REVIEW ARTICLE

PHAGE AS MEDICINE FOR BACTERIAL DISEASES

P. Lokesh[1], S. Sivasankara Narayani[2*]

[1]School of Bio-Sciences and Technology, Vellore Institute of Technology (VIT), Vellore, Tamil Nadu 632014, India
[2]Department of Microbiology, Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India.

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Address for Corresponding : *Dr. Sivasankara Narayani,* [2]Department of Microbiology, Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, INDIA. *Email:* sivasan91@gmail.com

ABSTRACT

The phage is a simple, extremely diverse, non-living biological entity consisting of DNA or RNA fenced within a protein capsid. As naturally occurring bacterial parasites, the phage is incapable of replicating independently and is ultimately dependent on a bacterial host for survival. Phage characteristically binds to specific receptors on the bacterial cell surface, inject their genetic material into the host cell, and then either integrate this material into the bacterial genome which is called "temperate" phages and those reproduce vertically from mother to daughter cell, or hijack the bacterial replication machinery to produce the next generation of phage progeny and lyse the cell is called as "lytic" phages. Upon reaching a perilous mass of phage progeny, which can be anywhere from a few to over 1000 viral particles, depending on ecological factors, the lytic proteins become active and hydrolyze the peptidoglycan cell wall, releasing novel phage to reinitiate the lytic cycle**.** Both antibiotics and phage function as an antibacterial that disrupts bacterial colonies through lysis or inhibition, so far, several key variances make each antibacterial more or less fitting depending on the situation. Adversative reactions to antibiotics include occurrences of anaphylaxis, nephrotoxicity, cardiotoxicity, hepatotoxicity, and neurotoxicity, as well as several gastrointestinal and hematological complications. Antibiotics, and phages tend to be specific to both species and strains. phages and phage-derived proteins for combating bacterial infections, specifically those of multidrug-resistant bacteria. The spread of antibiotic resistance genes carries a unique danger in that many antibiotics have diminishing efficacy against common infections. So, it's better to treat with the help of phages.

Keywords: Phage; Multidrug resistance; Pathogens; Antibiotics; Bacteria

INTRODUCTION

Bacteriophages are one of the most proficient substitutes for antibiotics against bacterial infections [1]. Phages are existing in every environment where bacteria exist, and there is at least one type of phage, more than one in most cases, to infect every strain of bacteria [2]. Before the development of antibiotics, bacteriophages were the choice of treatment against bacterial infections such as diarrhoea [3]. But soon after the starter of antibiotics, the use of bacteriophages in therapy was almost abandoned. [4]. The misuse and mismanagement of antibiotics led to the progress of antibiotic-resistant bacteria which is one of the most troublesome healthcare problems. In the post-antibiotic era, it becomes obligatory to combat antibiotic-resistant bacterial infections using alternate therapies as antimicrobial compounds are ineffective. Phage therapy receives renewed curiosity among phage researchers, and vital and practical studies on bacteriophages increased intensely, newly also together with clinical trials [3]. The investigation of bacteriophages for clinical or biotechnological purposes added increasing attention after the 2000s, particularly the isolation of virulent bacteriophages for the treatment of bacterial infections, and the research of phage banks for personalized phage delivery [4]. However, bacteriophages and their proteins have a crowd of applications in different fields, for clinical use, therapeutic phages are required to be characterized in much detail. Virulent phages undergo a lytic cycle that differs from that of the temperate phage which by a lysogenic cycle. During this cycle, the phage genome integrates into the host genome, which can present a benefit to the host as some prophages encourage host behaviour or virulence by encoding virulence factors such as poisons or antimicrobic resistance genes. [5,6,7]. Phages suitable for therapeutic purposes are usually lytic, where the produced phage progeny is released by lysis (the devastation of the bacterial cell envelope) after their replication inside the host bacterium [8]. With this specific quality, virulent phages can be used to slaughter the pathogenic bacteria present inside the human system, and more often phages are specific to specific bacteria. This review mainly emphasizes the phages in medicine for the treatment of bacterial diseases.

Antibiotic Resistance and Phage Therapy

Antibiotic resistance amid the pathogenic bacteria has made a universal emergency, instigating the quest for an alternate cure.

Bacteriophages were discovered over a century ago and are evidenced to be an effective alternative at the time of antibiotic medication catastrophe [9]. According to WHO's (2020) report the percentage of resistance to ciprofloxacin, an antibiotic that is used to treat urinary tract infections, diverse from 8.4 per cent to 92.9 per cent for *Escherichia coli* and from 4.1percentage to 79.4 percentage for *Klebsiella pneumoniae* in countries reporting to the global antimicrobial resistance and use surveillance system, in some countries, carbapenem antibiotics does not work in more than half of the patients administered for *K. pneumoniae* infections due to resistance and the resistance to fluoroquinolone antibiotics in *E. coli*, which is used for the treatment of urinary tract infections, is widespread. There are several countries in many parts of the world where this treatment is now unsuccessful for more than half of the patients. Colistin is the only last choice treatment for lethal infections caused by carbapenem-resistant *Enterobacteriaceae* which are *E. coli, Klebsiella*. Bacteria tolerant to colistin have also been detected in some countries and regions, triggering infections for which there is no active antibiotic treatment at present. The bacteria *Staphylococcus aureus* is a chunk of our skin flora and is also a communal cause of infections both in the public and in healthcare facilities. People with methicillin-resistant *Staphylococcus aureus* infections are 64% more likely to die than persons with drug-sensitive infections. Phage therapy practices are actively taking place in various Eastern European countries with effort work in Georgia and Poland being the most conversed. Several efforts have been made to investigate records of their development, testing, and applications; though, it is not vibrant what section of the applicable info is reachable due to soviet-era practices, military concerns, and language fences. phages into magistral provisions for human use has been legalized since 2018 [10]. Thus, it's the correct time to switch to Phages which appear to be better therapeutic agents as they have numerous advantages overtraditional antibiotics [11,12,13].

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Table.1 Recent advances of phage therapy in human medicine

Phage	Infection	Administration
PP1450, PP1777, and PP1792	*P.aeruginosa* prosthesis knee infection	Local Injection [14]
Pyophages and intesti phages	*Enterococcus faecalis* infected total hip arthroplasty	Oral suspension [15]
PM448	Recalcitrant *Staphylococcus epidermidis* prosthetic knee infection	Intra-articular [16]
Pyophage cocktail (Georgian pharmaceutical product registration number R-022600)	Urinary tract infections in patients undergoing transurethral resection of the prostate	Intravesical [17]
Cocktail of phages	Chronic nonhealing wounds s due to infection by AMR *Escherichia coli* (37.5%) followed by *Pseudomonas aeruginosa* (31.2%) and *Staphylococcus aureus* (31.2%), *Klebsiella pneumoniae* (12.5%), *Proteus species* (6.2%), *Citrobacter freundii* (4.1%), *Morganella morganii* (2.1%), and *A cinetobacter baumannii* (2.1%)	Topical application [18]
Three lytic phages of cocktail APC 1.1, JWDelta, JWT.	Pandrug-Resistant *Achromobacter xylosoxidans*	Nebulization [19]
Coli A11/58c, Kl 53N/1920, EF1/1679L, Ps1N/734, Coli 77/850	Chronic urinary and urogenital MDR bacterial infection caused by *E.coli, K.variicola, K.pneumoniae, P.aeruginosa*	Intravesical and intravaginal [20]
KpJH46Φ2 phage	Limb-threatening prosthetic knee *Klebsiella pneumoniae* infection	Intravenous [21]
Ab_SZ3 with z tigecycline and polymyxin	Carbapenem-resistant *Acinetobacter baumannii* Lung Infection	Nebulization [22]
AbW4878ø1, sulfamethoxazole/trimethoprim and tigecycline	MDR *A cinetobacter baumannii* respiratory infection	Intravenous and nebulized bacteriophage [23] therapy
Phage M1 along with meropenem and colistin, followed by ceftazidime/avibactam.	Fracture-related pan drug-*resistant Klebsiella pneumoniae* infection after long-term (>700 days)	Catheter administration [24]

The above research in Table 1 was carried out on humans. *P.aeruginosa* prosthesis knee infection was treated with PP1450, PP1777, and PP1792 phages where the patient got cured [14]. Pyophages and intestine phages were used against the *Enterococcus faecalis* infected total hip arthroplasty where the patient also got cured [15].

The patient who had also been ill with debilitating plastic anemia for more than 2 years, was recovering after receiving adjuvant bacteriophage therapy (PM448 phage), and also the recalcitrant *Staphylococcus epidermidis* prosthetic knee Infection was also cured [16]. Pyophage cocktail (Georgian pharmaceutical product registration number R-022600) was used against the urinary tract infections in patients undergoing transurethral resection of the prostate which resulted in the normalization of urine culture, measured by a quantitative microbiological urine test [17]. Cocktail of phages isolated from the hospital sewage, river Ganga, ponds, and sewer of the municipal corporation against chronic nonhealing wounds s due to infection by AMR *Escherichia coli* (37.5%) followed by *Pseudomonas aeruginosa* (31.2%) and *Staphylococcus aureus* (31.2%), *while Klebsiella pneumoniae* (12.5%), *Proteus species* (6.2%), *Citrobacter freundii* (4.1%), *Morganella morganii* (2.1%), and *Acinetobacter baumannii* (2.1%) where a total of 5 to 7 applications were made till the wound became free from infecting bacteria. The success rate of therapy was found to be 81.2% was obtained, of which 90.5% (19 out of 21) patients were nondiabetic and 74.1% (20 out of 27) diabetic patients. The wounds diseased with *Klebsiella pneumoniae* had relatively delayed healing [18]. Three lytic phages of cocktail APC 1.1, JWDelta, and JWT against the pan drug-resistant *Achromobacter xylosoxidans* due to the cocktail of phages treatment, the bacteria became favourable both susceptible to the applied phage cocktails [19]. Coli A11/58c, Kl 53N/1920, EF1/1679L, Ps1N/734, Coli 77/850 phage against the chronic urinary and urogenital MDR bacterial infection caused by *E.coli, K.variicola, K.pneumoniae, P.aeruginosa* there was a significant reduction in the bacterial load was observed [20]. A trend in the biofilm biomass reduction was distinguished after 22 hours of exposure to KpJH46Φ2 in the limb-threatening prosthetic knee *Klebsiella pneumoniae* infection [21]. Ab_SZ3 with z tigecycline and polymyxin treatment led to clearance of the carbapenem-resistant *Acinetobacter baumannii* lung Infection in the patient [22]. After treatment of AbW4878ø1 phage along with sulfamethoxazole/trimethoprim and tigecycline for a total of 35 days the health of the patient became good and discharged [23]. Phage M1 along with meropenem and colistin, followed by ceftazidime/avibactam was highly effective against the patient's *K. pneumoniae* strain in vitro, in 7-day mature biofilms and suspension [24].

Recent advances of phage therapy in veterinary medicine

Phages have also been effectively used for veterinary applications either to direct address infectious diseases in animals or for satisfying facts when about human clinical trials [25]. It is too significant to reveal that animal phage therapy trials do not have a similar degree of strict obstacles as human trials and are plausible to put a key pattern for phage therapy in the clinical setting. The earliest use of phages in veterinary medicine was conducted by D"Herelle in 1919, who showed their efficacy in averting and treating fowl typhoid (S. gallinarum) in six experimentally disease-ridden chickens [26].

Table 2. Recent advances of phage therapy in rats

Bacteriophage	Infection	Mode
A. baumannii phage	Dermal infection of MDR *A. baumannii* in diabetic rats	Topical application [27]
PaAH2ΦP, PaBAP5Φ2, and PaΦ13,	MDR *Pseudomonas aeruginosa* infecting lungs	Intraperitoneal injection [28]
ZCKP8 Phage	MDR Klebsiella pneumoniae infected rat wound	Topical application [29]
Bacteriophages	*Klebsiella pneumoniae* XDR strain	Oral gavage [30]
phage 2003, 2002, 3A, and phage K	*Pneumonia* due to MRSA	Nebulization, Intravenous Phages [31]

The above research in table 2 was carried out on the rats. A mischief of rats was infected with *MDR A. baumannii* and dared with bacteriophages. There was a substantial fall in disease, the phase of colonization, and injury shrinkage were noted in the phage dared group when related to the antibiotic-treated unrestrained diabetic rats also the control group. Swabs which were procured on day 2 exposed Gram-negative bacilli with more grade four neutrophils and MDR *A. baumannii*. The reduction in the inflammatory cells was detected on day four and six where the phages were administered after 48 hours, the bacterial load amplified on day 4, and got reduced on day six. On day 8 no bacteria were detected. Consequent swabs also did not disclose the existence of any bacteria or inflammatory cells, and no growth for MDR *A. baumannii*[27]. Phages PaAH2ΦP, PaBAP5Φ2, and PaΦ13 provided a significant survival benefit over the sub-efficacious dose of meropenem [28]. ZCKP8 phage exhibited the high therapeutic efficacy *in vivo* on the rat as it can treat full-thickness wounds disease-ridden with a *K. pneumoniae* clinical isolate, which was resistant to the multiple antibiotics [29]. Continued phage therapy for 28 days against *Klebsiella pneumoniae* XDR strain was found to be safe concerning animal haematology histopathology, body weight, feed intake, and behavioural parameters biochemistry [30]. The phage cocktail of equal 4 genetically unique phages called 2003, 2002, 3A, and phage K enhanced the animal survival and reduced MRSA burdens in tissues [31].

Table 3. Recent advances in phage therapy in mouse

Bacteriophage	Infection	Mode
Kp_Pokalde_002	Carbapenem Resistant *Klebsiella Pneumoniae*	Oral and intraperitoneal [32]
phiEF7H, phiEF14H1 and phiEF19G	*Enterococcus faecalis* - Endophthalmitis	Intra vitreously [33]
Lytic bacteriophages	The post-burn infections are caused by an opportunistic pathogen; *Pseudomonas aeruginosa*	Topical formulation [34]
Bacteriophage Cocktail	*Salmonella enterica serovar Typhimurium Burden*	Oral gavage [35]
Phage vB_PaeS-PAJD - 1	Murine mastitis by MDR *Pseudomonas aeruginosa*	Intramammary [36]
Virulent bacteriophage vB PaeP-SaPL	Multi drug-resistant *Pseudomonas aeruginosa PA-1*	Intra peritoneal [37]
Bacteriophage	Wild-type *Salmonella enterica* serovar Typhimurium	Oral gavage [38]
Pharr (P1), and ϕKpNIH-2	MDR *Klebsiella pneumoniae ST258*	Intraperitoneally [39]
TCUCAP1 Phages	MDR *Cutibacterium* infection	Intradermal injection [40]
S. aureus phage SaGU1 and S. epidermidis SE-4.	Atopic dermatitis with the suppressor of phage-resistant mutants	Applying topically [41]
Bacteriophage strain KPP10	*Pseudomonas aeruginosa* caused Pneumonia and Sepsis	Inhalation [42]
Lytic Phage SHWT1	MDR *Salmonella*	Oral administration [43]
vB_PaeP_PA01EW	*Pseudomonas aeruginosa* causing Pneumonia in Eight-week-old BALB/c mice	Intratracheal [44]

The above research in table 3 was carried in the mouse. The Kp_Pokalde_002 phage against carbapenem-resistant *Klebsiella Pneumoniae* there was a substantial drop of bacterial load (3^{-7} log10 CFU/ ml) in the blood and lung was observed in the treatment group [32]. phiEF7H, phiEF14H1 and phiEF19G phages lysed broad-range *E. faecalis*, including strains derived from endophthalmitis and mice got cured [33]. Lytic bacteriophages extracted from hospital sewage were collected from the third pond of sewage in Ghanem Hospital of Mashhad city and filtered by a membrane filter where the prepared ointment effectively prevented and treat the post-burn infections with no allergic reactions caused by an opportunistic pathogen, *Pseudomonas aeruginosa* which became complex due to its innate and attained resistance in mice [34]. They did not alter the intestinal microbiota of healthy mice and reduced microbiota perturbations induced by *Salmonella* [35]. The Phage vB_PaeS-PAJD – 1 protected the mice from mastitis infection by MDR *P. aeruginosa* [36]. Virulent bacteriophage vB PaeP-SaPL on 24 h post-inoculation single dose of a treated group the Multidrug-resistant *Pseudomonas aeruginosa PA-1* showed survival of 100 % (27 out of 27 mice) [37]. One purified phage filtrate which was obtained among the sewage samples with an average plaque size of 0.816 mm treatment demonstrated was more efficient against the test antibiotic ciprofloxacin as it selectively eradicated the Wild-type *Salmonella enterica* serovar Typhimurium infection [38]. The cocktail of phage Pharr (P1), a 40.6-kb podophage, and ϕKpNIH-2 (P2), a 49.4-kb Sipho phage, proved the extreme increase in survival against MDR *Klebsiella pneumoniae ST258* [39]. The mice inoculated with MDR *Cutibacterium* infection developed the inflammatory nodules After TCUCAP1 phage injection, the nodule size reduced which also decreased the manifestation of inflammatory marker IL-1β and apoptotic marker caspase-3 days [40]. *S. aureus* phage SaGU1 and S. epidermidis SE-4 possessed a sufficient efficacy against atopic dermatitis and that with the combinational use of probiotics and phages was effective in the treatment of *S. aureus*-associated atopic dermatitis [41]. Bacteriophage strain KPP10 had a protective effect against pneumonia caused by *P. aeruginosa D4.* Furthermore, the administration of phage at delayed time points of post infections and the survival rate in pneumonic mice got well improved [42]. Lytic Phage SHWT1 tolerated the pH and the temperatures and was able to effectively inhibit the growth of bacteria and biofilm caused by prevalent *Salmonella serovars.* Furthermore, phage SHWT1 exhibited lytic activity against the intracellular *Salmonella also* [43]. vB_PaeP_PA01EW effectively lysed the *Pseudomonas aeruginosa* causing pneumonia in eight-week-old BALB/c mice with a large surge of release after a short incubation time [44].

Table 4. Recent advances in phage therapy in other veterinary

Bacteriophage	Infection	Host	Mode
Cocktail of phage PP1450, PP1777, PP1902, PP1792 & PP179	Pneumonia caused by *Pseudomonas aeruginosa* during mechanical ventilation	Piglets	Mesh nebulizer [45]
Phage XC31	Yellow spot disease conchocelis (*Vibrio mediterranei 117-T6*)	*Pyropia haitanensis*	Application topically [46]
(Φ 16-izsam) and 39 (Φ 7-izsam)	Colonization of *Campylobacter jejuni*	Broiler Chickens.	Oral gavage [47]
20 vB_AsM_ZHF phages	*Virulent Aeromonas salmonicida subsp. masoucida*	Scophthalmus maximus	Intraperitoneal injection [48]
B_EcoM_SYGD1, vB_EcoP_SYGE1, and vB_EcoM_SYGMH1	Mastitis by AMR *Escherichia coli*	Cow	Intramammary injected [49]
Cocktail of three *Staphylococcus* phages fPfSau02, fPfSau03, and fPfSau04	MRSA	Healthy carrier pigs	Nares and skin [50]
IME-AB2 phage	Wound infections	Pigskin model	Applied topically [51]
KpG phage with streptomycin	*K. pneumoniae*	Zebrafish	Injected [52]
vB_ZEFP	MDR *Enterococcus faecalis Infection*	Ex Vivo Human Root Canal [53]	--
Cocktail of phages Escherichia phages EP1 and EP2 and also with cefotaxime	*Enterotoxigenic Escherichia coli*	*Galleria mellonella*	Injection [54]

Cocktail of phage PP1450, PP1777, PP1902, PP1792 & PP179 is used to treat Pneumonia caused by *Pseudomonas aeruginosa* during mechanical ventilation the piglet got cured [45]. Phage XC31The survival was 83%, exhibited higher photosynthetic ability and robust antioxidant capacity of *Pyropia haitanensis* to cope with the Yellow spot disease conchocelis (*Vibrio mediterranei 117-T6*) [46]. (Φ 16-izsam) and 39 (Φ 7-izsam) phage reduced C*ampylobacter* counts [47]. After the Injection of the 20vB_AsM_ZHF phage, they abridged the 24 mortalities in turbot challenged by *A.salmonicida subsp. Masoucida* [48]. Three phages B_EcoM_SYGD1, vB_EcoP_SYGE1, and vB_EcoM_SYGMH1 showed promised effect as antimicrobial agents especially when used as the cocktail to significantly reduce the number of bacteria, somatic cells, and inflammatory aspects lower the signs of mastitis by AMR *Escherichia coli* and accomplishes a similar effect as antibiotic treatment [49]. Cocktail of three *Staphylococcus* phages fPfSau02, fPfSau03, and fPfSau04 against MRSA There was no reduction in the MRSA levels in the sampled healthy carrier pigs [50]. The IME-AB2 phage reduced 90% bacterial count was achieved after the 4-hour treatment in the pigskin model [51]. *K. pneumoniae* infected Zebrafish were treated with phages alone was found to be 77.7% and also the combination of streptomycin showed a substantial 97.2% decline in CFU/ml [52]. vB_ZEFP phage is evidenced to be effective when used in a mixture with hypochlorite allowing for the use of dual therapies the phage has potential for effectiveness in the prevention of infection after root canal treatments [53]. The Single dose of EP1 cleared the *Enterotoxigenic Escherichia coli* infection while another phage EP2 was unable to eliminate the bacterial pathogen in *Galleria mellonella.* A phage cocktail consisting of EP1 together with EP2 protected the larvae from bacterial infection with a 100% larval survival rate. The phage-antibiotic synergy, a combination of phage with cefotaxime was done where there observed a 100% survival rate within 72 hrs., while there was no significant survival was observed in the control group [54].

Table 5. Comparison of bacteriophages and antibiotics

Bacteriophages	Antibiotics
Phages are bactericidal which extremely are efficient in killing their targeted bacteria	Several antibiotics are bacteriostatic which inhibit the growth of bacteria, instead of killing them (e.g., chloramphenicol)[55]
Production is easy and economical.	Manufacturing is complicated and costly.
Phages are an 'intelligent' drug. They augment at the infection site up until there are no further bacteria after that they are excreted out.	They are absorbed and thrown out from the body and they do not concentrate at the site of infection.
The pharmacokinetics of bacteriophage therapy is such that the primary dose increases exponentially if the sensitive bacterial host is available. In such cases, there is no need to direct the phages repeatedly.	Repetitive doses of antibiotics are required to treat bacterial disease.
The high specificity of bacteriophages permits the targeting of specific pathogens, without affecting advantageous bacterial flora.	Antibiotics reveal bactericidal or bacteriostatic effects not only on the cause of bacterial disease but also the microorganisms present in the body including the host normal microflora which lead to the patient's microbial imbalance and may lead to several side effects.
Because of phages specificity, their use is not likely to select for phage resistance in other (non-target) bacterial species	The broad-spectrum action of antibiotics may lead to resistant mutants of many pathogenic bacterial species.
Humans are exposed to phages all over life, and be able to bear them. No serious side effects have been described.	Various side effects, including allergies, bowel ailments, and secondary infections (yeast infections) have been described.
Phage-resistant bacteria remain vulnerable to other phages having an alike host range.	Endurance to antibiotics is not restricted to targeted bacteria.
Phages are found all over nature. This means that it is trouble-free to find new phages when bacteria become unaffected by them. Choosing a new phage (e.g., against phage-resistant bacteria) is a fast process and often can be accomplished in days.	Developing a new antibiotic against AMR bacteria is a long process and may take some years to achieve.
Phages may be counted as a good alternate for patients allergic to antibiotics.	If the patient is averse to an antibiotic, curing the disease is very difficult

CONCLUSION

On examining the isolated bacteriophage's behaviour and molecular studies in vitro, they can be used for the in vivo studies using some animal models and submission of the data to the regulatory bodies with proper approval they can be intended for human use. We can use a cocktail of phage, phage coupled with antibiotics, phage coupled with probiotic microbes, or phage with other inhibitory components so that the bacteria won't get resistant to bacteriophage. If bacteriophages were handled improperly, they may lead to phage-resistant bacteria. Thus, on proper examination bacteriophage can be intended to treat bacterial diseases which are a good alternative to antibiotics in day today's life.

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