



A review on re-emerging bacteriophage therapy in the era of XDR

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Abstract: In the present medicine world antibiotic resistance is one of the key threats to universal health coverage. Researchers continue to work hard to combat this global health concern. Phage therapy, an age-old practice during the early twentieth century, was outshined by the discovery of antibiotics. With the advent of widespread antibiotic resistance, phage therapy has again redeemed itself as a potential alternative owing to its adeptness to target bacteria precisely. Limited side effects, the ability to migrate to different body organs, a distinct mode of action, and proliferation at the infection site, make phages a profitable candidate to replace conventional antibiotics. The progressive outcome of numerous *in vitro* studies and case reports has validated the clinical efficacy of phage therapy. The bright perspective of using phages to treat bacterial infections has fueled enormous medical research to exploit their potential as therapeutics. The gaps in the information about phages and the lack of consent for clinical trials is major hurdle for consideration of phage therapy. Crafting phage therapy as a reality in medicine requires a coordinated effort from different fraternities. With this review, we aim to emphasize the importance of phage therapy in modern medicine. This review explains their historical journey, basic phage biology, cross-talk with the host immunity, obstacles with phage therapy, and their possible remedies. Comprehensive data on the various significant clinical trials of phage therapy has been presented. We evaluated the efficacy of antibiotics and phage therapy in part and in combination, along with recent progress and future perspectives of phage therapy.

Introduction

Antibiotic-resistant bacterial strains have become a major problem in contemporary medicine. Since antibiotics have established themselves as effective antibacterial medicines, they have adopted multiple forms. Consequently, the constant antibiotic pressure has led to the emergence of antibiotic-resistant bacteria, some of which are MDR (Multi Drug Resistant)-bacteria unable to be treated with multiple antibacterial drugs. Such circumstances have brought forth the post-antibiotic era, which began in the late 2000s (Loganathan *et al.*, 2021). A group of the most common multidrug-resistant bacteria has been abbreviated ESKAPE, an acronym for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species* (Rice, 2008). The multidrug-resistant (MDR) strains of

Mycobacterium tuberculosis, the causative agent of MDR-TB (Multidrug-resistant tuberculosis), a major public health issue around the world, is of grave concern (Palomino and Martin, 2014). These *Mycobacterium* strains are mostly resistant to rifampicin and isoniazid, two important drugs used to treat the disease. Since 2006, the presence of even more resistant strains of *M. tuberculosis* known as extensively drug-resistant (XDR)-TB has been recognized (Gandhi *et al.*, 2006). Even more recently, a troubling situation has emerged with the characterization of *M. tuberculosis* strains found to be resistant to all antibiotics tested, a situation known as totally drug-resistant (TDR)-TB (Velayati *et al.*, 2009; Udwardia *et al.*, 2012).

One of the effective therapeutic options for use against highly resistant and incurable bacterial infections is bacteriophage-therapy. The perspective of bacteriophage usage for therapeutics has regained interest in view of widespread antibiotic resistance and enhanced understanding of the significance of the human microbiome. The antibacterial nature of the phage promises chances of a successful post-antibiotic era (Cisek *et al.*, 2017).

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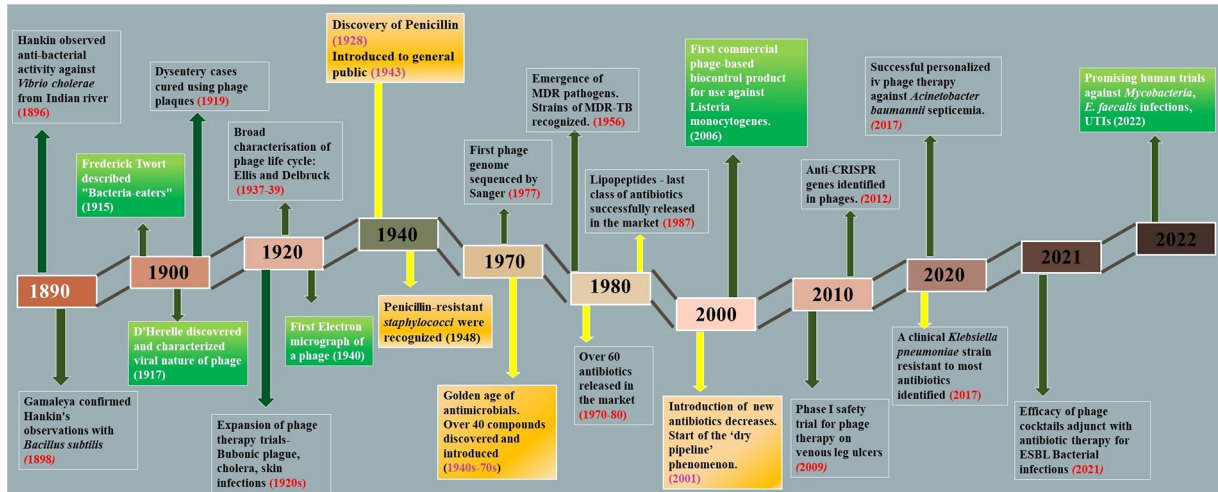


FIGURE 1. A timeline of notable developments in the history of phage research and phage therapy. The important milestones in the field of phage research and phage therapy are illustrated in the boxes. The high and low in the timeline represent the increase and decrease of research interest in phage therapy.

Bacteriophages were discovered over a century ago by two scientists, William Twort and Felix d'Herelle, independently. A bacteriophage is a common virus that feeds on bacteria (host) and may kill it in due course of time, serving as a means for releasing progeny phage. This discovery sparked controversy and excitement due to their unknown biology and potential for treating bacterial infections, respectively. Bacteriophages have since been known to be 'bacterial viruses' that infect specific bacterial strains and inhibit their growth, thus acting as a curative agent for bacterial diseases (Moelling *et al.*, 2018). A decade before the discovery of penicillin in 1928, bacteriophages were used to treat various bacterial infections. Physicians in countries like Georgia, Poland, and Russia continue to use phages as therapeutic agents and generate valuable practical experience from them (Kutateladze and Adamia, 2010). Myelnikov has elaborately explained in his publishing the adoption and survival of bacteriophage therapy (Myelnikov, 2018). In the United States, the incident of Tom Patterson, who was infected in Egypt by a resistant strain of *A. baumannii*, was brought back to the United States in critical condition and then cured by phages at the University of California, San Diego. This incident has spawned a number of articles and even a book in its praise. Several clinical trials are currently underway or will begin in the coming months. The number of scientific studies on the subject has increased significantly (Brives and Pourraz, 2020). Promising human trials against *Mycobacteria*, *E. faecalis* infections and urinary tract infections (UTIs), etc., are being carried out presently, providing the medicine world with hope for a better future in the regimen of antibacterial agents.

In this review, we highlight the significance of phage therapy in modern medicine, providing their history, mode of action as therapeutic agents, along with interaction with the host immune system. We have also delved into the obstacles with phage therapy and its solution, as provided by researchers. The review gives comprehensive data on the various significant clinical trials that have been done using the approach and the trials under progress. We evaluated

the efficacy of antibiotics and phage therapy in part and in conjunction. We also tried to highlight the recent progression made in phage therapy, and its future perspectives. The review also highlights the current scenario of phage therapy in India, battling with a multitude of cases of antibacterial resistance.

Golden history of phages

To begin with, bacteriologist Ernest Hanbury Hankin, in the year 1896, published in French in The Annals of the Pasteur Institute about a 'biological principle' capable of destroying the cultures of *Vibrio cholerae*. The 'principle' could pass through the Millipore filters that retained bacteria. He discovered them while he was appointed as the Chemical Examiner and Bacteriologist in the Central Provinces of India and found that they were present in the waters of the Ganga and Yamuna rivers in India (Fig. 1) (Wittebole *et al.*, 2014). In the year 1915, Frederick Twort, a British microbiologist, while trying to grow Vaccinia virus on agar media, observed a "glassy and transparent" transformation on agar plates where bacteria would not grow even when sub-cultured. This transformant could pass through a fine porcelain filter that would trap bacteria and could also break down the bacterial culture into granules. Before Twort could conduct further experiments, however, the First World War interrupted his career. In 1917, independent of Twort's research, Felix d'Herelle, who was a French-Canadian microbiologist, published a short paper about the research he carried out on the enteric bacteria of dysentery patients at the Pasteur Institute, describing the lysis of bacteria over multiple propagations. He coined the term bacteriophage (a bacteria-eater), justifying that there was some "invisible microbe that is antagonistic to the dysentery bacillus." Following this discovery, phages were used to cure cases of dysentery and clinical trials were expanded to experiment efficacy of phages on other bacterial infections like bubonic plague, cholera, and other skin infections. A major breakthrough occurred in 1939, when Ellis and Delbruck proposed the one-step growth curve for phage replication

(Fig. 1) (Cann, 2016). However, this golden period did not last long, as a major antibacterial compound penicillin was accidentally discovered by Alexander Fleming in 1928 and by the mid-to-late 1940s, it was made widely available for the general public. During the period of 1930s–40s, phage therapy saw a rapid decline (Fig. 1). Sooner, penicillin-resistant *Staphylococci* was also identified, but the discovery of many other antibiotic compounds continued. The development of sulfonamides (which were potent antibacterial agents) in Germany in the 1930s and the large-scale production and use of antibiotics in the United States from the 1940s onward coincided with the nearly complete departure of phage therapy in the following years (Myelnikov, 2018). More than 40 antibiotics were developed and made available for clinical use during the “golden period” of antibiotics, which lasted from the 1940s to the 1970s (Fig. 1).

During this period (1940–1970s), antibiotic resistance was viewed with minimal concern due to the rapid development of newer compounds, which fueled the cycle of antibiotic discovery. These discoveries somehow side-lined phage therapy and its trials. The second half of the 20th century was thus marked by a dramatic reduction in infectious diseases. It made antibiotics one of the greatest accomplishments in the history of medicine (Brives and Pourraz, 2020). However, beginning in the 1990s, a steady decline in the use of novel antibiotics made the effects of this cycle more obvious. The majority of newly released antibiotics seemed to be either modified or combined versions of previously recognized compounds, a phenomenon known as a “dry pipeline” in antibiotic research and development. The medicine world soon took another turn when bacteria started to become resistant to antibacterial compounds, and it became difficult to treat many mild to severe infections with common antibiotics. In 1956, the first MDR (multi-drug resistant) *Mycobacterium* was identified, and in 1987, the last antibiotic, Daptomycin of the class of lipopeptides, was discovered for the cure of serious gram-positive bacterial infections. Pathogens that were multidrug-resistant (MDR), extensively drug-resistant (XDR), and even pan-drug-resistant (PDR)-resistant to all current antibacterial agents were developed as a result of the continuous accumulation of antibiotic resistance traits by the host. The post-antibiotic era began with the emergence of ESKAPE pathogens. Persistent antibiotic usage has resulted in the emergence of MDR and XDR bacteria, rendering even the most effective drugs ineffective. This situation, described by the World Health Organization (WHO) as being a critical priority for research and development, calls for immediate development, standardization, and application of novel treatment options against infectious diseases, and phage therapy is once again gaining recognition. With the start of a new century, phage therapy got another chance to rekindle interest among scientists. Several attempts to develop phage-based biotechnologies in human health, animal health, or biocontrol in the agri-food sector occurred in the late 1990s and early 2000s (Fig. 1) (Kuchment, 2011). Phage therapy has been used occasionally in France and Belgium to treat patients in cases of therapeutic failure (Patey et al., 2018).

Over the past years, phage therapy has been under development again in laboratories and hospitals. Many successful trials have already been conducted and are still going on, which promise a high potential of phage therapy.

Phage biology

As a measure to combat multidrug-resistant infections, phage therapy is believed to be a potential adjunct to the existing conventional antibiotic treatments. The life cycle of a bacteriophage explains its antibacterial mode of action. It can infect its host chiefly in two ways-lytic and lysogenic (Fig. 2).

The distinction between lytic and lysogenic bacteriophages is based on how the replication cycle of a phage proceeds (Cisek et al., 2017). Phages bind to specific receptors on the bacterial cell surface and infuse their genetic material into the host cell. Following this, they can proceed with two mechanisms, the first one being lysogeny which involves the integration of the phage genome into the bacterial genome, and then, it re-produces vertically from mother to daughter cell. Besides lysogeny, a phage can undergo a lytic cycle, wherein it invades the host replication machinery to produce progeny phages and eventually lyse the cell. For therapeutic purposes, lytic phages are inevitably chosen. Some of these lytic bacteriophages use antimicrobial peptides-Amurins to suppress the synthesis of peptidoglycan, an important component of the bacterial cell wall (Woźnica et al., 2015). However, the majority of them use two sets of proteins to lyse the bacterial cell. These are Holins and Endolysins, which work synergistically to cause lysis, collectively called the Holin-lysin system (Fig. 3) (Loessner, 2005; Drulis-Kawa et al., 2015).

Amurin peptides are lytic antimicrobial peptides that are also known as DLAs (Direct Lytic Agents). Recently, both engineered and native DLAs with potent bactericidal activity against a variety of Gram-negative pathogens, including MDR and XDR *P. aeruginosa*, *K. pneumoniae*, and *Acinetobacter baumannii*, have been identified. In addition to their effectiveness, Amurins have been shown to clear biofilms and work synergistically with a variety of standard-of-care antibiotics *in vitro* (Schuch et al., 2022). Holins are involved in the process of triggering host cell lysis. Their function is to perforate the cytoplasmic membrane of the host (Fig. 3). This provides endolysins access to bacterial peptidoglycan. Hence, holins determine the time of bacterial lysis. They act at a specific time point, control bacterial murein accessibility for phage endolysin, and synchronize the activity of the holin-lysin system (Dewey et al., 2010; Shi et al., 2012). Enzymatic proteins called phage endolysins have the function of breaking down cell walls (Wang et al., 2000). They are utilized by bacteriophages to hydrolyze the peptidoglycan of infected bacteria (Fig. 3) (Schmelcher et al., 2012).

Endolysins carry out endopeptidase, amidase, glycosidase, or lytic trans glycosylase activities to degrade murein and destroy bacterial cells (Donovan and Foster-Frey, 2008; Nelson et al., 2012). They induce the release of progeny virions by acting at the end of the phage replication cycle (Fig. 3). Endolysins that target gram-positive bacteria have different structures than those targeting gram-negative

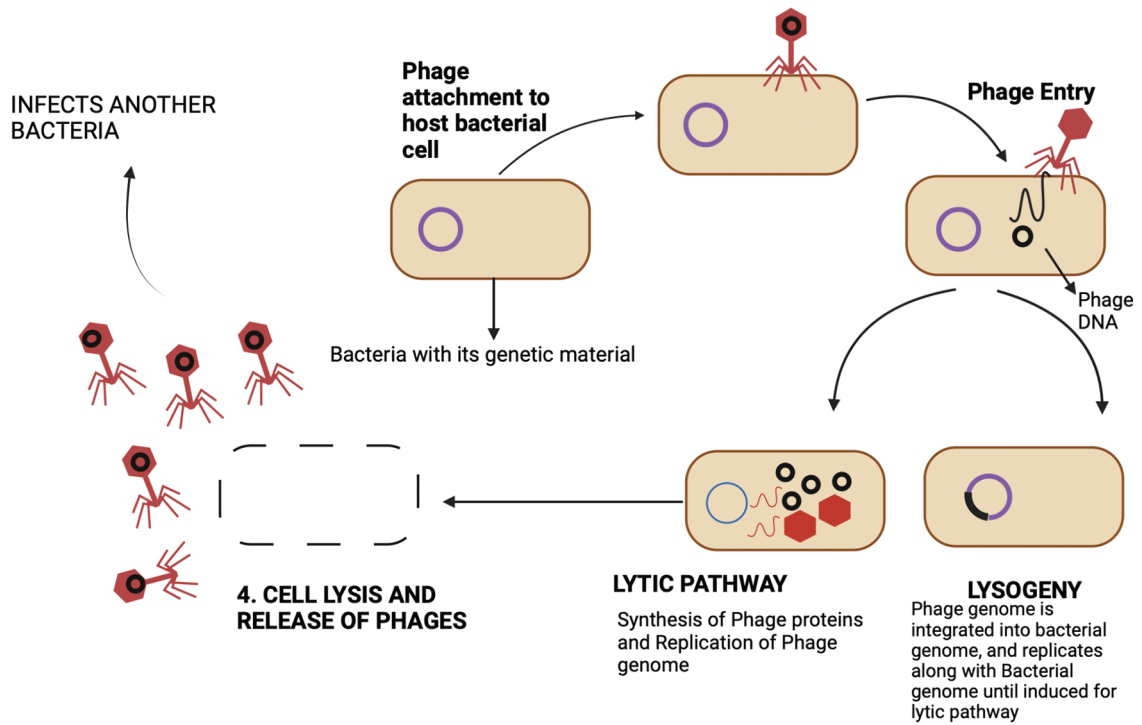


FIGURE 2. Bacteriophage life phases-Following the initial attachment of a bacteriophage to the host-cell receptor, the genetic material of the phage is introduced into the host cell, where it multiplies to produce many copies of the virus before lysing the cell wall and releasing progeny viruses to continue the cycle. This is known as the cycle of lytic replication. Alternatively, the lysogenic cycle can also be established by bacteriophages. In this case, the phage genome integrates into the host’s genome and multiplies along with it rather than giving rise to virus particles. While some lysogenic bacteriophages can be induced to enter the lytic cycle, others continue to be lysogenic.

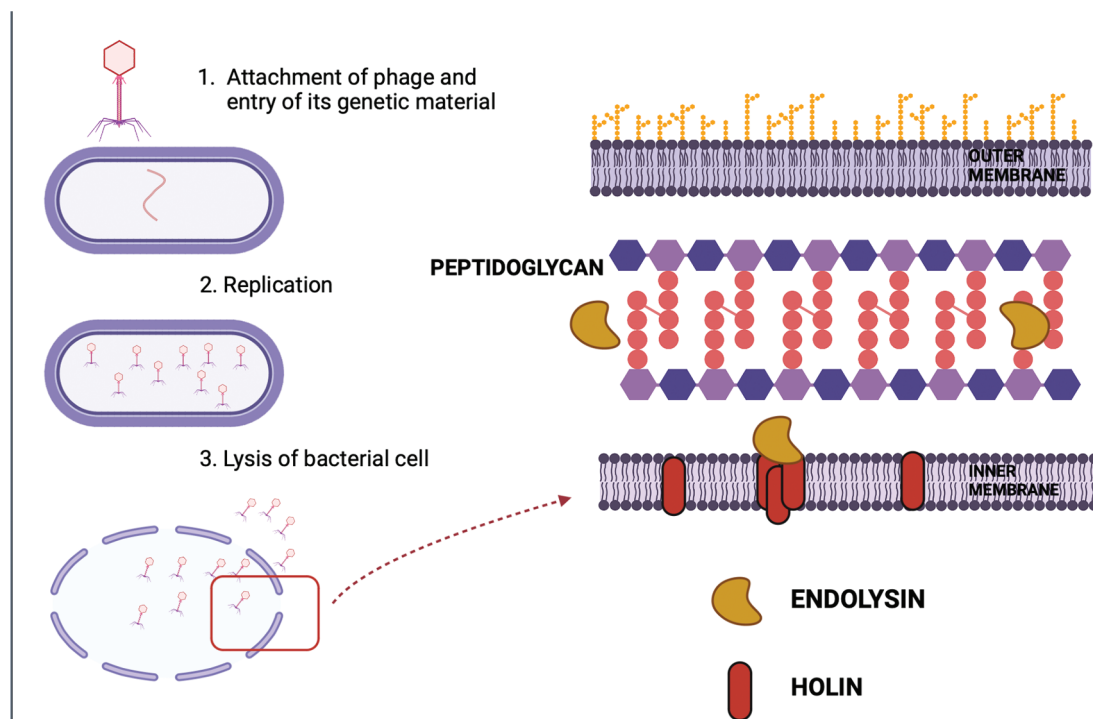


FIGURE 3. Overview of Holin-Endolysin mechanism of lysis: The figure depicts the mechanism of lysis of a bacterial cell. Two major proteins are involved in the process-Holin and Endolysin. Holins perforate the cytoplasmic membrane of the host and allow the endolysins to ultimately break down the peptidoglycan and lyse the cell.

bacteria, reflecting differences in enzyme targets’ cell wall architecture. Gram-positive bacteria have hydrolytic enzymes with two functional domains- the cell wall binding

domains (CBDs) and the enzymatically active domains (EADs). While EAD is the main catalytic site actually fueling the breaking of bonds in the peptidoglycan, CBD is

in place to avoid any collateral damage. Cell wall debris is tightly bound to it after the cell lysis to keep it from diffusing and destroying the cells that are not yet infected by phages (Borysowski *et al.*, 2006). Gram-negative bacteria, on the other hand, have an outer membrane in place that limits access to the peptidoglycan and prevents such collateral damage. Therefore, enzymes directed against them appear to be small globular proteins with only one domain, known as the enzymatically active domain (EAD). The holin-lysin system terminates the cycle of phage infection at a specific time point. The 'dual-start model' explains how the effect of endolysins on the bacterial cell wall is coordinated. According to it, the proportion of holin and its antagonist-antiholin, determines the time of bacterial lysis. The holin-antiholin ratio is maintained through the regulation of their translation. Loss of plasma membrane integrity occurs when there is an increase in the holin-antiholin ratio, which enables endolysins to enter the periplasm and eventually break down the host peptidoglycan, eventually lysing the bacterial cell (To and Young, 2014).

Bacteriophage receptors on the host cell surface

Phage-cell interactions are seen as a process with numerous sequential stages: phage adsorption on the host cell surface and entry of phage genome into the cell, intracellular synthesis of viral components and assembly of virions,

bacterial cell lysis, and phage re-lease (Rakhuba *et al.*, 2010). A particular bacteriophage strain is believed to be capable of infecting a narrow host range or microbial species. Such specificity in the interaction of the phage with the host cell is determined by the specificity of adsorption, which is in turn determined by the nature and the structural characteristics of receptors on the bacterial cell surface of both gram-positive and gram-negative bacteria (Fig. 4). The outer membrane of gram-negative bacteria is structurally distinct from the inner membrane and plasma membrane of gram-positive bacteria. There are two distinguishing characteristics- its high permeability, which is caused by increased levels of integral proteins forming transport channels (up to 20,000 per cell) (Nikaido, 2003), and the presence of a unique glycolipid lipopolysaccharide (LPS) in the external lipid layer, which is typically found only in gram-negative bacteria. Bacteriophage receptors could be proteins found in the membrane and at various LPS sites. In many cases, phages require both types of molecules for adsorption (Lindberg, 1973).

Outer membrane proteins are classified into five types: 1) structural proteins that interact with the peptidoglycan layer, 2) specific and non-specific porins that form membrane channels, 3) enzymes, 4) high-affinity substrate receptors, and 5) transport proteins that are involved in secretion. Bacteriophage binds with protein-LPS complex, and precipitation of this complex with Mg^{+2} leads to irreversible phage attachment (Datta *et al.*, 1977).

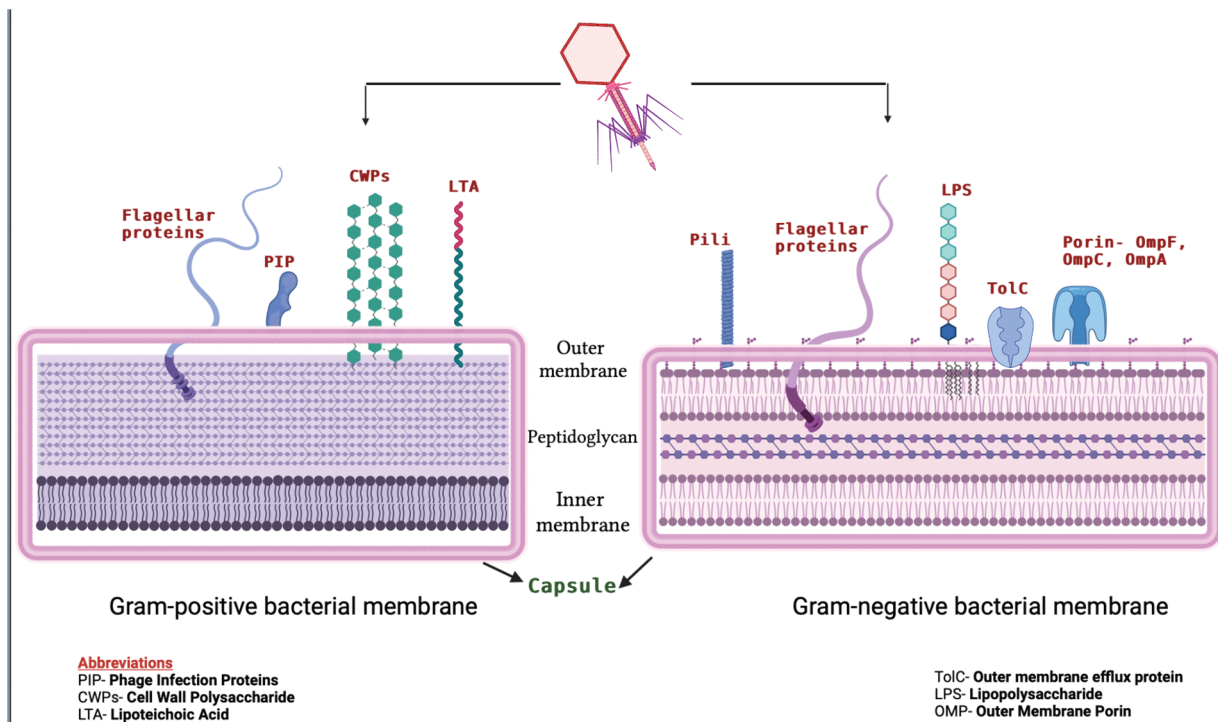


FIGURE 4. Schematic depiction of bacteriophage receptors on the surface of Gram-positive (left) and Gram-negative (right) bacteria. In the figure, Gram-positive and Gram-negative bacteria cell surface-associated virulent components that serve as phage receptors have been depicted. On the left, the receptors for Gram-positive bacteria are shown, which include surface-exposed LTA (lipoteichoic acid), PIP (phage infection proteins), and CWPS (cell wall polysaccharide). LPS (lipopolysaccharide) and CPS (capsular polysaccharide) are two surface-associated glycopolymers that Gram-negative bacteria (shown on the right) possess as some of their primary receptors for phages. A number of outer membrane proteins, including the porins-OmpC, OmpF, and TolC, have also been discovered to serve as the secondary receptor for the phages of Escherichia, Salmonella, and Vibrio.

Host specificity

The host specificity of phages varies greatly; some phages have demonstrated the potential to infect a wide variety of bacterial strains and even genera, while others are highly strain-specific (Koskella and Meaden, 2013). For example, some of the phages specific to *Clostridium* include-Susfortuna, CPS1, CPS2, phiCP39O, and phiCP26F, among others (Venhorst et al., 2022). According to Paolozzi and Ghelardini (2006), bacteriophage Mu can infect species of *Escherichia coli*, *Citrobacter freundii*, *Shigella sonnei*, *Enterobacter*, and *Erwinia*; the staphylococcal phage 812 infects 95% of the 782 strains of *S. aureus* and 43% of the other *Staphylococcus* species tested. Meanwhile, bacteriophage P-27/HP infects 60% of the 28 *S. aureus* isolates examined by Gupta and Prasad (2010). They are all characterized as polyvalent or having a wide host range. The majority of phages are exclusively infectious to bacteria that carry their complementary receptor, restricting the host range of a lytic phage. Typical lytic phages associated with human pathogens and the gut microbiome belong to the newly assigned class caudoviricetes (earlier in order caudovirales), which include all “tailed phages” of bacteria and archaea having double-stranded DNA genomes and *Microviridae*, which have single-stranded DNA genomes and are tailless (Turner et al., 2023). Traditionally, phage therapy has relied on lytic phages, which obligately kill their bacterial host. For treatment, lytic phages are combined into “phage cocktails,” which consist of various phages that have been shown to be effective against the target pathogen *in vitro*. To date, no serious adverse effects from phage therapy have been identified, justifying their use against bacterial infections. However, it is important to mention here that phages used in therapy should not contain any genes encoding virulence factors or protein toxins. The presence of such factors/genes can benefit the bacterial host by encoding virulence factors (such as diphtheria toxin, shiga toxin, and botulinum toxin), metabolic genes, and antibiotic resistance genes (such as β -lactamases). Toxin and virulence factor production is undesirable (Lin et al., 2017). Therefore, it is important to identify the most suitable phage for the treatment of an infection. Preclinical studies, both *in vitro* and *in vivo*, should prove that the phage meets all requirements for it to be developed into a pharmaceutical product (Manohar et al., 2019). There is considerable evidence that bacteriophages are part of the healthy human microbiota/virobiota, and numerous studies are being conducted to evaluate their role in the microbiome, which may imply that the human immune system does not perceive bacteriophages as a threat (Navarro and Muniesa, 2017; Minot et al., 2011).

Phage against Clinically Significant Pathogens

The first known therapeutic use of phages occurred in 1919 when d’Herelle and several hospital interns ingested a phage cocktail (later designated as Intesti phage) to check its safety. Later, they gave it to a 12-year-old boy with severe dysentery. With just a single dosage, the boy’s symptoms subsided, and he recovered completely within a few days. However, D’Herelle did not publish his conclusions until 1931. Time and again, phage therapy has been explored in

studies against a variety of clinically significant pathogens utilizing various animal models. Human trials for bacteriophage therapy have also been conducted, mostly at institutes in Eastern Europe. The most well-known of these institutes are the Eliava Institute of Bacteriophage in Georgia and the Institute of Immunology and Experimental Therapy in Wroclaw, Poland. The Eliava Institute has significantly used phages in the preclinical and clinical treatment of common bacterial pathogens such as *E. coli*, *S. aureus*, *P. aeruginosa*, *S. dysenteriae*, *Streptococcus spp.*, *Salmonella spp.*, *Enterococcus spp.*, and *Proteus spp.* (Kutateladze and Adamia, 2008). Some of the important promising clinical trials on humans and animal models against bacterial pathogens with their outcomes are listed in Table 1 in their chronological order, along with some ongoing trials. Clinical trials using various phage-bacteria combinations offer heterogeneous results. Results vary from some patients experiencing complete bacterial clearance while others remain without any impact (Vandenheuevel et al., 2015). The probable reasons behind some of the failures during phage treatment are discussed in different reports of randomized and double-blind clinical trials specifically carried out for this purpose. The enlisted reasons include-Ambiguity of intestinal bacterial infections caused by other co-infecting bacteria, interference by antibiotic treatments or too low titers of phages or target bacteria (Rose et al., 2014; Jault et al., 2019).

Immune System Response to Phage-therapy

Research and analyses on the interactions between bacteriophages and the immune system are crucial for the appropriate use of phage therapy as it carries a risk of immunological reactions. The location of the bacterial infection and the therapeutic phage administration site influences the immune response towards the phage (Reyes et al., 2012). Antibodies are known to be induced during oral administration of phages during therapy for bacterial infections caused by *Escherichia*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Staphylococcus* (Dabrowska et al., 2005). Anti-phage antibodies have been reported in the serum of many species (e.g., humans). These are indicative of the natural interaction of animals/humans with different types of phages (Smith et al., 1987). Cellular immunity, in addition to the humoral immune response, is crucial in the defense against phages. Since phages consist mostly of proteins and nucleic acids, they are inherently non-toxic. Additionally, the topical applications of phages during clinical studies have not shown any side effects as far as studies have been carried out (Wright et al., 2009), and so far, any immunological complications have not been reported after the administration of phages (Sarker et al., 2012). Overall findings point to the importance of testing each phage’s immune response, especially if intravenous therapy is being considered. A recent comparative study on the immunogenicity of two therapeutic bacteriophages-A3R and 676Z, active against *S. aureus*, has been carried out in murine models. When compared for their overall ability to induce specific antibodies, these two phages were able to produce IgM and IgG. Further, there were no enhanced

TABLE 1

Table enlists the details of phage therapy clinical trials and studies on animal models in chronological order

Causative agent	Model	Condition	Mode of administration	Phage/phage preparation used	Result	Ref.
<i>Shigella</i>	Human	Bacterial dysentery	–	<i>Shigella</i> phages	<i>Shigella</i> phages were successfully used for prophylaxis of bacterial dysentery	Babalova <i>et al.</i> (1968)
<i>Pseudomonas</i> , <i>Staphylococcus</i> , <i>Klebsiella</i> , <i>Proteus</i> , and <i>E. scherichia coli</i>	Human	Supportive Skin Infections	Oral/i.v.	Cocktail	Thirty-one patients having chronically infected skin ulcers were treated orally and locally with phages. The success rate was 74%	Cislo <i>et al.</i> (1987) Slopek <i>et al.</i> (1987)
<i>Shigella dysenteriae</i>	Human	Dysentery	Oral	–	All four treated individuals recovered after 24 h	Chanishvili, (2012)
<i>Vibrio cholerae</i>	Human	Cholera	Oral	–	68/73 survived in the trial group. 44/118 in the control group	Torabi <i>et al.</i> (2021)
<i>Vancomycin-resistant Enterococcus faecium</i>	Murine	Bacteraemia	Intraperitoneal (i.p.)	<i>Enterococcus</i> phages: ENB6 and C33	100% reduced mortality	
<i>Pseudomonas aeruginosa</i>	Murine	Gut-derived sepsis	Oral	KPP10 strain	Reduced mortality by 66.7%	Watanabe <i>et al.</i> (2007)
<i>Salmonella typhi</i>	Human	Typhoid	Oral	Intesti phage	In the cohort of 18577 children, phage treatment associated with a 5-fold decrease in typhoid incidence compared to placebo	Kutateladze and Adamia (2008)
<i>Antibiotic-resistant P. aeruginosa</i>	Human	Chronic Otitis	Oral	Bio phage-PA	Phage treatment was safe and symptoms improved in a double-blind, placebo-controlled Phase I/II trial	Watanabe <i>et al.</i> (2007)
<i>Escherichia coli</i>	Murine	Meningitis and Sepsis	i.p. or subcutaneous	Lytic phage, EC200PP	100% and 50% reduced mortality for meningitis and sepsis, respectively	Pouillot <i>et al.</i> (2012)
<i>MDR Vibrio parahaemolyticus</i>	Murine	Sepsis	I.p. and oral	Phage-pVp-1	92% and 84% reduced mortality for i.p. and oral routes, respectively	Fish <i>et al.</i> (2016)
<i>MDR Staphylococcus Aureus</i>	Human	Diabetic foot ulcer	Topical	<i>Staphylococcal</i> phage Sb-1	All six treated patients recovered	Jun <i>et al.</i> (2014)
<i>Methicillin-sensitive Staphylococcus aureus (MSSA)</i>	Human	MSSA prosthetic knee-joint infection	Into the joint	PP1493, PP1815, and PP1957 phage cocktail	Suppressive therapy, Beneficial with a clinically substantial improvement in function	Ferry <i>et al.</i> (2020)
<i>Klebsiella pneumoniae</i>	Human	Burn ulcers (ESBL bacterial infection)	<i>In vitro</i>	Three phages: PΦBw-Kp1, PΦBw-Kp2, and PΦBw-Kp3.	Isolated bacteriophages, in the form of phage cocktails, had a strong antibacterial impact and a broad-spectrum strategy	Torabi <i>et al.</i> (2021)
<i>E.coli</i>	Human	Urinary tract infection	Intravesical	Pyo bacteriophage	The treatment success rate was not different from that of antibiotic treatment/placebo	Leitner <i>et al.</i> (2021)
<i>Mycobacterium</i>	Human	Non-tuberculosis <i>Mycobacterium</i> (NTM) infections	Intravenous (i.v.)/ Aerosolization	Multiple phage cocktails	Cohort of 20 patients. 11/20 patients displayed symptom improvement or reduced bacterial presence. Five had inconclusive outcomes, and four exhibited no response to treatment	Dedrick <i>et al.</i> (2022)

(Continued)

Table 1 (continued)

Causative agent	Model	Condition	Mode of administration	Phage/phage preparation used	Result	Ref.
<i>Enterococcus faecalis</i>	Humanized mouse model	Alcohol-associated hepatitis	Oral	Cytolytic <i>E. faecalis</i> phages	Anti-inflammatory and tissue-restoring effects	Mendes <i>et al.</i> , (2022)
<i>P. aeruginosa coli</i>	humans	Cystic fibrosis	I.v.	Bacteriophage product-WRAIR-PAM-CF1	Ongoing	Clinical trial registration No. NCT05453578
Multi-drug target (<i>E. coli</i> , <i>P. vulgaris</i> , <i>K. pneumoniae</i>)	human	Acute tonsilitis	Nebulizer inhalation irrigation of the mucous membrane	Liquid pyobacteriophage complex	Ongoing	Clinical trial registration No. NCT04682964

specific immune responses to the proteins studied four phage proteins in the virions, with the emphasis on the tail sheath (Tm_pH) or external capsid head (M_cp) (Kaźmierczak *et al.*, 2022). These phages are routinely applied in patients at the Phage Therapy Unit, Poland for the treatment of *S. aureus* infections. In contrast to oral administration, a different trend is observed in the bloodstream and other internal organs, which are not native habitats for phages. Both adaptive and innate immunity are significantly stimulated when phages are administered intravenously. Phagocytes quickly eliminate certain phages from the blood and internal organs if there are no host bacteria for them. They are internalized and eradicated by reticuloendothelial system cells of the liver and spleen. It is interesting to note that specialized macrophages found in the liver, Kupffer cells can phagocyte phages four times greater than macrophages in the spleen. This increases the possibility that phages that are arrested in the spleen could trigger lymphocytes to produce antibodies, which can combat infections more effectively. Pathogenic bacteria are often eliminated by innate immunity, which is regarded as the first line of defense before the adaptive immune response is activated. According to studies, patients who received phage therapy showed a decline in the number of mature neutrophils in their peripheral blood, suggesting that an active innate immune response has been activated by the phages (Weber-Dąbrowska *et al.*, 2002). Phages are also capable of inducing certain antibodies (neutralizing antibodies) against them during phage therapy, which typically reduces its ability to kill the target bacteria *in vivo*. The factors which might affect the concentration of neutralizing antibodies include (a) The path of phage administration—topical and oral administration induce a slight increase in antibodies, and (b) and the dose regimen. Studies have indicated that anti-phage neutralizing antibodies are likely one of the most important factors responsible for the functional limitation of phage therapy (Smith *et al.*, 1987). It has been hypothesized that the production of neutralizing antibodies should not pose a major problem during the preliminary cure of acute infections. This is because the phage action dynamics are

quicker than the production of neutralizing antibodies by the host (Sulakvelidze and Alavidze, 2001). However, if anti-phage antibodies are still present when the second round of treatment is given, they could be a concern. One such immune response of a phage cocktail was observed on an 81-year-old immunocompromised patient with bronchiectasis and persistent *Mycobacterium abscessus* lung illness. The patient was given a six-month treatment with a three-phage combination active against the strain. Intravenous injection of phage to minimize infectious burden was safe and lowered sputum load tenfold within one month in this case study. However, *M. abscessus* counts re-emerged after two months as the patient generated a powerful IgM- and IgG-mediated neutralizing antibody response to the phages, which was later linked with poor therapeutic efficacy (Dedrick *et al.*, 2021).

This issue can be resolved in three different ways. It may be considered to (a) administer the phage again, (b) increase the phage concentration, or (c) administer other phages because resistance varies from one phage to another. Along with the development of anti-phage neutralizing antibodies, the level of non-neutralizing antibodies IgM and later IgG also increases, further strengthening the response of the immune system following subsequent jabs of certain types of phages (Biswas *et al.*, 2002; Capparelli *et al.*, 2010).

Challenges to Phage Therapy

Until now, phage therapy is not a chosen alternative for therapeutic treatments against bacterial infections, and its efficacy is still debatable (Sulakvelidze and Alavidze, 2001). Some of these challenges were recognized as early as the 1930s. It was recognized that the prior determination of phage specificity against bacterial strains is indispensable; there was a doubt if the efficacy of phage treatment was sustained over time or not as some of the pharmacokinetic experiments revealed that the body rapidly removed the phages via spleen. Studies by Luria and Delbruck in 1943 exhibited that bacteria could evolve to develop resistance against phages (Kortright *et al.*, 2019); moreover, some

earlier studies demonstrated that laboratory experiments of phages with bacteria did not coincide with *in vivo* results. Hence, the experiment suggested that the *in vitro* efficacy of phage therapy was good, but not *in vivo*. Most of these doubts were resolved by Smith and Huggins pioneering experiments with Phage R in 1982, moreover, they also proved phage therapy to be more effective than antibiotic therapy. Using mice as their experimental model, they showed that a single dose of phage R was equivalent to eight doses of streptomycin against K1+ *E. coli* (Smith and Huggins, 1982). However, there are still some unresolved challenges to be addressed, such as:

Selection of the 'appropriate' phage

The phages that can be used as therapeutic tools have to be carefully selected such that they are efficient in killing the target bacteria without altering much the environments where they are used (Merabishvili *et al.*, 2009). The following phage characteristics are generally ensured- the phages should obligately follow the lytic cycle of infection, there should not be storage problems associated with them, i.e., they should be easy to store, there should be carefully studied data about its efficacy and safety beforehand, and in the most ideal situations, the genome should be completely sequenced to ensure that there are no undesirable genes present that code for any toxins (Gill and Hyman, 2010). Moreover, we have to be careful enough not to select transducing phages for therapy (Abedon and Thomas-Abedon, 2010). Transduction is the phenomenon of the transfer of genetic material from one bacterium to another via a bacteriophage. If we use a lytic bacteriophage for therapy that has a transducing potential, there would be chances of it delivering antimicrobial genes from one bacterium to another and various other clinical complexities. One should therefore be cognizant of the transducing potential of a phage before using it for therapy (Doub, 2021). Some of the additional characteristics that may as well be accounted for are the morphology of the virion and its protein profiles (Skurnik and Strauch, 2006).

Narrow host range

Bacteriophages show high specificity against the bacterial strains that it infects and kills (Sybesma *et al.*, 2018). This specificity, although advantageous with respect to its mode of action, narrows the range of hosts that it can act against and limits the treatment only against those bacterial strains whose susceptibility to specific phages is known priorly.

Phage therapy is an 'unfamiliar' concept

One of the greatest challenges to phage therapy is it being an unfamiliar and new concept in the Western Medical Establishment. Lesser clinical trials are responsible for the Western countries to be reluctant in using phage therapy (Sybesma *et al.*, 2018). The preclinical research data has shown that phage therapy can be used effectively against a wide range of bacterial infections, but clinical trials are yet to confirm and establish this compared to the fewer experimental proofs that we have. Therefore, the insufficiency of knowledge about using the phages in therapy stands as a barrier to be used by scientists in

treatment protocols currently. More trials and studies in the future will help know about phage therapy better with all the pros and cons established.

Bacteria evolving to be phage-resistant

One of the major challenges to phage therapy is the possible evolution of bacteria to become resistant to phages forming bacteriophage-insensitive mutants (BIMs) (Pires *et al.*, 2020). It has been observed in the course of studies over the last few years that phage-resistant mutants emerge almost frequently, and it is unavoidable (Oechlin, 2018). Bacteria have evolved a wide range of strategies to prevent lytic phage infection, and phages have developed an equally diverse range of ways to overcome this resistance. For bacteria, it includes modification or loss of receptors, in addition to the incorporation of phage DNA into the clustered regularly interspaced palindromic repeats/CRISPR associated system (CRISPR/Cas) system (Labrie *et al.*, 2010; Bernheim and Sorek, 2020). On the other hand, phages have developed to become capable of identifying new or altered receptors as well as possessing anti-CRISPR genes (Koskella and Brockhurst, 2014). BIMs have been observed in some *in vitro* studies (Fu *et al.*, 2010; Le *et al.*, 2014). In a study by McGee *et al.* (2023), *Escherichia coli* cultures were infected with phage strains that were not previously encountered in order to determine bacterial resistance to phage across several strains. It was discovered that phage-resistant *E. coli* mutants that developed resistance to a particular phage strain were also resistant to phages with similar adsorption strategies. Mutations that confer phage-resistant phenotypes are usually those involved in LPS structure/synthesis (McGee *et al.*, 2023). However, some studies have shown that the *in vitro* observations of resistance do not always coincide with *in vivo* observations (Oechlin *et al.*, 2017).

Key to Challenges of Phage Therapy

- Bacterial resistance is a critical challenge in the effective development of phage therapies. Research conducted by Yehl *et al.* (2019) identified host-range-determining regions (HRDRs) in the T3 phage tail fiber protein through natural evolution and structural modeling and devised a high-throughput approach for genetically engineering these regions using site-directed mutagenesis. This method, inspired by antibody specificity engineering, creates deep functional diversity while minimizing disturbances to the overall tail fibre structure, resulting in synthetic "phage-bodies." It was demonstrated that altering HRDRs results in phage-bodies with different host ranges, and that certain phage-bodies may limit bacterial growth *in vitro* for a long period of time by inhibiting bacterial resistance. This strategy seems as a potential approach for the development of next-generation antimicrobials that reduce resistance development and can be applied to various viral scaffolds for a wide range of applications Yehl *et al.* (2019).

Dedrick *et al.* published a study that described the case of an adolescent with cystic fibrosis and a widespread *Mycobacterium abscessus* infection which was treated with a three-phage cocktail post bilateral lung transplantation. Genome engineering and forward genetics were used to

develop effective lytic phage variants that eliminated the infectious *M. abscessus* strain. Intravenous phage treatment was well-tolerated and linked to objective clinical improvements such as sternal wound healing, improved liver function, and significant clearance of infected skin nodules.

- Using phage cocktails in place of a single phage for therapy (Pirnay *et al.*, 2011). The goal is similar to antiviral/cancer combination therapy: to target numerous, distinct mechanisms used by bacteria for infection, such as to improve bacterial killing and minimize/delay resistance mechanisms of bacteria (Altamirano and Barr 2021). Post-Coronavirus Disease of 2019 (COVID-19), gut dysbiosis was observed as a common side effect where bacteriophage cocktails proved to be an efficient way for restoring the normal balance of the microbiota. A study was carried out by Zurabov *et al.* for the rehabilitation of 30 patients following COVID-19, wherein two complex phage cocktails targeting multiple bacterial species were used. The real-time efficacy of the bacteriophages was determined against the clinical strain of *K. pneumoniae*. The use of phage cocktails for two weeks demonstrated safety and the absence of adverse effects. This study serves as a foundation for a larger and more extensive investigation of the usage of phage cocktails and their effects on the different parts of the microbiome.

- Using a different phage against the bacteria in place of the phage against which the resistance has been developed—this is simple with phages owing to their ubiquitous nature and abundance (Rohde *et al.*, 2018).

- Using phages and antibiotics together (Tagliaferri *et al.*, 2019).

Weighing pros and cons of antibiotics and phage therapy

Bacteriophages, as well as antibiotics, are antibacterial entities/substances that disrupt/inhibit the growth of bacteria by different modes of action. Bacteriophages employ their lytic cycle to lyse the bacterial cells, whereas antibiotics disrupt certain physiological processes of bacteria, such as cell wall or protein synthesis. Antibiotic resistance is a grave concern in the healthcare system, prompting researchers to seek new or modified medications. However, developing a new antibiotic is a challenging task that can take years, and the likelihood of bacteria developing resistance to newly discovered antibiotics will always be an unanswered question (Venkatesan, 2021). Recent studies for finding alternatives to antibiotics have sparked interest in phage therapy. Bacteriophages have once again become the center of attention as a potential therapeutic approach for antibiotic-resistant bacterial infections (Pirnay *et al.*, 2018). Phage therapy and antibiotic therapy share certain characteristics, such as—both have to be administered at neutral pH environments, the success of therapy in each case is dependent on the time of initiation of treatment, the immune system of the patient plays an important role in treatment in both the cases, and there are chances that bacteria may become resistant to both phages and antibiotics (Loc-Carrillo and Abedon, 2011). Despite the shared purpose, there are certain striking differentiating features between the two.

Specificity

In contrast to antibiotics, phages are highly specific in terms of infecting only particular species and strains of bacteria. This specificity of phages could be both positive as well as negative. On the positive side, being specific against bacteria, the incidents of collateral damage are greatly minimized. They only lyse the target bacteria and do not perturbate the natural microflora. Antibiotics, on the other hand, have been reported to have notorious secondary outcomes such as *C. difficile* infection and antibiotic-associated diarrhea. Moreover, their disruption in the gut microbial community can also lead to increased chances of diabetes, asthma, and obesity. Even with various advantages, this specificity limits phage therapy in certain ways. For instance, this therapy will not be useful in incidents where more than one strain of bacteria has to be targeted, as in the case of burn wounds. Moreover, phages will only have a narrow spectrum of action in contrast to broad-spectrum antibiotics (Elbreki *et al.*, 2014).

Safety

The wide use of antibiotics over the years has also helped to document the various adverse effects that they might have caused. This data helps us to determine the safety of a drug in the treatment of a bacterial disease. Some of the complications related to antibiotic treatment are nephrotoxicity, anaphylaxis, hepatotoxicity, cardiotoxicity, and neurotoxicity, as well as a number of gastrointestinal and hematological complications (Granowitz and Brown, 2008). In contrast, phage therapy has only lately achieved recognition, and there is little evidence related to its safety. However, administering phages orally is generally considered safe (Bruttin and Brüßow, 2005). Despite this, there are some studies that demonstrate that phages can become a part of blood circulation after their translocation across the intestinal epithelium (Górski *et al.*, 2006). Such an immunological response implies the potential to cause a harmful reaction in people with compromised immunity (Borysowski and Górski, 2008). However, other researchers doubt and say that it is unlikely for phages to generate such a reaction.

Biofilm penetration

Another point that gives phage therapy an advantage over antibiotic therapy is the capability of phages to degrade extracellular polymeric substances (EPS) and diffuse the bacterial biofilms and hence giving them access to the bacteria. The phage disperses the biofilm with the aid of the enzyme EPS depolymerase that is present on the outer surface of its capsid. Antibiotics, on the other hand, are not equipped to treat biofilm-based bacterial infections. Higher doses are required in order to penetrate the dense biofilms, even then it does not guarantee complete eradication. The formation of biofilms on medical devices such as catheters, prostheses, and lenses by *P. aeruginosa*, *L. monocytogenes*, and *Staphylococcus epidermidis* can now be eliminated by phage treatment (Lin *et al.*, 2017). Additionally, an alternative to broaden the lytic spectrum of phages can be combining phages with other antimicrobial treatments or other phages as well, as seen in phage cocktails (Kutter *et*

al., 2010). An interesting point of advantage of phage therapy over antibiotic therapy is that even a broad-range phage cocktail will have greater specificity when it comes to its mode of action in comparison to that of the narrow spectrum antibiotics (Goodridge, 2010).

Recent Developments in Phage Therapy

Bacteriophages were utilized to treat patients suffering from various bacterial infections in Russia, Poland, and Georgia primarily during the time period of the 1920s–50s, soon after their discovery by D'Herelle in 1917. Lately, more countries joined, including Switzerland, Belgium, France, and the USA (Reardon, 2014). Our enhanced knowledge about genetics, pharmacology, immunology, and phage biology has upheld this re-established interest in phage therapy. The minimum suggested regulatory prerequisite for the therapeutic use of phages includes phages having a stringently lytic cycle, proven antimicrobial effect against the desired pathogen, and exclusion of endotoxins and contaminated bacterial debris. These major aspects involved in phage therapy have now been standardized to maximize treatment efficacy (Young and Gill, 2015). The Phage Therapy Centre of Hirsfeld Institute of Immunology and Experimental Therapy in Wroclaw, Poland, provides experimental antibacterial phage therapies to its patients against several pathogenic microbes including *Acinetobacter*, *Burkholderia*, *Escherichia*, *Klebsiella*, *Proteus*, *Enterobacter*, *Enterococcus*, *Pseudomonas*, *Shigella*, *Staphylococcus*, and *Salmonella*, among others. According to the Centre's data, 35%–50% of patients treated in the center had favorable therapy outcomes. Each therapy is a combination of a few bacteriophage strains to boost antibacterial action and reduce the likelihood of developing phage resistance (Viertel *et al.*, 2014). The potential of phages in the treatment of several human infections is still being studied at various levels. The US National Institutes of Health (NIH) has initiated a phase I/II trial with study no. -NCT04287478 intended to evaluate bacteriophage therapy in patients suffering from urinary tract infections in December 2020 with around 150 participants. This study is estimated to be completed by 2023.

A French company, Pherecydes Pharma, dedicated to precision phage therapy for the cure of resistant and/or complex bacterial infections, has announced the enrollment of the first patient in the PhagoDAIR Phase II clinical trial in June 2022. This is the world's first initiative of phage therapy for osteoarticular infections of prosthetic devices infected by *S. aureus*. The study will also be conducted in European countries and intends to include 64 patients having infected hip joint or knee with *S. aureus*. The patients will be divided into two groups: control group and phage therapy. Patient's receiving phage therapy will be given anti-*S. aureus* phages active on their strain accompanied by suppressive antibiotic therapy. The evaluation process will start 12 weeks after the administration of phages, and will continue for 2 years. By the end of 2023, the preliminary results are anticipated, and the evaluation process will continue until the first half of 2025. Based on the initial outcomes of the Phase II trial,

Pherecydes Pharma is preparing for a Phase III trial, which could start in 2024. Advancements in the gene-editing tool CRISPR/Cas have led to the achievement of new possibilities in phage therapy. One such example is a bioengineered phage able to deliver a CRISPR/Cas Programme to disrupt the gene for antibiotic resistance and can also eliminate antibiotic-resistant plasmids (Yosef *et al.*, 2015). These phages can be sprayed onto hospital surfaces to minimize the incidence and dissemination of antibiotic resistance genes. Although the domain of bio-engineered phages is still nascent, it will definitely spawn many valuable innovations like the one mentioned above.

Phage Therapy in India

The concept of phage therapy in India is in its juvenile stage and will take some more years of research and development to establish itself as a medicinal procedure. However, the country has been encountering antimicrobial resistance for decades, and there seems to be no promising alternative to tackle it. After the COVID-19 pandemic, the crisis has amplified, with many more bacteria augmenting the list of MDRs. In 2018, over 500,000 new cases of rifampicin-resistant tuberculosis were reported worldwide. Seventy-eight percent of these patients had multidrug-resistant tuberculosis. According to the Global Tuberculosis Report 2019, India accounted for 27% of the total global burden in 2018. A recent study on terminally ill hospitalized COVID-19 patients in India reports the emergence of various gram-negative bacteria to be resistant to various broad-spectrum antibiotics, including Carbapenem, during secondary infections (Vijay *et al.*, 2021). Carbapenem is a broad-spectrum antibiotic and is considered to be an unfailing drug treating bacterial infections. Antibiotic research has reached a roadblock where bacteriophage therapy seems to be an alternative treatment. The three experimental studies initiated by Banaras Hindu University, India, on clinical trials of phage therapy, showed the effectiveness of topical phage preparations in chronic wound healing. Gupta *et al.* (2019) conducted a clinical study that demonstrated the importance of bacteriophage therapy in chronic wounds caused by antibiotic-resistant bacteria. The study included twenty patients who had chronic non-healing ulcers for more than six weeks. Within a few weeks, a significant improvement in the form of complete wound epithelization could be accomplished (Gupta *et al.*, 2019). A study by Patel *et al.* (2021) was carried out with forty-eight patients having at least one full-thickness wound that did not heal in 6 weeks with conventional wound treatments. Positive results were observed with significant improvement in wound healing in >82% of the patients by the end of three months of follow-up. Moreover, the study also suggested that specialized phage therapy is similarly effective whether the patient is diabetic or not. In diabetic patients, however, healing was relatively delayed (Patel *et al.*, 2021). Both studies show that topical phage therapy resulted in complete clinical wound healing in patients who were refractory to traditional therapy. Furthermore, the antibiotic resistance status of the bacteria suspected of being involved in the wound had no impact on the outcome of the therapy.

Another recently published non-randomized case-control study on the impact of bacteriophages on the healing process of infected acute traumatic wounds by [Bhartiya et al. \(2022\)](#) has shown impressive results. When compared to the control, i.e., conventional therapy, the average number of days needed for total granulation of wounds, achieving sterility, and time of recovery, was lessened by half ([Bhartiya et al., 2022](#)). Because phages are highly specific for their bacterial hosts, phage cocktail preparations typically ensure a broader spectrum of activity and decrease the probability of the development of bacterial mutants resistant to phages. As a result, all of these studies used a bacteriophage cocktail as therapy. GangaGen, an Indian pharmaceutical company, is testing ectolysin encoded by phage P128 against methicillin resistant *S. aureus* (MRSA), and they are in Phase 2 of their clinical trials. More such trials in the future will pave the way for revolutionary medicine against drug-resistant bacteria in India ([Channabasappa et al., 2018](#)). In 2021 a case report was published in which an Indian patient suffering from an inflammatory disorder of the prostate gland known as Chronic Bacterial Prostatitis (CBP) showed recuperation in his symptoms by phage preparations from the Eliava Institute Georgia. Earlier, the CBP conditions were difficult to resolve even after multiple antibacterial treatments ([Johri et al., 2021](#)). Several such clinical trials are required in India to demonstrate the safety and efficacy of data generated in preclinical experiments of phage therapy before they can be reliably converted into clinical practice.

Conclusion

As we are moving ahead in modern medicine to provide a better healthcare system to mankind, the escalation of antibiotic resistance is dragging our feet back into the dark ages of medicines. According to a 2014 UK Government-commissioned Review on Antimicrobial Resistance, AMR might kill 10 million people annually by 2050 ([O'Neill, 2014](#)). WHO, numerous other organizations, and researchers concur that the development of AMR is a critical issue that necessitates a global, coordinated action plan to address. Bacteria that are multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) are extremely lethal. The global upsurge of antibiotic resistance increases the likelihood of returning to the clinical equivalent of the pre-antibiotic era and necessitates the development of new groups of antimicrobials that could improve the efficacy of currently used drugs. The development of new antibacterial drugs that can facilitate a more strategic treatment approach without affecting the beneficial natural microbiota can be made possible by the promising but challenging approach of phage therapy. In the post-antibiotic era, phage therapy is the best natural complement. The mode of action of bacteriophages is different from that of antibiotics, which makes antibiotic-resistant bacterial strains susceptible to phage infection. This is a significant advantage of phage therapy. Numerous phage therapy clinical trials in phase I and phase II have shown that there aren't many severe safety issues with phages. The effectiveness of phage

therapy depends on the concentration of phages (the dose) administered at the infection site as well as on their capacity to target and kill bacteria, inhibit bacterial development, and eradicate the infection. This has been demonstrated in animal studies. Several Gram-positive bacterial infections, including MRSA and VRE, are still incurable. Despite the availability of last-resort antibiotics, the mortality rate for these drug-resistant bacterial infections remain high. Physicians, funding bodies, governments, and policymakers must not overlook the use of phage therapy to treat such infections. The use of phage-antibiotic combinations is becoming more popular in phage research, but implementation is debilitated by a lack of proper implementation. Though there are a limited number of clinical trials which are in process, and only some are in phase II, there is enough evidence from Poland and Georgia that bacteriophages are effectual therapeutic agents. In India, clinical trials have been conducted by Banaras Hindu University (BHU), and have shown promising results. However, constraints such as a limited host range, bacterial resistance to phage, manufacturing costs, and challenging dose finding/delivery methods must be addressed. Recent advancements in synthetic genome assembly and viral genome engineering can be used to develop phage with remarkable pharmacological activities. The advancement in technology, such as next-generation sequencing and high-throughput screening, have augmented the likelihood of genome analysis and phage characterization. Many studies and clinical trials conducted worldwide have shed more light on bacteriophage therapy as an effective substitute. Though phages would not reinstate antibiotics, they are the best complement for the non-antibiotic era.

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