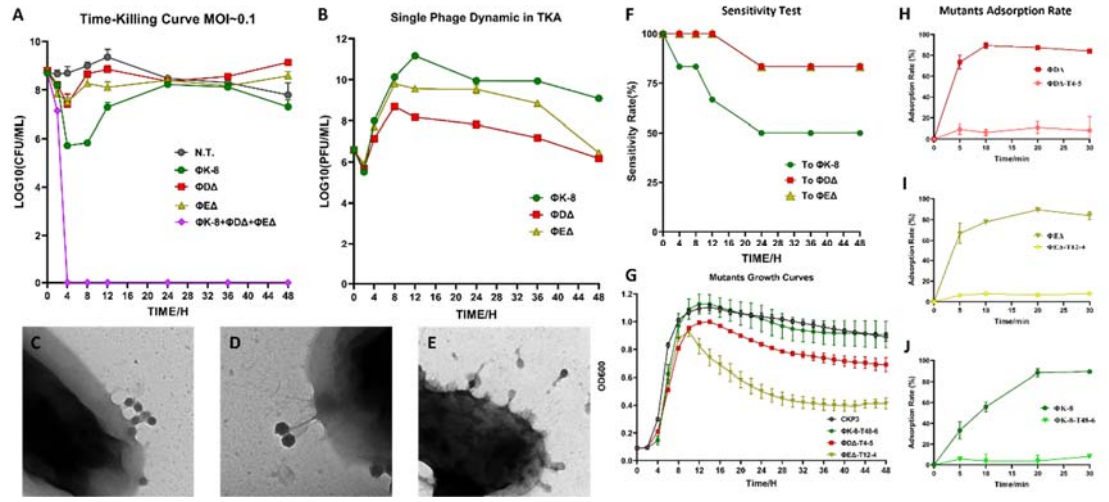

Fig 2. A synergistic three-phage cocktail achieves sustained bactericidal activity by constraining resistance evolution.