



Therapeutic Potential of Intravenous Phage as Standalone Therapy for Recurrent Drug-Resistant Urinary Tract Infections

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ABSTRACT Recurrent urinary tract infections (rUTI) are common in kidney transplant recipients (KTR) and are associated with multidrug resistance and increased morbidity/mortality. Novel antibiotic alternatives to reduce UTI recurrence are critically needed. We describe a case of rUTI due to extended spectrum beta lactamase (ESBL) *Klebsiella pneumoniae* in a KTR that was treated successfully with 4 weeks of adjunctive intravenous bacteriophage therapy alone, without concomitant antibiotics, and with no recurrence in a year of follow-up.

KEYWORDS phage, rUTI, kidney transplant, bacteriophage, ESBL

Urinary tract infections (UTIs) occur frequently in kidney transplant recipients (KTR), adversely impact allograft function, are associated with multidrug resistance (MDR), and cause significant morbidity (1–5). Recurrent UTI (rUTI) is common in KTRs and are related to persistent nidus and/or gut and urinary biome persistence (6, 7). Antibiotic treatment of asymptomatic bacteriuria does not prevent future recurrence (2), and alternatives are critically needed.

There is increasing interest in using lytic bacteriophages (phage) for the treatment of MDR infections, though they are generally used with concomitant antibiotics (8–12). Two recent KTR cases suggest efficacy in the eradication of MDR pathogens, although effect of the phage was unclear, as it was used with concomitant antibiotics (13, 14). In a third case, the patient underwent native nephrectomy that was thought to be the infection reservoir (15). For the first time, we describe the clinical course of a KTR with a complicated rUTI that was treated with adjunctive phage alone, without concomitant antibiotics.

CASE PRESENTATION

The patient was a 70-year-old female with combined liver/kidney transplantation in March of 2020 for nonalcoholic fatty liver disease and diabetes-related kidney disease. She developed recurrent transplant pyelonephritis, bacteremia, and cystitis, mainly due to *K. pneumoniae* (including from extended spectrum beta lactamase [ESBL] producing isolates) and she required multiple hospital admissions and multiple courses of antibiotics over the next 1.5 years. She did not have a chronic indwelling urinary catheter or a ureteral stent, and she did not require intermittent self-catheterization. She was postmenopausal and maintained on vaginal estrogen. Imaging was negative for

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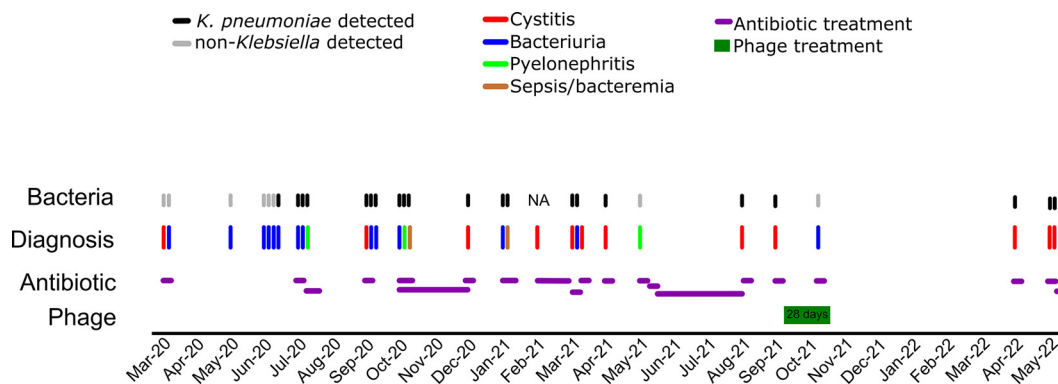


FIG 1 Clinical course and treatment administration. Clinical episode diagnoses from March of 2020 to May of 2022 are represented as colored lines. The microbiology analysis of the urine culture at each clinical episode is indicated as either black (*K. pneumoniae* was identified) or gray (non-*Klebsiella* was identified). The administered antibiotics are shown as orange bars, with the smallest bar width representing a five-day treatment course. The phage treatment lasted for 28 days and is indicated by a green box.

renal calculi, indwelling hardware, or cysts. The patient suffered several episodes of acute kidney injury when admitted with sepsis and bacteremia and eventually developed chronic kidney disease. Her initial immunosuppression consisted of steroid induction that was followed by maintenance with tacrolimus, mycophenolate mofetil, and prednisone. The mycophenolate was changed to sirolimus in April of 2020, and the overall tacrolimus trough goal was reduced from 8 to 10 ng/mL to approximately 5 ng/mL from May of 2020, onwards. The patient had several ESBL *K. pneumoniae* isolates that were variably resistant to ciprofloxacin, fosfomycin, nitrofurantoin, and trimethoprim-sulfamethoxazole, as noted in Table S2.

CHALLENGE QUESTION

How can we reduce the risk of future UTI, infection-associated hospitalization, and further kidney damage in this patient?

1. Long term antibiotic suppression with IV ertapenem (the patient had previously broken through oral suppression with nitrofurantoin and fosfomycin with the development of resistance)
2. Fecal microbiota transplant
3. Chemical prophylaxis with methionine
4. Phage therapy

STRATEGY AND OUTCOME

We treated her with phage in an effort to eradicate ESBL colonization and prevent further infections. As we aimed to eradicate colonization and not treat an acute infection, we used adjunctive phage alone, without concomitant antibiotics (all previously published phage experience in transplant recipients has been with concomitant antibiotics).

A 4-week course of phage was initiated in September of 2021 with a three-phage cocktail that was administered intravenously (IV) twice daily, with regulatory approval from the FDA (single patient eIND 27811) and the local institutional review board (UCSD IRB number 20-0163) as well as signed informed consent.

The clinical course, microbiological results, and treatment (antibiotics or phage) are detailed in Fig. 1 and Table S1. The patient had four symptomatic *K. pneumoniae* UTIs in the 6 months prior to phage initiation and none in the following 6 months. The first *K. pneumoniae* UTI after the phage treatment occurred 206 days after the phage initiation but with no recurrence of ESBL strains by a one-year follow-up. Postphage *K. pneumoniae* infections were susceptible to oral antibiotics, and the patient has not required IV antibiotics since.

TABLE 1 Characteristics of phages included in the treatment cocktail

Phage name	Source	Isolation host	Morphology	Accession number	Genome size (bp)	Total protein-coding genes	ICTV taxonomic subfamily, genus, species ^a
Metamorpho	Texas A&M University	<i>K. aerogenes</i> ATCC 13048	Myophage	MT701588	171,475	287	<i>Tevenvirinae; Jiaodavirus</i>
Mineola	Texas A&M University	<i>K. pneumoniae</i> clinical isolate (ST258)	Myophage	MH333064	166,130	276	<i>Tevenvirinae; Jiaodavirus; Jiaodavirus JD18</i>
pKp20	Monash University	FADDI-KP057	Myophage	OP331213	165,762	271	<i>Tevenvirinae; Jiaodavirus; Klebsiella virus JD18</i>

^aICTV taxonomy placement is based on the nucleotide similarity that was determined via BLASTn against the NCBI nt database.

PHAGE SELECTION

Descriptions of materials, methods, and production are provided in the supplementary appendix. After screening 111 *Klebsiella* phages against 4 ESBL *K. pneumoniae* isolates, 3 lytic phages (Table 1) that demonstrated the effective inhibition of host isolates (Fig. 2) were selected.

PHARMACOKINETICS

Following the first dose, infective phage particles were detectable in the patient’s serum collected at 15 min postinjection, indicating their rapid distribution into the systemic circulation. A gradual decrease in phage titers was observed with a calculated half-life of 0.4 h (Fig. 3A). We did not detect infective phage particles in urine; however, urine collection was not timed to phage administration. We spiked live phage into

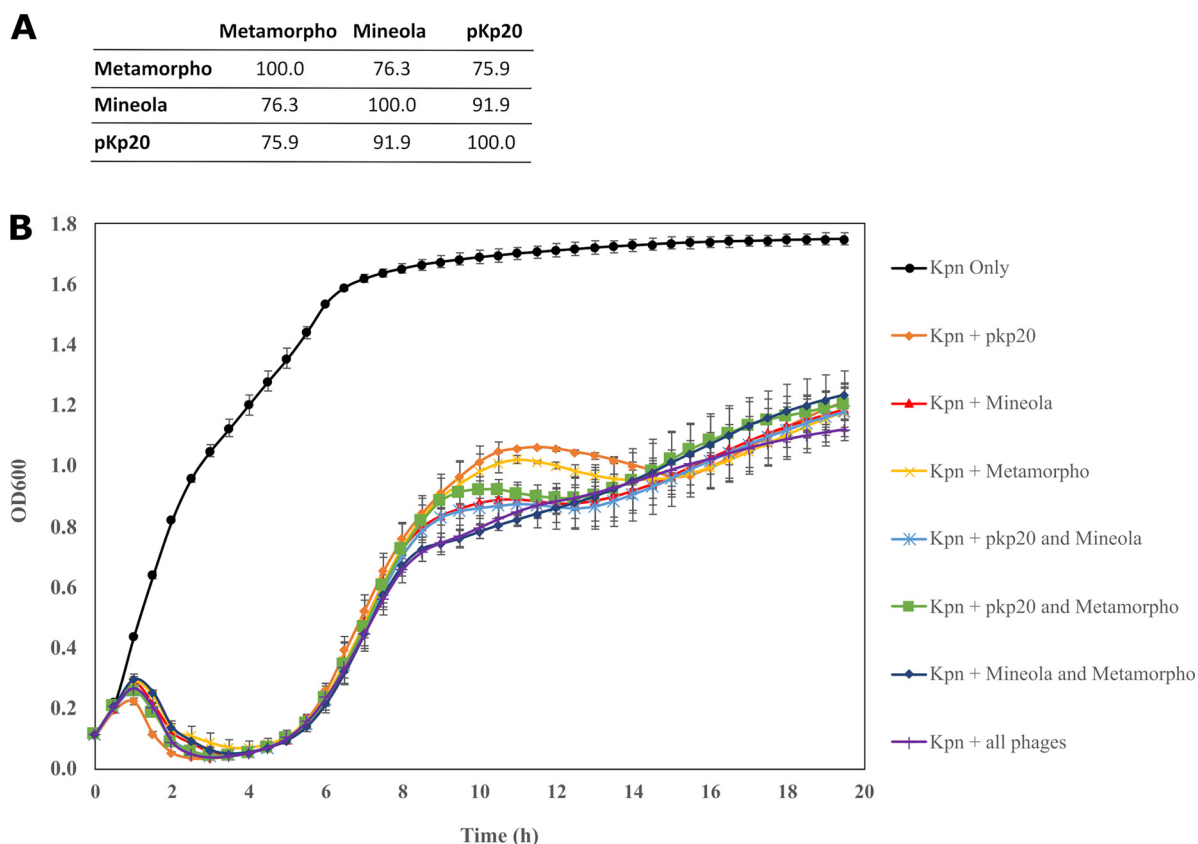


FIG 2 Characterization of individual phages and phage cocktails. (A) DNA sequence relatedness of the three phages, showing the pairwise percent DNA sequence identities, as determined by ProgressiveMauve. (B) Inhibition activity of individual and phage cocktails (of two phages and of three phages) against the patient isolate (from April of 2021) at a multiplicity of inhibition of 0.01 over a 20 h period. The error bars represent the standard deviation (SD) from two independently repeated experiments.

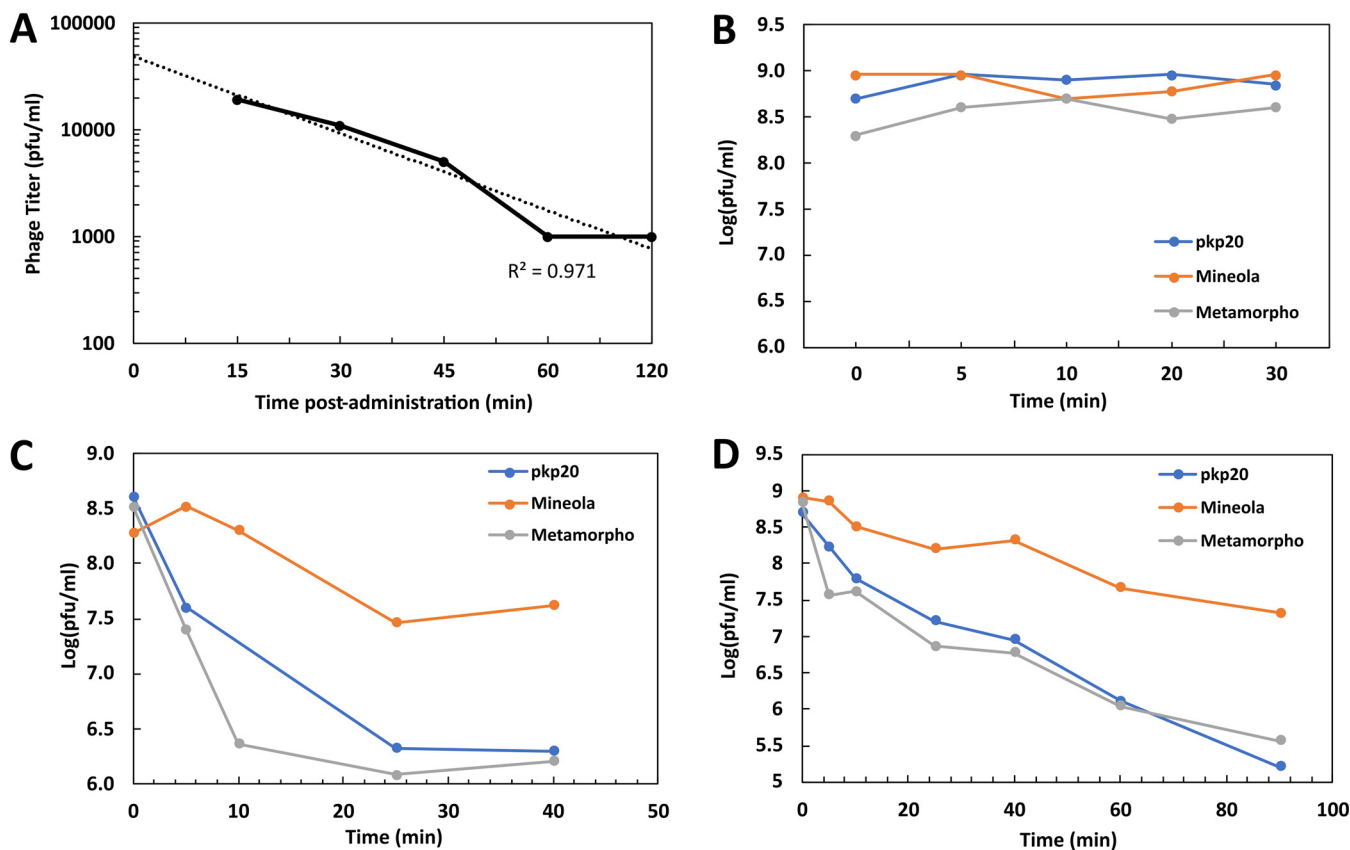


FIG 3 The phage pharmacokinetics on day 1 and the serum neutralization during treatment. Following the IV phage injection (at 5×10^9 PFU/dose) on day 1, the patient serum samples were collected at different time points (15, 30, 45, 60, and 120 min), and the infective phage titers were determined (A). Serum samples were also collected on (B) day 1, (C) day 8, and (D) day 15 to test their neutralization effects on phages by spiking phages to serum to approximately 10^9 PFU/mL and then determining the titers after incubation to assess the viable phages.

urine samples and determined the titers of recoverable phages, and the results indicated that urine did not inactivate the phage.

SERUM NEUTRALIZATION

The patient developed a partial serum neutralization response to phages from day 8, onwards (Fig. 3B–D). Serum neutralization was variable toward different phages, with the weakest neutralization being observed against the phage Mineola (half-life of 0.18 h on day 8 and 0.29 h on day 15) and the greatest neutralization being observed against the phage pKp20 (half-life of 0.09 h on day 8 and 0.16 h on day 15). All three phages demonstrated an extended half-life in day 15 serum samples, compared to those observed a week earlier.

DIVERSITY OF *K. PNEUMONIAE* IN URINE

Among 20 *K. pneumoniae* isolates that were collected between 06/2020 and 05/2022, 15 were ESBL (Table S2). Genome sequencing revealed 5 distinct *Klebsiella* sequence types (which correlate with 5 different phylogenetic clades clustered based on genome-wide comparison), encoding 4 identifiable capsule locus types and a variety of AMR genes (Table 2; Table S3; Fig. S1). Genome sequencing and antibiograms suggested that the infections were independent occurrences rather than a chronic infection caused by a single organism.

URINE MICROBIOTA DURING PHAGE TREATMENT

The total microorganism counts in the urine ranged between 10^3 and 10^4 CFU/mL. 16S gene identification and metagenomic analyses revealed a bacterial population dominated

TABLE 2 Phage susceptibility and genome sequence characteristics of selected *Klebsiella* isolates^a

Isolate ID	Date of isolation (month-year)	Sensitivity to phage			Best matched locus	Sequence type	Phylogenetic clade ^b	AMR genes in genome	ESBL	Number of antibiotics resistant to ^c
		Metamorpho	Mineola	pKp20						
B8142334	Sep-20	Z	R	R	KL102	307	A	18	Yes	9
B9236220	Oct-20	R	R	R	KL102	307	A	18	Yes	11
B9236223	Oct-20	Z	R	R	KL102	307	A	18	Yes	11
C2042673	Jan-21	R	R	R	KL30	3647	E	5	No	2
C4023081 ^d	Mar-21	S	S	S	KL21	Novel	C	21	Yes	12
C5012365	Apr-21	S	S	S	KL21	Novel	C	19/19	Yes	13
20210909B1 ^e	Sep-21	Z	Z	Z	KL17	1015	B	6	No	1
September to October of 2021: <i>Klebsiella</i> directed phage treatment										
D7091294	Apr-22	Z	Z	S	KL7	6	D	3	No	1
D8232378	May-22	R	R	R	NA	NA	NA	NA	No	0

^aZ, clear zone was observed at high phage concentrations (>10⁸ PFU/mL), but there was no plaque formation at lower dilutions; R, resistance (no observable clearing at any phage concentration); S, sensitive with EOP comparable to that of the propagation host; ESBL, extended-spectrum beta-lactamase; NA, not available.

^bDetermined via whole-genome comparison. See supplementary data.

^cOut of a total of 19 types of antibiotics tested.

^dPhage propagation host.

^eIsolated 7 days prior to phage treatment.

by species of *Streptococcus*, *Corynebacterium*, *Enterococcus*, and *Anaerococcus* (Tables S4 and S5). *Klebsiella* spp. were not identifiable in day 8 samples but were identified in samples from day 15, 22, and 29 at extremely low levels (0.16%, 0.19%, and 0.39%, respectively, of the total population). Metagenomic data could not be obtained for the day 1 sample due to DNA extraction failures. After the phage treatment was completed, monthly surveillance urine samples did not test positive for *Klebsiella* for a period of 6 months until April of 2022.

DISCUSSION

We report a case of intravenous phage that was used alone for the targeted eradication of ESBL *K. pneumoniae* colonization in an immunosuppressed patient with recurrent infections. We demonstrate the safety and potential utility of this approach as a means of addressing the common problem of rUTI.

The cycle of rUTI was resolved for almost 6 months following phage initiation, and targeted ESBL strains have not been observed to date (>1 year out). The patient developed UTIs from day 206 onwards, but these were due to new *K. pneumoniae* isolates that were antibiotic-sensitive, as was revealed via sequencing and antibiotic susceptibility testing (isolates from April and May of 2022) (Table 2), and they were easily treated with oral antibiotics, leading to a significantly improved quality of life.

While ESBL *K. pneumoniae* isolates commonly caused pre-phage UTI, whole-genome sequencing revealed that these were successive infections by at least four different bacterial strains over the preceding year; this phenomenon was noted by others, as well (16). The isolation of multiple strains complicated attempts at phage therapy, as phages tend to be strain-specific; genetic engineering and the development of broad host-range phages may overcome this issue. Metagenomic analyses of urine samples indicated that *Klebsiella* was absent or present at minimal levels during the phage therapy. Post-phage *K. pneumoniae* isolates represented a new infection and not a recurrence of previous isolates. We hypothesize that the phage targeted a gut/urinary reservoir of the novel ESBL clones that were identified in previous UTI episodes; unfortunately, we were not able to collect stool specimens to test this hypothesis. A recent study demonstrated that the relative gut abundance of *Escherichia* and *Enterococcus* species were independent risk factors for bacteriuria and UTI in KTR; a strain analysis established a close alignment between gut and urinary species in the same subjects (17).

The host immune response, as evidenced by partial phage inactivation by the patient's serum, was observed 1 week after phage administration, which is consistent with the results of previous reports, despite the patient's immunosuppression regimen (18–20). In contrast to one study, in which complete inactivation of the phage was

observed from week 2, onwards (19), our data showed lower rates of phage inactivation in the serum from day 15 versus day 8, and the reason for this is unclear. Clinical success, despite *in vitro* serum neutralization, suggests that shorter courses of phage therapy may be effective. At this point, it is unclear from the literature whether or not serum neutralization effectively limits further treatment. We have previously reported several successful outcomes with repeat courses of phage therapy, including one case with serum neutralization present at day 0 of phage therapy (10, 18, 19). Alternatively, a case with clinical deterioration that was coincident with the antibody response to phage was also published (21).

In conclusion, we demonstrate that standalone phage therapy without concomitant antibiotics in the setting of rUTI in a KTR was associated with a reduction in UTI frequency and a lack of recurrence of the targeted ESBL *K. pneumoniae* isolate. This strategy will need to be assessed in prospective clinical trials.

Data availability. The complete genome sequences of the *K. pneumoniae* phages Mineola, Metamorpho, and pkp20 were deposited in GenBank under the accession numbers [MH333064](#), [MT701588](#), and [OP331213](#), respectively. Genome sequences of the bacterial strains are deposited under Bioproject [PRJNA941389](#).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.03 MB.

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