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Research Article

First Use of Phage Therapy in India for the Treatment of a Life-Threatening, Pan-Drug-Resistant *Klebsiella pneumoniae* Periprosthetic Joint Infection

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Abstract

We are reporting a prosthesis-associated infection caused by pan-drug-resistant *K. pneumoniae*, culminating in septicaemia. A phage cocktail at a concentration of 1 × 109 PFU/mL was used to irrigate the implant site. The elevated sepsis and renal dysfunction markers returned to normal within 72 hours of the phage therapy. *Acinetobacter baumannii* could appear twice when *K. pneumoniae* became sparse at the wound site, which was treated with the customised phage cocktails. The patient fully recovered with the ongoing local application of the phage cocktail for 152 days. This is a unique case reporting phage therapy for septicaemia on compassionate grounds in India.

Keywords: Bacteriophage; *Klebsiella pneumoniae*; Colistin; Biofilm; Septicemia; Orthopaedic-Implant

Introduction

Biofilms, a complex community of bacteria encased in a protective matrix, are implicated in 60-80% of human bacterial infections, significantly complicating antibiotic treatment even when the antibiotics have been proven effective *in-vitro*. Biofilms form on various surfaces-living tissues such as tooth enamel, lungs, skin and the gastrointestinal tract, as well as non-living surfaces like medical implants (catheters, heart valves, joint prostheses). These biofilms present challenges due to their 10- to 1,000-fold higher antibiotic resistance compared to planktonic bacteria, alongside their enhanced ability to resist phagocytosis, antimicrobial factors and external stressors. Environmental factors, including temperature, pH, nutrient availability, oxygen levels, osmolality and coexisting bacteria, further complicate their eradication, making biofilms a significant obstacle in clinical and natural environments.

The situation becomes particularly dire when Multidrug-Resistant (MDR) or Pandrug-Resistant (PDR) bacterial pathogens, such as *K. pneumoniae*, form biofilms [1-3]. *Klebsiella pneumoniae* is a formidable pathogen due to its extensive repertoire of over 100 mobile Antimicrobial Resistance (AMR) genes, often carried on plasmids and Mobile Genetic Elements (MGEs) such as transposons and integrons [4]. The 2024 GLASS-EAR report highlighted the global emergence of hypervirulent *K. pneumoniae* (hvKp) carrying carbapenemase genes, detected in at least one country across all six WHO regions. These strains are particularly concerning due to their ability to cause severe, invasive infections in both healthy and immunocompromised individuals, coupled with resistance to last-line antibiotics [5-8]. The high resistance rates of *K. pneumoniae* to third-generation cephalosporins

(80% to 100% globally) and a median resistance rate of 17% to carbapenems underscore the growing threat posed by Multidrug-Resistant (MDR) and Pan-Drug-Resistant (PDR) strains, particularly in hospital settings [9]. The alarming rise in colistin resistance among *K. pneumoniae*, as highlighted by Uzairue, et al., significantly complicates treatment, especially for infections involving Pan-Drug-Resistant (PDR) strains on artificial implants, as noted by Shadkam, et al., Colistin, a last-resort antibiotic, is critical for treating Multidrug-Resistant (MDR) and carbapenem-resistant *K. pneumoniae* infections [10,11].

In such cases, prompt removal of the infected implant is often the only viable option to prevent progression to life-threatening septicemia, as antibiotics offer no benefit against PDR strains [12]. The delayed removal of infected implants in cases of carbapenem- and colistin-resistant *Klebsiella pneumoniae* infections significantly increases the risk of systemic infections, such as septicemia, with increased 28 days mortality rates [13].

Bacteriophage therapy offers a promising alternative to antibiotics, as phages can effectively target and eliminate bacteria, irrespective of their resistance to antibiotics or their state within biofilms. This therapy is particularly advantageous for combating *K. pneumoniae* infections, especially those involving Multidrug-Resistant (MDR) or Pan-Drug-Resistant (PDR) strains and biofilms, where antibiotics such as colistin and carbapenems often fail. These viruses specifically penetrate biofilms, targeting and lysing bacteria, thus providing a highly selective approach that circumvents nearly all traditional antibiotic resistance mechanisms.

We are herein reporting a case of septicaemia with an orthopaedic implant primarily infected with pan-drug resistant K. pneumonaie. A female, now aged 60 years, was met with an accident as a pillion rider on a road with craters and waterlogging in 2009. She had a fall on boulders and fractured her right hip joint. Only 3-4 mm of bone was left attached to the pelvic girdle. She was advised to go for a hip joint replacement. The hip implant surgery was done, but it failed to provide relief. Three years later, she consulted another surgeon, who performed a fresh hip joint replacement in 2012. This time, she could walk comfortably after 3 months of physiotherapy. She was on regular six-month follow-ups. She met with another accident in the year 2021; while riding an e-rickshaw, the vehicle overturned and an iron rod fell right on the implant site of the thigh. Since then, pain and swelling started at the implant site. Her discomfort and pain increased while walking since the beginning of 2023. Later, she was diagnosed with a cricket ball-sized cyst near the implant. The cystectomy was executed on October 22, 2023. Her wound got infected with *K. pneumoniae*. The infecting strain was resistant to all the available antibiotics, including colistin. Her general condition was deteriorating with the signs and symptoms of septicaemia. Her blood examinations revealed procalcitonin, 5.25 ng/mL; CRP (quantitative), 240.1 mg/mL; blood urea, 121mg/dl; serum creatinine, 3.5 mg/dl with total leukocyte counts 28740 /µl with a differential count of polymorphs 87%, lymphocyte 10%, eosinophil 1% and monocyte 2%. The blood examination showed low haemoglobin, 8.4 g/dL. The pus discharge was about 150 to 200 mL per day.

On December 2, her scientist son visited us. After proper consent to use bacteriophage on compassionate grounds, we provided him with a cocktail of three well-characterised bacteriophages with broad-spectrum lytic activity against *K. pneumoniae* for empirical use. The pus sample was collected for bacterial isolation. The phage cocktails were pushed inside the stitched wound. The patient was shifted to the ICU to monitor for serious adverse effects, if any, arising during the therapy. The therapy course is presented in Table 1.

Date	**Total leucocyte count/µL differential leukocyte counts**	**Hemoglobin (g/dL)**	**CRP (mg/dL)**	**Blood urea (mg/dL)**	**Creatinine (mg/dL)**	**Phage therapy schedule** phage therapy given(φ)
01.12.2023	28740 N 87%, L 10%, M 2%, E 1%	8.4	240.1	121	3.50	**Serum procalcitonin 5.25 ng/mL**
02.12.2023	**21000**	**-**	**-**	**-**	**-**	***(φ) Phage therapy started***

(Day 1)						***empirically***
03.12.2023 *(Day 2)*	28000	**-**	**-**	**-**	**-**	(φ)*K. pneumoniae* isolated.
04.12.2023 *(Day 3)*		**-**	**-**	**-**	**-**	(φ) bacterial isolate resistant to colistin and imipenem. All the phages lytic to the *K. pneumoniae* isolate
05.12.2023 *(Day 4)*	15800	**-**	**-**	**-**	**-**	(φ) pus discharge decreased to about 5 mL only
06.12.2023 *(Day 5)*	15460	**-**	**-**	**70**	**1.90**	(φ) Huge serous discharge (200 mL/day)
07.12.2023 *(Day 6)*	12800	**-**	**-**	**55**	**1.30**	(φ) Serous discharge, Few colonies of *K. pneumoniae* but abundant growth of *Acinetobacter species* like colonies
08.12.2023 *(Day 7)*	N 88%, L 12%, M 2%, E 3%	10.1	71.4	46	1.2	(φ) The new isolate could be identified as *A. baumannii* and was subjected to 10 different phages specific to *A. baumannii* available to us. **Serum procalcitonin 0.298 ng/mL**
09.12.2023 *(Day 8)*	13730	10.1	**-**	36	1.0	(φ) at 9.00 am *A. baumanni*-specific Phage cocktail of 3 phages in the volume of 2.00 mL (1×10^9PFU/mL) mixed in 20 mL saline was used to irrigate the wound site at 6.45 pm. A mild febrile reaction lasting for 2h was observed
10.12.2023 *(Day 9)*	17900	8.4	**-**	**-**	**-**	(φ) Both phage cocktails were applied separately at an interval of 12 h. No febrile reaction was observed.
11.12.2023 *(Day 10)*	13500	9.6	**-**	**-**	**-**	(φ) Both phage cocktails were applied separately at an interval of 12 h.
(Day 11) 12.12.2023	10750	9.9	39	30	0.70	(φ) Both phage cocktails were applied separately at an interval of 12 h.
13.12.2023 *(Day 12)*	8130	9.2	40.7	20	0.70	(φ) Both phage cocktails were applied separately at an interval of 12 h, Serous discharge coming from the wound site
14.12.2023 *(Day 13)*	8770	9.4	40	20	-	• (φ) • *baumannii* did not grow. However, a few colonies of *K. pneumonaie* still growing

15.12.2023 *(Day 14)*	13170	9.9	67.4	20	-	• (φ) • On suspicion because of urinary symptoms, urine was cultured on 12.12.2023; the sensitivity report indicated *Enterococcus faecium* and only sensitivity was observed with Linezolid. • The antibiotic was started with a dose of 600mg twice daily. • The phage cocktail for *A. baumannii* was stopped.
16.12.2023 *(Day 15)*	7710	9.1	55.5	-	-	• (φ). • Serous discharge present
17.12.2023 *(Day 16)*	8000	9.1	24.75	-	-	• (φ) • The treating surgeon closed the wound as the blood parameters were normal. • However, serous discharge was still coming out. • The surgeon put a cannula for the inoculation of the phage cocktail.
18.12.2023 *(Day 17)*						• (φ) • serous discharge present
19.12.2023 *(Day 18)*	6540	9.3	16.20	-	-	• (φ) • a few colonies *of K pneumoniae*. • *A.baumannii* did not grow
20.12.2023 *(Day 19)*						• (φ) • *K. pneumoniae*, a few colonies were still growing.
21.12.2023 *(Day 20)*	8350	-	26.50	-	-	• (φ) • The Tab Linezolid was stopped • The patient was shifted to her home with a cannula (Padrauna, Deoria, Uttar Pradesh).
24.12.2023 *(Day 23)*	10900	9.4	43.69	-	-	• (φ) • No antibiotic was given. • Visually, the stitches were looking good.
25.12.2023 *(Day 24)*	9650	9.6	23.81	-	-	• (φ) • No growth of *K. pneumoniae* from serous discharge.
26.12.2023 *(Day 25)*	9650	-	29.88	-	-	• (φ) • Advised to apply phages at the interval of 48h in 10 mL

						volume only at the concentration of 1× 10^9 PFU/mL.
29.12.2023 *(Day 28)*	8400	-	25.89	-	-	• (φ) • Plenty of serious discharge is coming out.
01.01.2024 *(Day 32)*	6890	-	9.98	-	-	• (φ) • There was growth of *Acinetobacter baumanni* again, which was sensitive to Chloramphenicol and Imipenem.
06.01.2024 *(Day 36)*	7300	9.0	7.63	-	-	• (φ) • Phages against *A. baumannii* were prepared and transported to the patients.
08.01.2024 *(Day 38)*						• (φ) • Phages for *A. baumanii* were pushed into the wound site daily, • while *K. pneumoniae* phages were on alternate days.
11.01.2024 *(Day 41)*	8180	9.40	18.83	-	0.86	• (φ) • Phages for *A. baumanii* were pushed into the wound site daily, • while *K. pneumoniae* phages were on alternate days.
13.01.2024 *(Day 43)*						• (φ) • Twenty millilitres of oily serous fluid was collected from the wound site and submitted for culture
18.01.2024 *(Day 48)*						• (φ) • *K. pneumoniae,* a few colonies grew, • but no *A. baumanni.*
19.01.2024 *(Day 49)*	8160 N 61.3%, L 30%, M 2.8%, E 5.5%,	9.00	16.87	-	-	• (φ) • A fresh cannula was placed in the wound and both cocktails were pushed to the cannula at the gap of 12 h. • *K pneumoniae* was continued on an alternate basis. • *A. baumanni.* Phage cocktail was stopped.
27.01.2024 *(Day 57)*	7590	9.70	16.70	-	-	• (φ) • Seventy milliliters of discharge could be collected

30.01.2024 *(Day 60)*						• (φ) • About 40 ml of discharge could be collected. The volume of the phage cocktail inoculated was 10 mL, while the discharge coming out was more. • The number of *K. pneumoniae* colonies was 3 in 0.01 ml volume. • *A. baumanni* did not grow.
31.01.2024 *(Day 61)*	7250	-	33.6	-	0.83	• (φ) • The patient has had a severe dry cough for 3 days. • The drainage fluid volume was 20 mL. • The fluid is found sterile now. • However, it was advised to instill a phage cocktail on an alternate day because of the implant and suspected biofilm.
06.02.2024 *(Day 67)*	-	-	18	-	-	• (φ) • About 25 mL of fluid is still coming out
15.02.2024 *(Day 75)*	9170	9.60	15.35	-	-	• (φ) • Four serous discharge samples collected on different days and preserved at 4^0 C were received today and subjected to culture.
20.02.2024	7900	-	28	-	-	• (φ) • Since *A. baumanni* did not grow in the previous 5 samples collected on 5 consecutive days, we stopped phages against it. • However, we continued the phage cocktail against *K. pneumoniae.* • All three phages in the cocktail were still effective against the *K. pneumoniae* isolate. • The fluid seems reactionary due to inflammation, as suggested by the treating surgeon.
07.03.2024					0.93	• (φ)

						• The serous discharge was found to be sterile
15.03.2024	8500 N 70%, L 23.6%, M 2.2%, E 5.6%,	9.90	30.70	-	-	
22.03.2024	7270	10.6	21.28	-	-	
01.04.2024						The fluid was sterile on cultures for five different consecutive days of collection. The phage application over the wound's surface continued on an alternate-day basis for the next 15 days. The drainage tube was removed.
01.04.2024						The 7 wound swabs collected on alternate days fere found sterile.
17.04.2024	***Phage therapy was continued for four months, eighteen Days***					**The phage application was stopped.**
19.04.2024						No discharge from the stitch site. However, the collection of fluid (70 ml) could be seen on the USG examination**.**
16.05.2024	-	-	11	-	-	The serous discharge has become scanty and swabs from the wound surface were sterile on repeated cultures. The fluid collection could be seen in ultrasonography. Aspiration was tried but could not be aspirated as it was quite thick. The treating surgeon predicted it as sterile fluid collection, leading to a mild inflammatory response and suggested no intervention. The phage therapy was stopped.
16.06.2024	9500	-	17	-	-	The patient was fine. The wound site is completely healed. She gained weight and is mobile by herself.
16.07.2024						The fluid collection has regressed to 4.34 mL on the USG examination.

Table 1: Outlines of the protocol for administering treatment regularly during the first 16 days and subsequently, along with the relevant laboratory findings, to assess the progress of the phage therapy.

Materials and Methods

Isolation of Bacteria from Clinical Specimens

The patient's clinical samples (pus and urine) were inoculated on McConkey agar, blood agar and CLED (Cystine Lactose Electrolyte Deficient) media and incubated at 37°C overnight. The bacterial isolate was identified using standard microscopy and test substrates. The determination of antibiotic susceptibility for different isolates was assessed using the disk diffusion

(Kirby-Bauer method) or broth microdilution (colistin) methods, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020).

Isolation of K. pneumoniae-Specific Bacteriophages

The *K. pneumoniae* (KpnBHU109) specific bacteriophages were isolated from the sewage drainage system of the University Hospital and Trauma Centre, BHU, Varanasi, using a slightly modified version of the previously described method [14]. Table 2 shows the accession number on NCBI and none of the phage genomes had antibiotic resistance or known virulence genes.

Bacteriophage Cocktail Against A. baumannii

We have a collection of 10 bacteriophages against *A. baumannii* isolates as an outcome of a completed research project. The phages were checked against the *A. baumannii* isolates and 3 actively lytic phages were selected for therapeutic purposes. The phages were purified and preserved for ready use. These phages were not sequenced.

S. No.	Bacteriophage	Genome size (bp)	GC content (%)	Protein	GenBank accession #
1	ΦKpnBHU1	40410	54	51	OL979478
2	ΦKpnBHU2	42251	53.7	40	OL979479
3	ΦKpnBHU3	43437	54.1	48	OL976437

Table 2: Bacteriophage genome data submitted to GenBank.

Results

We immediately provided a cocktail of 3 bacteriophages having broad-spectrum lytic activity against *K. pneumoniae* for empirical use on December 2, 2023. The pus sample was subjected to culture daily. The patient was shifted to the ICU to monitor any serious adverse effects. The phage cocktails were pushed inside the stitched wound through a catheter. The course of phage therapy is given in Table 1. The appearance of different bacterial species during phage therapy is shown in Fig. 1 and blood parameters are shown in Fig. 2.

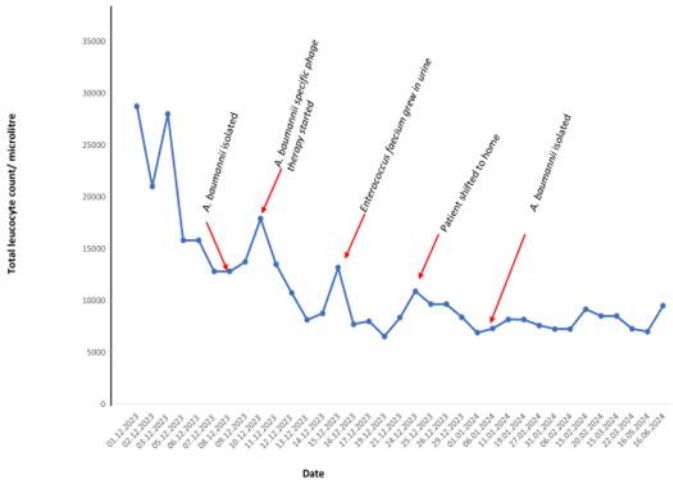

Figure 1: The graph represents the appearance of *K. pneumoniae*, *A. baumannii* and *Enterococcus faecium* from the patient sample during the phage therapy.

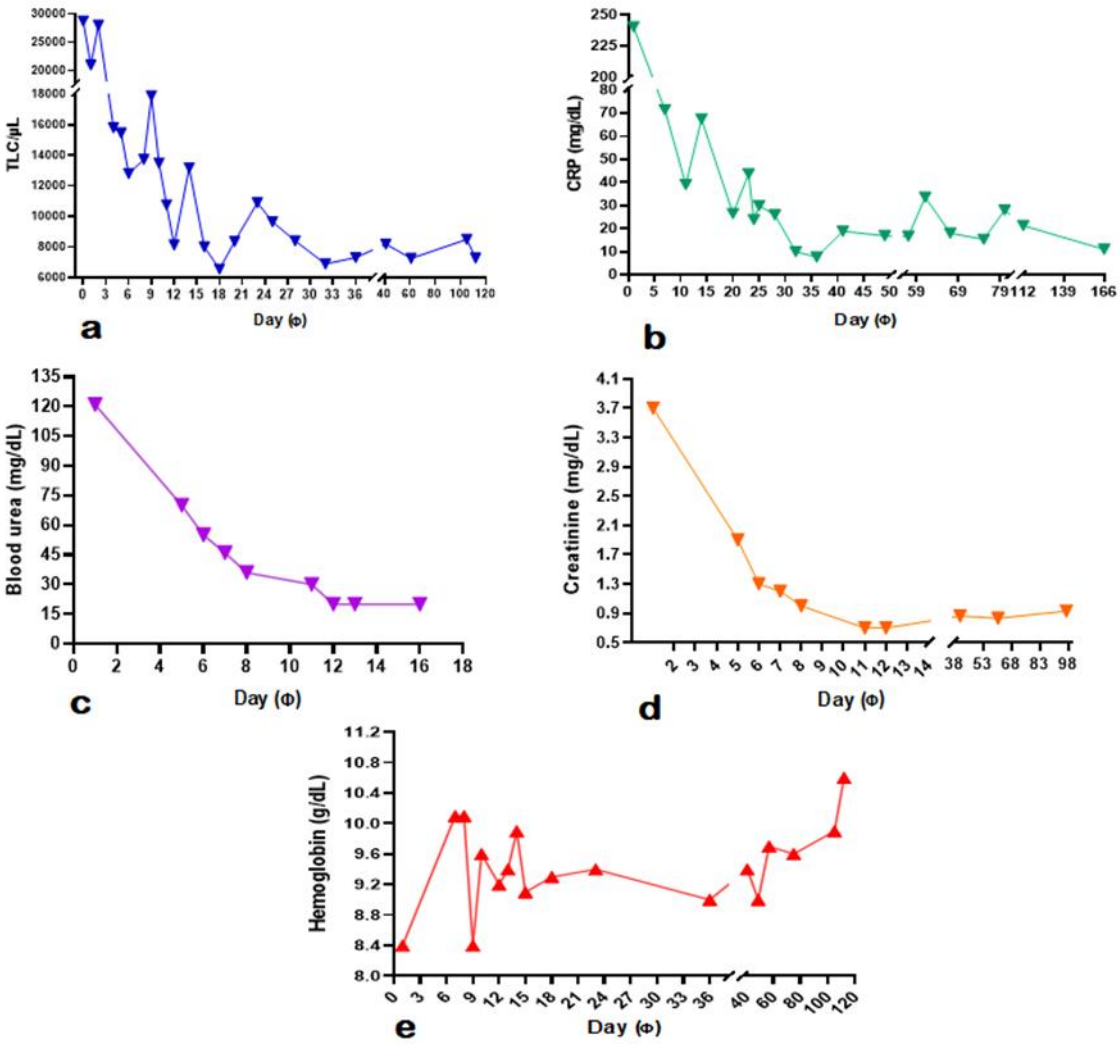

Figure 2: The graph of blood parameters during the phage therapy. a) Total leucocyte count; b) C-reactive protein; c) Blood urea; d) Serum creatinine and e) Haemoglobin.

Discussion

Concerning the efficacy, safety and immune neutralisation of phage therapy, we undertook several *in-vitro* and preclinical studies. We carried out an *in-vitro* experiment on phage-antibiotic synergy, emphasizing its role in eradicating biofilms. The synergy is optimal when the antibiotic is administered 6-8 hours after the phage cocktails [15]. The first preclinical study involved inducing a wound on rat skin and establishing an infection with Pseudomonas aeruginosa. This infection was successfully treated using a bacteria-specific phage cocktail in an *in-vivo* wound model [16]. In the rabbit osteomyelitis model, acute and chronic *Staphylococcus aureus*-induced osteomyelitis were established by creating a crater near the distal end of the femur, which could be cured using a cocktail of seven phages. Both forms of osteomyelitis were successfully cured [17]. We further investigated the use of phage therapy to treat S. aureus-infected orthopaedic implants in a rabbit model, demonstrating the successful eradication of the bacteria [18]. Similarly, to address urinary tract infections, including chronic UTIs, we developed the model in mice and rats by inoculating the bacteria via the urethra to establish the infection, followed by determining the safe dose, dosage schedule and route for effective phage use. We conducted these preclinical studies on *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Enterobacter spp*. A concentration of 109 PFU per dose administered through the urethra was the most effective, requiring only two doses to cure the infection [19,20].

In human studies, soft tissue-related chronic wound ulcers, we treated cohorts of patients, including a case series and a case-control study of phage cocktail. We successfully used customized phage cocktails for both diabetic and non-diabetic patients [21,22]. In a recent study, a double-blind, randomised controlled trial of phage therapy for chronically infected wounds resulted

in a cure rate exceeding 92% [23]. Additionally, we conducted a case-control study using customised phage cocktails on acute, large, road traffic accident-contaminated wounds. We reported a one-third reduction in costs, length of hospital stays and healing without fibrosis, attributable to quicker recovery. In this study, both groups underwent standard surgical therapy, while one group received additional treatment through the local application of customised phages [24].

We further conducted many preclinical studies to treat septicaemia in patients infected with MDR bacterial strains. The bacterial species involved in inducing septicemia in the mouse model encompassed all organisms in the ESKAPE group, namely Enterococcus species, S. *aureus, A. baumannii, K. pneumoniae, P. aeruginosa* and *Enterobacter cloacae*. We could decide the safe doses and route of administration of phage cocktail in such cases. It was found that for treating septicaemia, one should start with a minimal dose of phage cocktail, with continuous monitoring of the patient's vitals (blood pressure, SpO2 and temperature rise) to avoid endotoxic shock due to sudden bacteriophage-induced bacterial lysis [14,18,25,26]. We also screened the phage therapy on a surrogate model of acute enteric fever (septicaemia) and chronic typhoid carriage, using a surrogate pathogen *Salmonella Typhimurium*. Additionally, we noted the successful eradication of chronic carriage [27]. We also searched for adverse reaction and toxicity studies. We observed that neutralising antibodies rise late, usually after three weeks of phage inoculation in rabbits [28]. We administered various concentrations of phages to several rats orally, ranging from very low to very high. It was intriguing to note that no adverse effects existed [29]. All these *in-vitro*, *in-vivo* (preclinical and clinical studies have been given in Table 3.

In-vitro Studies

S. No	Infection Syndrome	Target bacteria	Clinical outcome	Reference
1	Biofilm	*Staphylococcus aureus* and *Pseudomonas aeruginosa*	The Bacteriophage incorporated with chitosan microparticles enhances the antibacterial activity.	[34]
2	Biofilm	*Acinetobacter baumannii*	The developed formulation exhibited excellent antibiofilm eradication potential *in-vitro* and effective wound healing after topical application.	[35]
3	Biofilm	Colistin resistant-*Klebsiella pneumoniae*	This study showed that combining phage ΦKpnBHU3 (1×10^9 PFU/mL) with the sub-inhibitory concentration of colistin (12.2μg/mL) produced a synergistic antibacterial effect and successfully eradicated planktonic and biofilm forms of *K. pneumoniae*	[15]

In-vivo (Preclinical) Studies

S.No	Infection Syndrome	Animal (Species/Strain)	Route of administration	Target bacteria	Clinical outcome	Reference
1	Wound infection	Swiss albino mice	Subcutaneous	*Pseudomonas aeruginosa*	The bacteriophage cocktail significantly reduces the bacterial load in the wound site.	[16]
2	Chronic osteomyelitis	Rabbits	Intraperitoneal	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Phage therapy resulted in complete wound healing along with site sterilization.	[17]
3	Chronic	Rabbit	Intralesional	Methicillin-Resistant	Phages	[18]

	Osteomyelitis			*Staphylococcus aureus*	successfully eradicated the MRSA biofilm formed on the metal implant.	
4	Intestinal infection	Female/male Swiss albino mice	Oral	MDR *Klebsiella pneumoniae*	Complete eradication was observed in 6 days of administration of bacteriophage therapy.	[36]
5	Septicemia	Female/male Swiss albino mice	prophylactic	*Pseudomonas aeruginosa*	No mortality could be observed with reduced dose of cocktail, that is, 10^8, 10^9 and 10^{10} PFU administered 6 hours after bacterial challenge.	[25]
6	Neutralizing antibody response	Rabbit	Subcutaneous	*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella Typhi* and *Staphylococcus aureus.*	Complete neutralization of bacteriophages could be seen between 3 and 5 weeks after immunization.	[28]
7	Septicaemia	Female/male Swiss albino mice	Intraperitoneal	colistin-resistant *Acinetobacter baumannii*	No mortality was observed with reduced doses of the cocktail (10^6 and 10^5 PFU/ml).	[26]
9	Septicemia	Female/male Swiss albino mice	Intraperitoneal	Colistin resistant-*Klebsiella pneumoniae*	A single dose of 10^5 PFU/mouse protects the mice from fatal outcomes at any stage of septicemia.	[14]
10	Acute toxicity	Male/Female Charles Foster rats	Oral	*XDR Klebsiella pneumoniae*	No adverse effect was observed in any of the experimental as well as in the control animals	[37]
11	Gastrointestinal disease	Neonatal goats	Oral	Enteropathogenic *Escherichia coli* (EPEC)	The administration of bacteriophage therapy completely eradicated the	[38]

					EPEC from the Neonatal goats	
12	Acute and chronic Gastrointestinal infection	Swiss albino mice	Intraperitoneal and Oral	*Salmonella* Typhi	The oral feeding of phage cocktail completely cured the carrier state within 7 days of feeding.	[27]
13	Fish infection	*Pangasius buchanani*	Intramuscular and water immersion	*Aeromonas hydrophila*	Intramuscular dose of 10^4 PFU/fish and water immersion 10^6 PFU/mL results in complete cure.	[39]
14	Urinary Tract Infection	Female Charles Foster rats	Urethral	*Escherichia Coli*	The two doses of phage cocktail 10^8 and 10^7 PFU/ml resulted in the complete cure of UTI	[19]
15	Biofilm-Mediated Burn Wound Infection	Female/male Wistar rats	Topically	MDR *Klebsiella pneumoniae*	Improved wound contraction in 28 days with reduced inflammation	[40]
16	Urinary Tract Infection	Female Swiss albino mice	Urethral	Colistin resistant-*Klebsiella pneumoniae*	The two doses of 10^5 and one dose of 10^9 PFU/mouse resulted in the complete cure of UTI	[20]

In-vivo (Clinical) Studies

S.No	Infection Syndrome	Patients	Route of administration	Target bacteria	Clinical outcome	Reference
1	Chronic Nonhealing Wound	20 Male/Female patients	Topical	*Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*	Seven patients achieved complete healing on day 21 with no adverse effect	[21]
2	Chronic Nonhealing Wounds	48 Male/Female patients	Topical	*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Proteus* species, *Citrobacter freundii*, *Morganella morganii* and *Acinetobacter baumannii*	A cure rate of 81.2% could be obtained, of which 90.5% (19/21) patients were	[22]

					nondiabetic and 74.1% (20/27) diabetic.	
3	Traumatic Wounds	54 Male/Female patients	Topical	*Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Enterococcus faecalis*	A significant and rapid improvement was observed in wound healing in cases then control group	[24]
4	Chronic Wound Infections	30 Male/Female patients	Topical	*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* species, *Citrobacter freundii*, *Morganella morganii* and *Acinetobacter baumannii*	A total of 93.3% of the wound became sterile in 39 days (median sterility time), followed by complete healing by the end of 90 days in the phage group	[23]

Table 3: Showing studies done at our centre before taking this first case of life-threatening septicaemia.

After conducting the aforementioned *in-vitro*, preclinical and clinical studies, we approached phage therapy with great caution. We obtained the necessary consent from the patient and the treating physicians for a septicemic patient with a primary infection focus caused by pan-drug-resistant *K. pneumoniae*, associated with a hip implant wound. Phage therapy was initiated on compassionate grounds and the improvement was miraculous. On the 4th day, after three doses of a specific phage cocktail, the discharge of frank pus ceased. The procalcitonin level decreased from 5.23 ng/mL to 0.298 ng/mL. The total leukocyte count was from 28740/μL to 15460/μL and the polymorph percentage was reduced from 87% to 72%. The other marker of acute inflammatory response, CRP, decreased from 240.1 mg/mL to 71.4mg/mL. The deteriorating kidney function showed a decrease in blood urea from 121 mg/dL to 70 mg/dL and a reduction in serum creatinine from 3.5 mg/dL to 1.0 mg/dL. It is worth noting that the bacteria were continuously detected for nearly 100 days during phage therapy. It indicates the longevity of the biofilm on the implant and no antibiotic can be used for such a prolonged period, even if it is found to be effective, without risking the development of resistance and adverse effects on the human microbiome [1,2,30]. The breaking down of the bacterial cell wall and EPS layer of the biofilm through the action of phage enzymes (glycan depolymerase, etc.) allows the diffusion of the bacteriophages, while the antibiotics cannot execute the same [31,32]. We can exploit this action of bacteriophages to use their synergy with antibiotics [33].

It was intriguing to note during therapy that the *A. baumannii* could be isolated when the CFU came down. It is quite likely that *K. pneumoniae* might have an inhibitory effect on *A. baumannii in-vivo*. It is worth mentioning that *A. baumannii* appeared twice. The second appearance of the bacterium occurred 31 days after the start of *K. pneumoniae*-specific phage therapy and a single course of the phage cocktail was likely inadequate for eradicating A. baumannii. This implies that in cases of mixed bacterial infections, phages targeting them should be administered for a sufficiently long duration if the disease is chronic.

All three phages used in the cocktail were lytic against the last time isolated bacteria. This observation implies that a cocktail of the phages prevents the development of mutants. Administering the phage cocktail at a high dose may induce neutralizing antibody formation. Fortunately, the antibody could be detected at the end of the therapy at a very low titre, i.e. <1:40 only. The reason might be that these phages are part of the natural phage biome of the body and colonized the patient during early childhood [28].

The patient was transferred to the ICU with the concern that a sudden release of bacterial endotoxins at high levels could lead to shock-like conditions. However, only a mild febrile reaction was observed after the first dose when the cocktail was administered. The toxin may be released in significant amounts with the first dose to induce fever; however, later on, due to a reduction in CFU, the amount of endotoxin released could be minimal. This finding suggests that localised infections may be treated with higher doses of the phage cocktail; however, in a severe septic state, the doses of the phage cocktail may need to be gradually increased [26]. Although many questions about the clinical efficacy and safety of bacteriophage therapy remain unanswered, particularly regarding cases involving Multidrug-Resistant (MDR) or Pan-Drug-Resistant (PDR) infections, several studies have already been conducted on safety, immunogenicity, dosing, routes of administration, treatment courses for specific infections, molecular characterization of each phage for clinical use, phage-antibiotic synergy and pharmacokinetics and pharmacodynamics. However, several carefully treated case reports are needed in clinical studies to support the safety and efficacy of phages and convince regulatory bodies to facilitate the large-scale application of phage therapy. This first Indian report treating life-threatening septicaemia bears great potential for phage therapy in chronic biofilm-associated infections with MDR/PDR in humans.

Conflict of Interest
The authors have declared no conflict of interest.

Data Availability Statement
The original contributions in the present study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

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Consent
Patient consent for phage therapy was taken.

Authors Contribution
GN: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing - review & editing. ANS: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing - original draft, Writing - review & editing. GP: Formal analysis, Writing - original draft, Writing - review & editing. SP: Resource. GKR: Resource.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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