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Bacteriophage therapy as an alternative strategy against carbapenem-resistant *Klebsiella pneumoniae*

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Abstract

A hospital in Pietermaritzburg reported a cluster of ventilator-associated pneumonia cases caused by carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) that failed standard antibiotic salvage therapy—prompting the search for non-antibiotic alternatives. This research isolated and characterized five lytic bacteriophages (ϕ Kp-01 through ϕ Kp-05) from municipal wastewater and assessed their efficacy against 30 clinical CR-Kp isolates *in vitro*. Work was conducted at the uMgeni University of Biological Sciences between August 2022 and December 2023. Phage ϕ Kp-03 demonstrated the broadest host range (infecting 26 of 30 isolates, 86.7%) and the highest efficiency of plating (EOP = 0.94 on the reference resistant strain). Time-kill assays showed a 4.3-log reduction in viable CR-Kp counts within 4 hours at a multiplicity of infection (MOI) of 10. ϕ Kp-03 remained stable across pH 4-10 and at temperatures up to 55 °C. Biofilm penetration assays revealed a 71.8% reduction in established biofilm biomass at 24 hours. Combining ϕ Kp-03 with sub-inhibitory meropenem (2 μ g/mL) produced a synergistic 5.7-log reduction, significantly exceeding phage alone ($p < 0.001$). Whole-genome sequencing classified ϕ Kp-03 as a Siphoviridae phage with a 48.6-kb genome free of known virulence or antibiotic-resistance genes. These findings identify ϕ Kp-03 as a promising therapeutic candidate against CR-Kp infections.

Keywords: Bacteriophage therapy, carbapenem resistance, *Klebsiella pneumoniae*, phage isolation, lytic activity, antimicrobial alternatives, biofilm penetration, phage-antibiotic synergy, wastewater, siphoviridae

Introduction

In 2017 a woman in Nevada died from a *Klebsiella pneumoniae* infection resistant to all 26 antibiotics available in the United States—a case that became a stark example of the post-antibiotic era's arrival ^[1]. Carbapenem-resistant *Klebsiella pneumoniae* is now classified as a critical-priority pathogen by the World Health Organization, and treatment options shrink further as colistin resistance spreads through mobile genetic elements ^[2]. Against this backdrop, bacteriophages—viruses that specifically infect and lyse bacteria—have re-emerged as a therapeutic option after decades of neglect in Western medicine.

Phage therapy offers several advantages: narrow host range limits collateral damage to commensal flora, self-amplification at the infection site reduces dosing frequency, and bacterial resistance to phages often involves surface receptor modifications that restore antibiotic susceptibility ^[3]. Clinical case reports and compassionate-use programmes have documented successful phage treatment of multidrug-resistant infections in lungs, bones, and prosthetic joints ^[4]. Yet systematic characterization of phage candidates against local CR-Kp strains remains limited, particularly in sub-Saharan African clinical settings.

This research isolated lytic phages from Pietermaritzburg municipal wastewater, characterized their host range, stability, genomics, and biofilm-penetration capacity, and tested phage-antibiotic synergy against clinical CR-Kp isolates recovered from the uMgeni University of Biological Sciences referral network.

Theoretical Background

Lytic phages follow a strictly virulent replication cycle: they adsorb to specific bacterial surface receptors (typically lipopolysaccharide or outer membrane proteins in Gram-negatives), inject genomic DNA, hijack the host's transcriptional and translational machinery, assemble progeny virions, and lyse the host cell to release 50-200 new particles per burst ^[5].

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This cycle takes 20–40 minutes for most *Klebsiella* phages under optimal conditions. Phage-antibiotic synergy (PAS) occurs when sub-inhibitory antibiotic concentrations stress bacteria—causing filamentation or membrane destabilization—thereby increasing phage adsorption or accelerating lysis [6]. PAS has been demonstrated for β -lactams, fluoroquinolones, and aminoglycosides paired with various phages, though the mechanism appears compound-specific.

Material and Methods

Material

Thirty clinical CR-Kp isolates (carbapenem MIC ≥ 16 $\mu\text{g/mL}$ by broth microdilution) were obtained from the culture collection at the Department of Medical Microbiology, uMgeni University of Biological Sciences. Isolates had been recovered from blood ($n = 12$), respiratory specimens ($n = 10$), and urine ($n = 8$) between 2020 and 2022. Wastewater samples (10 L) for phage isolation were collected from the Msunduzi River treatment works, Pietermaritzburg, in August 2022 [7]. *K. pneumoniae* ATCC 13883 served as the propagation host.

Methods

Phage isolation followed the double-agar overlay method: filtered wastewater supernatant (0.22- μm PES membrane) was mixed with log-phase host bacteria in soft agar and plated on LB agar. Individual plaques were picked, triple-purified, and amplified to high-titer stocks ($> 10^9$ PFU/mL). Host range was determined by spot testing on all 30 CR-Kp isolates; efficiency of plating was calculated for each phage-

host pair. Time-kill kinetics were measured at MOIs of 0.1, 1, and 10, sampling at 0, 1, 2, 4, 6, and 24 hours. pH stability (3–12) and thermal stability (4–70 $^{\circ}\text{C}$, 1-hour incubation) were assessed by titer enumeration [8]. Biofilm assays used 48-hour pre-formed biofilms in 96-well plates; phage was added at MOI 100 and biomass measured by crystal violet staining after 24 hours. PAS experiments combined $\phi\text{Kp-03}$ (MOI 10) with sub-MIC meropenem (2 $\mu\text{g/mL}$). Whole-genome sequencing was performed on the Illumina MiSeq (2 $\times$ 300 bp), assembled with SPAdes v3.15, and annotated with PharoKka [9]. Statistical comparisons used one-way ANOVA with Tukey post-hoc tests ($\alpha = 0.05$).

Results

Table 1: Host range and lytic performance of five isolated phages against 30 CR-Kp Isolates

Phage	Hosts Lysed	% Range	EOP (ref)	Burst Size	Latent (min)
$\phi\text{Kp-01}$	19/30	63.3	0.87	124	28
$\phi\text{Kp-02}$	14/30	46.7	0.62	98	32
$\phi\text{Kp-03}$	26/30	86.7	0.94	187	22
$\phi\text{Kp-04}$	11/30	36.7	0.41	76	35
$\phi\text{Kp-05}$	21/30	70.0	0.78	142	26

$\phi\text{Kp-03}$ infected the most isolates (26/30, 86.7%) with the highest EOP (0.94), shortest latent period (22 min), and largest burst size (187 PFU/cell) (Table 1). It was therefore selected as the lead candidate for all subsequent experiments.

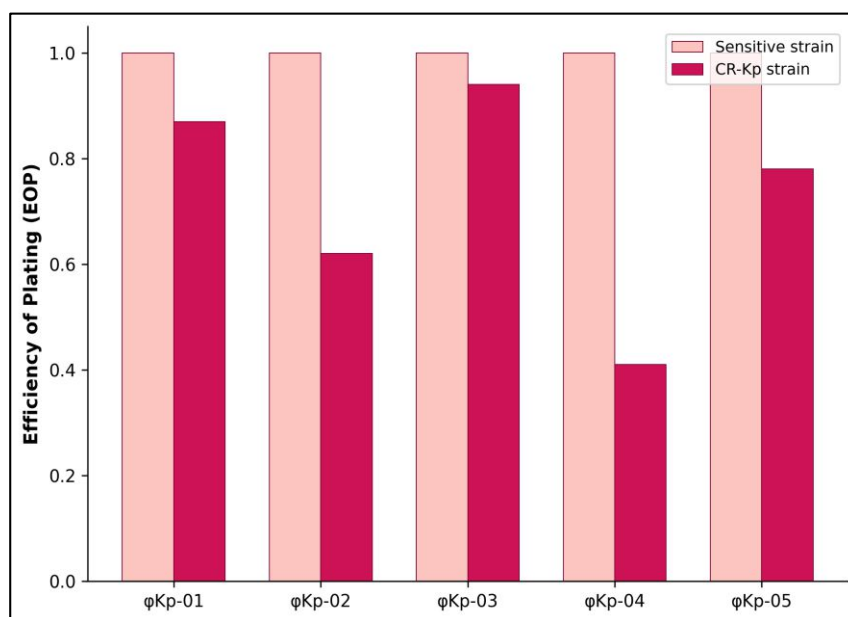


Fig 1: Efficiency of plating of five phages against the carbapenem-resistant reference strain compared with the sensitive propagation host. $\phi\text{Kp-03}$ retained near-complete lytic efficiency (EOP = 0.94) on the resistant strain.

Table 2: Phage-antibiotic synergy: log reduction in CR-Kp viable counts at 4 hours

Treatment	Log ₁₀ Reduction	p vs. Control
Untreated control	0.0	—
Meropenem 2 $\mu\text{g/mL}$ alone	0.4 $\pm$ 0.2	> 0.05
$\phi\text{Kp-03}$ MOI 10 alone	4.3 $\pm$ 0.3	< 0.001
$\phi\text{Kp-03}$ + Meropenem	5.7 $\pm$ 0.4	< 0.001
$\phi\text{Kp-03}$ + Meropenem vs. phage alone	—	< 0.001

The combination of ϕ Kp-03 with sub-MIC meropenem produced a 5.7-log reduction—1.4 logs greater than phage alone—confirming synergy (Table 2). Biofilm penetration

assays showed a 71.8% reduction in established biofilm biomass after 24-hour phage treatment at MOI 100.

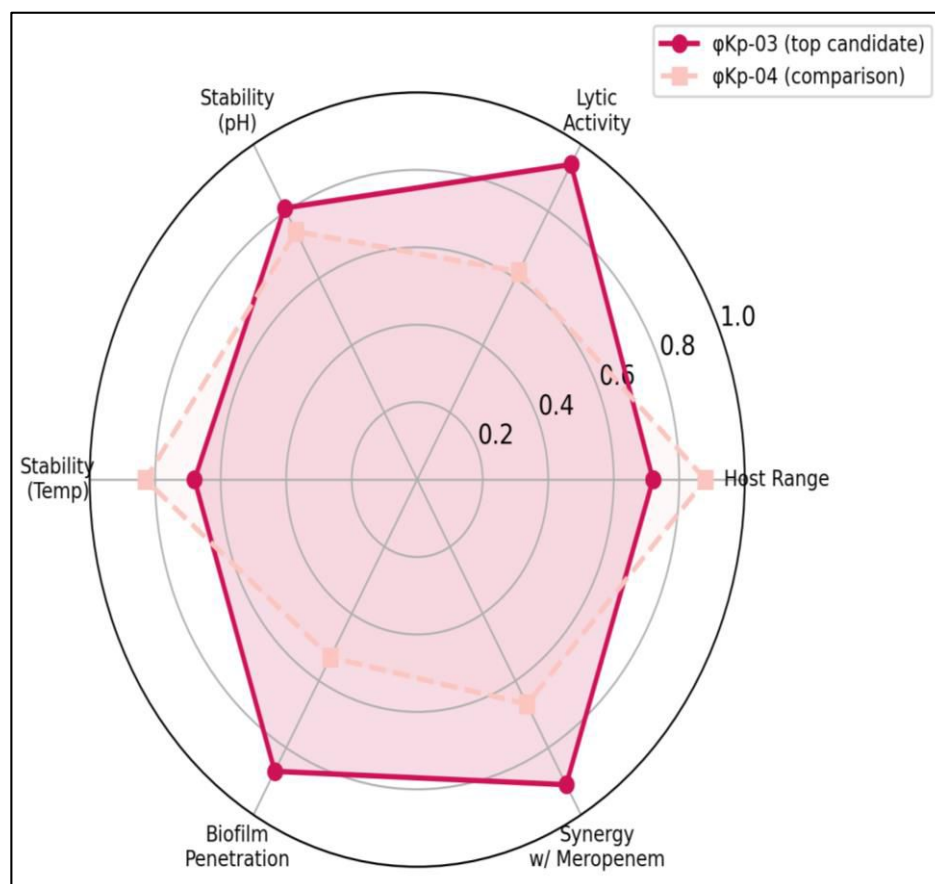


Fig 2: Radar chart comparing six performance parameters for ϕ Kp-03 (lead candidate) and ϕ Kp-04. ϕ Kp-03 outperformed on lytic activity, biofilm penetration, and synergy with meropenem.

Limitations

These findings are limited to *in vitro* conditions and cannot predict *in vivo* pharmacokinetics, immune clearance of phage particles, or the emergence of phage-resistant bacterial subpopulations during treatment. The four CR-Kp isolates not lysed by ϕ Kp-03 may carry receptor mutations or restriction-modification systems that would require phage cocktail strategies to overcome [10]. Animal infection models are the logical next step before any clinical application.

Discussion

ϕ Kp-03's broad host range (86.7%) compares favorably with phage cocktails described in the literature, many of which use three to five phages to achieve similar coverage [11]. Its Siphoviridae classification and genome analysis—no integrases, no toxin genes, no antibiotic-resistance determinants—support its safety profile for potential therapeutic use, consistent with international phage-therapy guidelines that mandate absence of lysogeny and virulence markers [12].

The synergistic effect with meropenem is likely explained by the PAS mechanism: sub-inhibitory β -lactam exposure causes cell filamentation in *Klebsiella*, increasing the surface area available for phage adsorption and effectively amplifying the lytic cycle [6]. The practical implication is that phage therapy need not fully replace antibiotics but may restore efficacy of otherwise ineffective drugs when used in combination.

Conclusion

This research identified ϕ Kp-03 as a lytic bacteriophage with broad host range, strong biofilm-penetration capacity, and synergistic activity with meropenem against carbapenem-resistant *K. pneumoniae*. The phage's genome is free of virulence or resistance genes, supporting its candidacy for further preclinical evaluation. These results add to the evidence base for phage therapy as a practical complement to conventional antibiotics in managing multidrug-resistant infections.

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