Bacteriophage Treatment

Subjects: Virology

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The rapid and ever-growing increase in numbers of antibiotic resistance pathogens calls for alternative treatment options. Bacteriophage represents a well-suited option due to its adaptability and diversity. The review highlighted the significance of phage therapy and addressed the work needed in the clinical, experimental, regulatory, and manufacturing fields to emphasize phage's value as an antibacterial agent. Additionally, the review discussed effective implementation methods of phage therapy against infections such as antibiotic- phage combination therapy. Finally, although phage therapy has multiple challenges, undertaking it will improve treatment outcomes.

Keywords: phage therapy ; S. aureus ; K. pneumoniae ; P. aeruginosa ; E. faecalis ; combination therapy ; Bacteriophage targets

1. Introduction

Bacteriophages are non-living biological entities consisting of genetic material (DNA or RNA) enclosed within a protein capsid capable of infecting and replicating within bacterial cells [1]. They were first identified in 1915 by Frederick Twort and considered the most abundant organism on Earth, with estimated numbers ranging from 10^{31}–10^{32} [2]. Bacteriophages play significant roles in microbial dynamics, physiology, evolution, and therapeutics [3]. They are naturally occurring bacterial parasites, depending on the bacterial host for survival, and are incapable of reproducing independently. They replicate through two primary life cycles, the lytic cycle, where phages infect and rapidly kill their bacterial host, or the lysogenic cycle, where they either integrate their genome into the infected host cells (prophages) or exist as plasmids within the bacterial host [4].

The emergence of antibiotic resistance organisms poses a fundamental threat to public health worldwide. Because of that, phage therapy presents a promising alternative approach to combat emerging pathogens [5]. Bacteriophage (phage) therapy is defined as administering whole lytic phage or purified phage particles directly to a patient to lyse the bacterial pathogen that is causing the infection [6]. Although the practice of phage therapy has been around for a century, the idea of therapeutically using phages against bacterial infections has recently gained attention in response to the emergence of antibiotic resistance pathogens [3]. Advantages of using phages as treatment include (i) high specificity with most phages infecting only a single species of bacteria, (ii) low natural toxicity as they kill targeted bacteria without disrupting the host's normal flora or human cells, (iii) are unlikely to induce cross-resistance to antibiotics [7], (iv) anti-biofilm activity with bacteriophages in contrast to most antibiotics being able to penetrate and disperse existing biofilms and in some cases even prevent further biofilm formation [8], and (v) the presence of massive untapped natural repertoire of diverse bacteriophages offering numerous treatment options and potential combination cocktails. Phage therapy is effective against both antibiotic resistance and antibiotic sensitive pathogens. Their activity is not simply bacteriostatic but rather bactericidal, thus eradicating their target pathogens and thereby preventing bacterial evolution towards resistance [9]. Additionally, phage therapy also reverses antibiotic resistance and restore susceptibility to various classes of antibiotic in *Pseudomonas aeruginosa* [10].

2. Study Characteristics

Our initial search yielded 1082 articles (*S. aureus*; 531, *K. pneumonia*; 125, *E. faecalis*; 72, and *P. aeruginosa*; 354 articles). After reviewing and eliminating studies based on inclusion and exclusion criteria, we critically reviewed the full-text manuscripts of 29 studies. The specific details regarding infection and treatment, including the outcome for these studies are listed in Table 1. Most studies (75.9%) investigated the use of single phage rather than cocktails. In 5 studies, phages were used combined with antibiotics as a therapy approach; all others tested phages as a monotherapy. Animal/biofilm models used in the studies received phage doses ranging from 10^4 to 10^{10} plaque-forming units (PFU) per dose. The duration of phage treatment follows up in models ranged from one hour to thirty days.

3. Characteristics of Model Used

Out of the selected 29 studies, 18 studies used multiple in vivo models that were used to address and test the hypothesis, such as Albino mice (5.6%), BALB/C mice (50%), Swiss webster mice (5.5%), Sprague-Dawley rats (11.2%), rabbit (5.5%), C57BL/6 mice (11.2%), Wistar rats (5.5%), and 5.5% of the selected articles used unspecified mice. These models' average age ranged from one month to twenty weeks, with females being the predominant gender (eight studies used male mice). For the remaining eleven studies that assessed biofilm/bacterial growth, researchers used in vitro models in usually 96 well polyester tissue culture plates and test flasks to conduct their experiments. Animal or biofilm models were infected with *S. aureus* (24.1%), *K. pneumonia* (17.2%), *E. faecalis* (20.7%), and *P. aeruginosa* (27.6%). Two studies investigated both *S. aureus* and *P. aeruginosa* and one study observed the effect of phage therapy on *K. pneumonia*, *S. aureus,* and *P. aeruginosa.* Although the administration of phage therapy as a cocktail or single phage was done intraperitoneally in nine studies, five studies evaluated a novel intranasal approach as an alternative administration method for pneumonia treatment, one study each investigated the intravenous approach and oral delivery respectively, and in two studies a subcutaneous injection or through the skin mode was used for phage delivery. One study tested both intraperitoneal and intragastric inoculation. In the eleven exclusive in vitro studies, phages were added directly into the in vitro models.

4. Phage Isolation and Purification Protocol

Isolation and purification of bacteriophages are critical steps that can determine phage's utility for therapy. Although the basic and the most common method used for phage isolation is the enrichment procedure, some researchers isolate phage by directly plating environmental samples and look for plaque-forming units or spot culturing. For the enrichment procedure, the environmental source samples are rid of endogenous bacteria by centrifugation or filtration and then incubated with the target bacterial sample to assess for the presence of phage(s) [11].

In the 29 studies reviewed, bacteriophages were isolated majorly from sewage (55.2.%), and they were mainly detected and purified using a double-layer agar plate (DLA) and picking single plaques and re-plating multiple times. Phage concentration and purification majorly started with an enrichment step/culture step involving incubation with host bacteria, followed by centrifugation to eliminate debris and bacteria and or filtration or chloroform treatment to remove residual bacteria. Precipitation of phage particles in certain studies was achieved using polyethylene glycol, and the phage's activity in the supernatant was confirmed by spot assay/DLA on different agars specific for the host bacteria. Purified phages were stored in aliquots of phage buffer and glycerol or Luria-Burtani (LB) broth at 4 °C for temporary storage or at −80 °C for extended storage. Protocol variations between reviewed studies include centrifugation speed ranging from 3000× *g* to 8000× *g*, precipitant used, the addition of cesium chloride (CsCI), which blocks bacterial lysin activity, and the phage storage solution used.

5. Effectiveness of Phage Therapy as a Treatment Option

Among the studies analyzed, phage therapy's effectiveness was investigated predominantly in three clinical manifestations, pneumonia, biofilm, and bloodstream infections such as bacteremia and sepsis. In the 29 reviewed studies, 28 unique phage and phage cocktails were used to target pneumonia (seven studies), biofilms (nine studies), bacteremia (six studies), and sepsis (one study) and one study that analysis the role of phages in both biofilm and bacteremia model. In the sections ahead, these three prominent clinical manifestations will be discussed further, and the effect of phage therapy in these will be addressed.

5.1. Pneumonia

Among the seven studies that evaluated phage therapy against pneumonia, three studies targeted *P. aeruginosa* and two studies each evaluated *S. aureus* and *K. pneumoniae* respectively [12][13][14][15][16][17][18]. All seven studies showed the effectiveness of phage therapy against *P. aeruginosa, S. aureus,* and *K. pneumoniae* with a remarkable reduction in bacterial cell population and a significant improvement of survival to varying degrees in a dose-dependent matter. In one of the studies evaluating multiple phages, via intranasal approach to administer phage therapy against pneumonia, two of the nine tested phages had an insignificant improvement of mice's health and the bacteria were able to develop resistance, which occurred spontaneously 24 h post *P. aeruginosa* infections [15]. Out of the seven studies, only one study assessed the effect of combination therapy of phage with antibiotics (teicoplanin) administered intravenously to target *S. aureus.* The combination therapy did not improve the treatment outcome as no synergistic or antagonistic effects were

observed [18]. Four studies reported phage therapy's effect on cytokines level (TNF-a and IL-6), and a significant reduction was observed in the phage treated model compared to the control [13][14][16][18]. One study reported lowering of Lower IFN-γ, TNF-a, IL-1α, and IL-8 by phage therapy [17].

5.2. Biofilm

Nine studies assessed phage as a treatment option against biofilm formation. Among these studies, one article targeted biofilm produced by *P. aeruginosa,* two by *S. aureus,* three by *E. faecalis,* and one by *K. pneumoniae*. One study tested the effect of phages on dual infections caused by *S aureus* and *P. aeruginosa* and one tested their effect on both organisms individually [19][20]. In all the reviewed studies, bacteriophage was active against both planktonic cells and biofilm, showing a bactericidal effect and detachment activities against tested pathogens. Out of the nine studies, two evaluated the effect of combination therapy of phage with antibiotics against *S. aureus* using vancomycin/rifampicin, and teicoplanin, respectively [20][21]. The first *S. aureus* study illustrated a synergistic effect between vancomycin/rifampicin, and bacteriophage when phage was administered directly to tissue culture plates at a low concentration of phage (10^5 PFU/mL) and vancomycin (6 mg/L)/rifampicin (MIC of 0.016 mg/L) [21]. At higher concentrations, no synergy was seen. In the second study against *S. aureus,* teicoplanin administration combined with phage resulted in no visible biofilm in in vivo experiments [20].

Additionally, although the administration of teicoplanin in combination with phage degraded biofilm produced by *S. aureus*, the method of phage (intravenous) and antibiotics (Intraperitoneal) administration was different, which lacked justification in the paper [20]. One of these studies also tested the effect of combination therapy of phage with imipenem, cilastatin, and amikacin against *P. aeruginosa* in an in vivo biofilm model [20]. In this study, although both bacteriophage and bacteriophage plus antibiotic combination reduced bacterial load in the animal, they did impact biofilm thickness. Another study compared the activity of phages vs. antibiotic (tetracycline) against *S. aureus* and *P. aeruginosa* dual infection biofilm where tetracycline showed superior anti-biofilm activity than the tested phages [19].

Additionally, an *E. faecalis* study illustrated that a phage isolated from wastewater was able to target and degrade the biofilm at first, but then the bacteria were able to develop resistance against the selected phage. This was indicated by the resistance mutation in the enterococcal polysaccharide antigen gene (epa), which is necessary for the infectivity of phage [22].

Although all reviewed studies showed significant degradation and bactericidal effects on biofilm, the mechanism by which phage can disperse biofilm is yet to be explained. Possible mechanisms include the expression and secretion of depolymerizing enzymes by phages that degrade extracellular polymeric substances (EPS) or because of potentially high capacity of phages that allow it to infect metabolically inactive persister cells found within biofilms. These are topics that need further investigation.

5.3. Bloodstream Infections: Bacteremia and Sepsis

From the 29 reviewed studies, five articles assessed the impact of phage therapy on bacteremia in *P. aeruginosa* (16.7%) [23], *E. faecalis* (16.7%) [24], *K. pneumoniae* (33.3%) [25][26], and *S. aureus* (16.7%) [27], and one tested the efficacy of phage against sepsis model specifically against *E. faecalis* [28]. One particular study tested both host specific phage against *K. pneumoniae* and *P. aeruginosa* infections individually and the combined phage cocktail against both single and polymicrobial infections caused by these two bacteria [29]. All studies showed high efficacy of phage treatment by rescuing the model used from death caused by bacteremia. Among the six studies, two evaluated the effect of delayed treatment on the bacteremia model used. The results illustrated that delayed treatment could reduce the protection rate as seen by differences in survival rate between page therapy at 10 min (100% survival) vs. 1 h (12.5% survival) after infection in one of the studies [26] and between survival rate at 4 h (90% diabetic mice and 100% non-diabetic mice) and survival rate at 8 h (90% diabetic mice)/20 h (0% non-diabetic mice) in the other study [28]. In the other study which tested delayed treatment effect, a negative impact on the health score of the animals was seen in response to treatment delays (18 h after bacterial challenge), but this did not impact the survival rate with the eventual recovery of health back to normal within four days .

In comparison to antibiotics, one study that tested oxacillin efficiency vs. phage on *S. aureus* infection reported significantly less viable bacteria when phage was administered vs. control in contrast to antibiotic (oxacillin) treatment . Of interest, no studies noted adverse events with phage administration, and only one study data reported the emergence of bacterial resistance against the tested phage . Most of these studies did not evaluate the combination of phage therapy with antibiotics or critically evaluate resistance development and associated mechanisms. Only one study reported phage therapy's effect on cytokines level and identified statistically significant lower TNF-α, MCP-1, IL-10, and IL-6 upon phage

therapy. Only one study out of the 29 reviewed studies evaluated phage therapy's effect on *E. faecalis* infections' sepsis model. In this study, both the phage as well as the phage lysin and both phages and the endolysin reduced bacterial load and protected mice from lethal challenges (survival rate of 60–80%).

6. Phage Safety and Efficacy

Among the 29 reviewed studies, many investigated the phage's safety and efficacy in successfully reducing bacterial growth and enhancing clinical outcomes without significant side effects. Despite the studies using different dosages, phage administration routes, and different infection models, no adverse effects were reported.

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