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REVIEW ARTICLE

Phage therapy: a promising cure for bacterial infections in humans

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Abstract

Phage therapy uses bacteriophages (viruses) that eliminate bacteria, as a substitute to antibiotics to cure bacterial infection. With the significant increase of antibiotic-resistant strains, bacterial infections have become a very challenging global health concern. In contrast to antibiotics, phage therapy has various benefits, like its narrow host range that selectively targets specific bacterial strains while not affecting beneficial microbiota. Phages can be conveniently isolated and produced on a large scale, potentially making phage therapy a cost-effective alternative for bacterial illness. The history of the phage therapy started in early 20th century when it's potential for combating bacterial infections was discovered. With the discovery of antibiotics, its popularity declined in Western world, but it remained in use in Eastern Europe. The ever-increasing antibiotic resistance against antibiotics, has again renewed our interest in phage therapy. Phages have a complex life cycle involving the lytic and lysogenic cycles. Phage therapy utilizes various mechanisms, including inhibition of cell wall biosynthesis in growing bacterial cell by phages with small genome and production of several protein like holin and endolysin (that lyse the cell membrane of the host bacteria) and modulation of host immune reaction by phages with large genome. Promising results of phage therapy have been found while treating bacterial infections caused by multidrug-resistant bacterial strains. However, issues such as phage resistance and immune responses are required to be addressed. Despite these challenges, phage therapy has proven to be viable approach to combat bacterial infections, especially against antibiotic resistance bacterial strains. There is need for further study and development in field of phage therapy for realizing its full potential in clinical practice.

Keywords: Phage therapy, Antibiotic Resistance, Bacterial Infection, Bacteriophages, Cost-effective Treatment, Beneficial Microbiota, Selective Targeting

1. Introduction

Bacterial infections are a primary contributor to mortality death globally. For the past several decades, antibiotics have been primarily used for treatment of bacterial infections. But with the developments of antibiotic resistance bacterial strains, its treatment has become a significant challenge in the recent year (Laxminarayan et al., 2013; World Health Organization, 2014). Phage therapy has been proved to be alternative treatment option in such cases. Phage therapy utilizes the ability of phages (bacteriophages, a type of virus) to specifically infect and kill bacterial cell. Before the emergence of antibiotics, phages were actively being utilized for the treatment of bacterial infection (Summers, 2001). With the global application of antibiotics, phage therapy, however, fell out of favor in the Western world. But in Eastern Europe, it has remained to be practiced where it has been a part of mainstream clinical practice over many years (Żaczek et al., 2020).

In the recent time, scientist has shown a renewed interest in use of phage therapy to cure bacterial infection in humans, as an alternative option or supplementary to antibiotics. It has various of advantages against antibiotics, including their narrow bacterial host range, which allows for targeted killing of specific bacterial strains while leaving the beneficial microbiota intact (Gorski and Weber-Dabrowska, 2005; Chan et al., 2013). Additionally, phages can be easily isolated and produced in large quantities, making them a potentially cost-

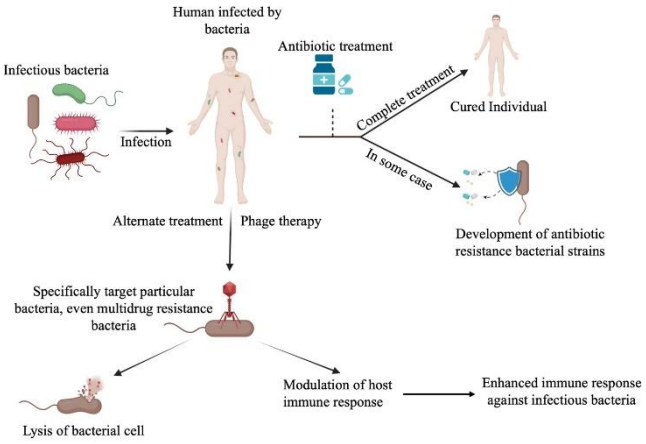

Figure 1. Graphical abstract: Phage therapy presenting a potential treatment for bacterial infections in humans. This figure was made by using BioRender (https://www.biorender.com).

effective treatment option (Loc-Carrillo and Abedon, 2011; Lin et al., 2017; Nagel et al., 2022).

Studies has indicated effective and safe application of phage therapy for the clinical treatment of multidrug-resistant (MDR) bacterial infections in humans (Jennes et al., 2017; Schooley et al., 2017). However, there are also many challenges linked with phage therapy, including development of phage resistance, the need for precise identification and matching of phages to the bacterial strains causing the infection, and regulatory hurdles linked with the clinical application of phages in human treatment (Golkar et al., 2014; Schooley et al., 2017).

Despite these challenges, phage therapy holds promise as a viable approach to cure bacterial infections in humans, particularly in the time of the ever-increasing threats of resistance against important antibiotics. In this review article, we have aimed to describe precisely about the fundamentals of phage therapy, including life cycle of phages, their mode of action, their potential advantages and shortcomings. Additionally, we also elaborate the current status of phage therapy research, including its potential applications in the bacterial infection treatment and apparent challenges that must be overcome for its widespread adoption in clinical practice.

2. History and current prospective of phage therapy

Phage therapy, application of bacteriophages to treat of bacterial infection, has a history started in the early 20th century. In 1915, Frederick Twort discovered that a virus could infect and kill bacteria, and Félix d'Herelle autonomously discovered bacteriophages in 1917 and coined the term "phage" (Carlton, 1999; Sulakvelidze et al., 2001). Application of phage therapy became popular during the 1920s and 1930s in Eastern Europe, particularly in the Soviet Union. It was used for the treatment of many bacterial infections, including typhoid fever, dysentery, and wound infections, with reported success rates as high as 80% (Sulakvelidze et al., 2001). In the West, however, the advent of antibiotics to treat bacterial infections during 1940s ushered to a decline in interest in phage therapy, as antibiotics were seen as a more reliable and easier-to-use alternative. Eastern Europe continues to employ phage treatment and other parts of the world, but research and development of phage therapy lagged behind that of antibiotics in the West (Kutter and Sulakvelidze, 2004; Sharma et al., 2016). In recent decade, clinical application in the phage therapy has been renewed as a prospective substitute to antibiotics, especially in the time of rising resistance against important antibiotics. It has been applied successfully to treat several bacterial infections, including MDR bacterial strains infection (Schooley et al., 2017; Dedrick et al., 2019). However, there are still few challenges to overcome, including issues related to phage selection, delivery, and regulatory approval (Fauconnier, 2019).

3. Fundamentals of phage therapy

3.1. Phages and their life cycle

Phages, previously known as bacteriophages, are a class of viruses that are proved to infect and kill bacteria specifically. These are most diversified and abounding biological entities on earth, with roughly estimated population of 10^{31} (Ackermann, 2007; Dion et al., 2020). Phages play crucial role in ecology of microbial communities, influencing abundance, diversity, and evolution of bacterial populations.

Phages mainly shows two type of life cycles; lytic cycle and lysogenic cycle.

A. Lytic Cycle: In this cycle, the phages infect a bacterium, reproduces rapidly, and causes lysis of host bacterial cell, or burst open, releasing new phage particles. The steps involved in the lytic cycle of phages are as follows:

1. Attachment: This is first step in the lytic cycle, in which the phages are attached to the host bacterial cell through specific receptor on its surface. The receptor is usually a specific molecule on the bacterial surface, such as a protein or a sugar. The attachment is mediated by the tail fibers and is highly specific (Sharma et al., 2016; Lin et al., 2017).

2. Penetration: After attaching to the surface of host bacterial cell, DNA of phage is injected into the bacterial cytoplasm using its tail and the basal plate. The tail contracts, and the basal plate acts like a hypodermic needle, resulting in injection of phage DNA into bacterial cytoplasm through the cell wall and cell membrane. This method of DNA injection is still to be understood precisely, but researches have shed some light on the role of different phage proteins involved in this process (Zinke et al., 2022).

3. Replication: After entry inside host bacterial cell, phage DNA start controlling bacterial machinery in such a way that bacterial cell facilitates phage DNA replication. The phage DNA administers the host cell to produce all the necessary enzymes and proteins for the phage replication. The replication of phage DNA is highly efficient and can reach rates of up to 2000 base pairs per second (Loenen and Murray, 1986; Sharma et al., 2016).

4. Assembly: As the phage DNA replicates, newly synthesized phage particles are assembled inside the host bacterium. The phage head is assembled first, and then the tail fibers are added. Finally, the new phage particles are encased with the phage DNA. The assembly process is tightly regulated by the phage proteins, and any defect in the process can result in non-infectious phage particles (Casjens and Hendrix, 2005).

5. Lysis and release: When the host cell is unable to contain the increasing number of phage particles, it lyses or bursts open, releasing the newly formed phages into the environment to infect other host cells. The process of lysis is mediated by the phage lytic enzymes, which degrade the bacterial cell wall and membrane (Young, 2014).

B. Lysogenic cycle: This cycle is more complex than lytic cycle. In this, phage integrate its DNA into the main genome of host bacterium and phage DNA replicates as integral part of the host DNA. The lysogenic cycle has the following steps:

1. Attachment and penetration: The first steps of the lysogenic cycle are similar to lytic cycle. Phage attaches to the surface receptor of host bacterium and injects its DNA into the cytoplasm of host bacterium. The process of penetration is facilitated by the same proteins as in the

Table 1. Phage therapy and mode of action

Mode of Action	Description	References
Lysis of bacterial cell by inhibition of cell wall peptidoglycan biosynthesis	Phages with the small genomes like RNA phage Qb or Coliphage ΦX174 utilizes single effector protein, which inhibit peptidoglycan synthesis in growing bacterial cell. This action ultimately results in bacterial cell lysis.	Bernhardt et al., 2001a, b; Sulakvelidze et al., 2001; Bernhardt et al., 2002, Catalão et al. 2013.
Production of holin and endolysin proteins	Coordinated action of holin and endolysin protein result in the bacterial cell lysis, where holin protein form pore in cell membrane and endolysin digest cell wall of bacteria.	Bernhardt et al., 2002; Schmelcher et al., 2012; Catalão et al., 2013.
Modulation of host immune response	Some phages induce the production of pro-inflammatory cytokines and activate phagocytic cells, and some decrease pro-inflammatory cytokines and chemokine depending upon immunological balance to enhances the host immune response and aids in the clearance of the infection.	Eriksson et al., 2009; Petrovic Fabijan et al., 2020; Souza et al., 2023.

lytic cycle (Wommack and Colwell, 2000; Sulakvelidze et al., 2001; Young, 2014).

2. Integration: Once inside the cytoplasm of host bacterium, phage DNA is integrated into the main genome of host bacterium. This process is accomplished through the activity of integrase enzyme, which catalyzes the recombination of phage DNA into the host genome. The integrated phage DNA is now known as prophage (Howard-Varona et al., 2017).

3. Replication and cell division: The prophage replicates along with the host genome during cell division, and the daughter cells inherit the prophage DNA. The prophage remains dormant in host bacterium, and no phage particles are produced.

4. Induction and lytic cycle: Under certain conditions, such as exposure to UV radiation or certain chemicals, the prophage may be induced to exit the genome of host bacterium and initiate usual lytic cycle. During the lytic cycle, the phage multiplies in large number and ultimately lyses the bacterial host cell to release new phage particles. The process of induction is regulated by the phage-encoded proteins, which can sense the host's physiological state and respond accordingly (Clokie et al., 2011; Howard-Varona et al., 2017).

Phages have been widely researched which resulted in discovery of many applications in various fields, such as biotechnology, medicine, and food safety. Phages have been clinically utilized to control or eliminate bacterial infections in animals and humans, to decontaminate food, and to manipulate bacterial populations in industrial processes (Lu and Koeris, 2011).

Thus, phages are viruses that are proved to infect and kill bacteria specifically and play critical function in the ecology of microbial communities. In lytic cycle, phage replicates rapidly and lyses the bacterial cell, resulting in the release new phage particles. In lysogenic cycle, phage insert its DNA into the bacterial chromosome and reproduces as a component of the bacterial genome. The prophage remains dormant until it is induced to get separated from the genome of bacterial host and enter in the lytic cycle (Campbell and Reece, 2005; Howard-Varona et al., 2017). Phages have numerous applications in various fields and hold promise for the future development of new therapies and technologies.

3.2. Phage therapy and mode of action

Phage therapy can be defined as curative application of bacteriophages for the treatment of specific bacterial infections. It has been applied for over a century, primarily in Eastern Europe and erstwhile Soviet Union. There was a period of decline due to discovery and wide use of antibiotics, but it regained scientific and clinical interest in recent years due to ever increasing problem of antibiotic resistance (Sulakvelidze et al., 2001).

The mode of action of phages in therapy is multifaceted and can differ depending on the specific phage-bacterium interaction. However, there are some prevailing mechanisms by which phages. To escape from the host cell, phages employ two different strategies. In First, phages (like bacteriophage λ) with large dsDNA genome encode holin and endolysin protein. The coordinated work of these protein results in the lysis of host bacterial cell. Holin protein oligomerizes to form pores in bacterial cell membrane through which endolysin protein is secreted in periplasmic space. This endolysin digest cell wall of host bacterium by cleaving the peptidoglycan (Bernhardt et al., 2002; Schmelcher et al., 2012; Catalão et al., 2013). In second, phages (like RNA phage Qb or Coliphage ΦX174) with small genome utilizes single effector protein, which inhibit particular step of biosynthesis of cell wall peptidoglycan in growing bacterial cells. Incomplete cell wall synthesis results in the host cell lysis (Bernhardt et al., 2001a, b; Bernhardt et al., 2002, Catalão et al., 2013) (Table 1). In addition to bacterial cell lysis, phages can also modulate the host immune response. Some phages are known to induce production of pro-inflammatory cytokines and activate phagocytic cells, such as neutrophils and macrophages (Eriksson et al., 2009; Petrovic Fabijan et al., 2020). Phagocytic activity is also supported by decrease in reactive oxygen species by phages (Souza et al., 2023). In some case phages have shown to induce anti-inflammatory response or decrease in pro-inflammatory chemokines and cytokines. Thus, phages may induce an anti-inflammatory or pro-inflammatory response depending upon the ultimate immunological balance to enhance the host immune response and aid in the clearance of the infection (Table 1).

3.3. Benefits and challenges of phage therapy

Phage therapy has various benefits over antibiotics, including their specific or narrow host range, which allows for targeted killing of specific bacterial strains while leaving the beneficial microbiota intact (Chan et al., 2013; Lin et al., 2017; Khan et al., 2022). Additionally, phages can be easily isolated and produced in large quantities, making them a potentially cost-effective treatment option (Harper et al., 2014). Phage therapy has shown its significance in the treatment of several types of bacterial infections, including infections arising due to MDR bacterial strains (Adebayo et al., 2017; Lin et al., 2017; Arumugam et al., 2022; Durr and Leipzig, 2023) (Table 2). In a study of patients suffering with chronic otitis resulted by MDR *Pseudomonas aeruginosa*, phage therapy resulted in remarkable decrease in bacterial load and clinical improvement in majority of patients (Wright et al., 2009). Similarly, in a case report of patients with MDR, *Acinetobacter baumannii* infection, phage therapy led to complete clearance of the infection (Schooley et al., 2017).

However, there are few challenges need be addressed while applying phage therapy to cure bacterial infection. In some cases, host bacterium develops resistance against phage which possess potential challenge in phage therapy. This can occur through a range of mechanisms, like mutations in phage receptors or the production of phage-resistant variants (Chan et al., 2013) (Table 2). To address this challenge, phage cocktails that target multiple bacterial strains or phages with different mode of action can be used. Also, there is need for precise identification and matching of phages to the host bacterial strains causing the infection, and regulatory hurdles associated with the application of phages in human medicine (Brüssow and Kutter, 2005; Pirnay et al., 2011) (Table 2). These challenges highlight the need for continued detailed research and development in the area of phage therapy to overcome these obstacles and to fully realize the potential of this method for the clinical treatment of bacterial infections (Table 2).

Another challenge is the emergence of immune responses against the phage itself. In some cases, patients may develop antibodies against the phage, which can limit the efficacy of treatment (Petrovic Fabijan et al., 2020; Hibstu et al., 2022). However, this can be addressed by selecting phages with low immunogenicity or by using phages in combination with immunomodulatory agents (Eriksson et al., 2009; Petrovic Fabijan et al., 2020; Souza et al., 2023).

4. Applications of phage therapy

4.1. Bacterial infections treatable by phage therapy

Phage therapy has shown promising results to cure of various bacterial infections. Phage therapy also targets pathobionts in diseases like inflammatory bowel disease (IBD) showing immune modulation which reduces bacterial load without antiphage resistance in IBD (Pessina et al., 2023) (Figure 2). Another example is successful recovery of patient from urinary tract infections (UTIs) by *Escherichia coli*. In a clinical trial, a phage cocktail was administered to patients with UTIs caused by antibiotic-resistant *E. coli*, resulting in a complete cure in 80% of cases (Sarker et al., 2012) (Figure 2).

Phage therapy has also been investigated as a possible cure for respiratory infections, such as, those caused by bacterial species, *Pseudomonas aeruginosa* (Marza et al., 2006; Chang et al., 2018; Chang et al., 2022) (Figure 2). It has been suggested as a substitute to reinstate intestinal eubiosis in the absence of effective treatments because of immunomodulatory and bactericidal effects of phages against pathogenic bacteria, including invasive adherent *Escherichia coli* in Crohn's disease and *Clostridioides diffcile* in ulcerative colitis (Gutiérrez and Domingo-Calap, 2020).

Although antibiotic treatments are given to a patient, COVID-19 children who develop secondary bacterial infections (SBIs), as a consequence of coronavirus infections, have much worse outcomes. Phages, which naturally destroy bacteria, are thought to be a possible alternative for antibiotics in the clinical treatment of bacterial infections in lungs. But their application in treating SBIs during viral pandemics like COVID-19 is not well understood (Wu et. al., 2022).

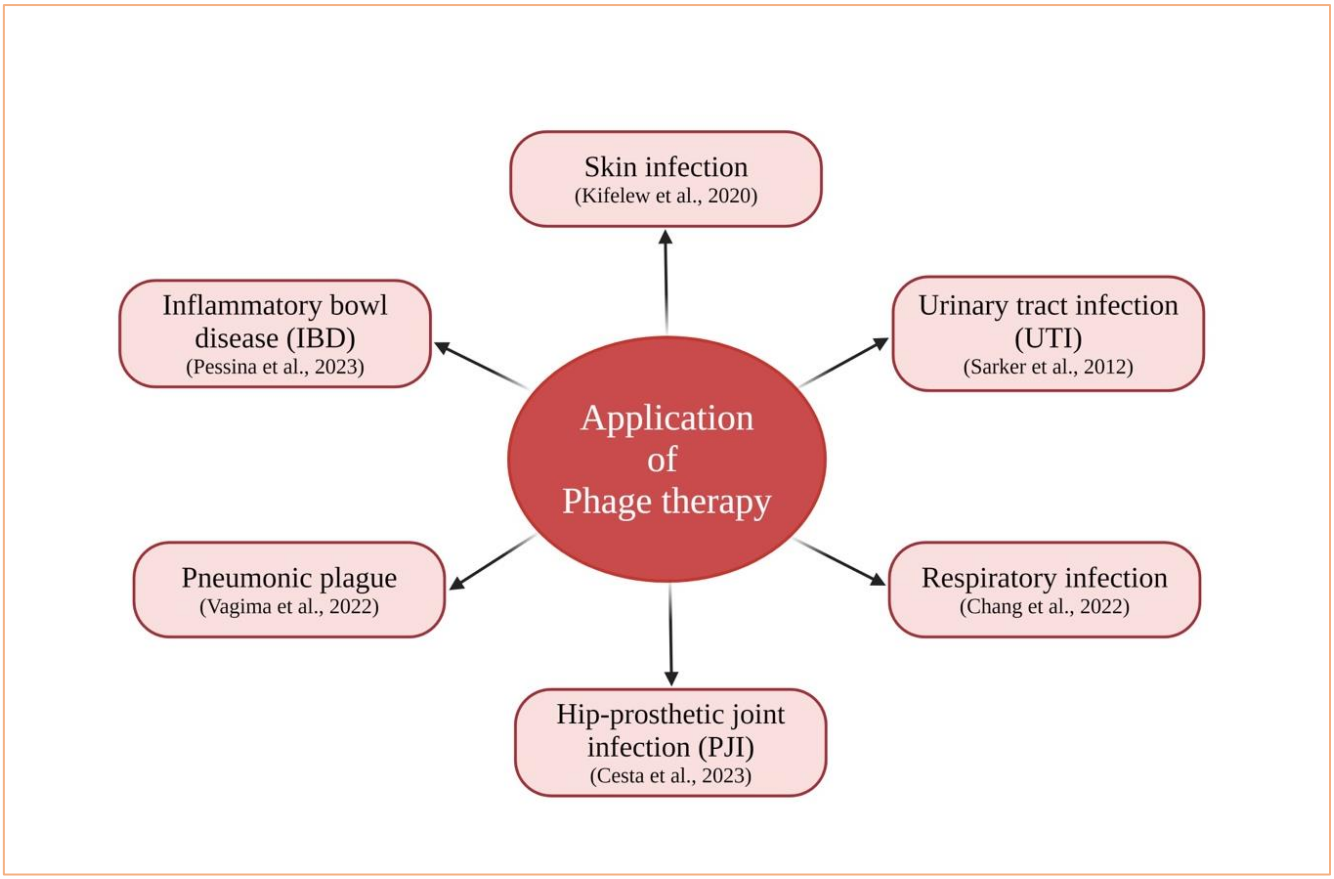

Figure 2. Application of Phage therapy. This figure was made by using BioRender (https://www.biorender.com).

The COVID-19 patients who developed secondary bacterial infection, about 75% of them were treated with antibiotics during coronavirus infections.

The over use of broad-spectrum antibiotic contributes the development of antimicrobial resistance (AMR) which ultimately give negative affects to recovery of such patients due to the specific characteristics of the gut-lung axis. Phage therapy can be applied as an effective alternative treatment to treat COVID-19 patients because it does not contribute to AMR development and give positive impacts to the treatment (Khan et. al.,2022).

4.2. Potential use in multi-drug resistant infections

Phage therapy has again secured attention of researchers in recent years due to ever increasing occurrence of antibiotic-resistant bacterial stains. Antibiotic resistance is an important health concern globally, and there is an urgent need for alternative treatments. Phage therapy has the prospect to be a highly effective and targeted treatment for MDR infections because phages can target and kill bacteria specifically without harming body's own cells.

Phage therapy has been proved to be successful in treating bacterial illnesses caused by MDR strains in several studies. For example, in a study, a patient with a severe MDR *Acinetobacter baumannii* infection was successfully treated with a cocktail of phages (Fish et al., 2016). Phage therapy has also been investigated as a possible remedy for infections resulting from other MDR bacterial strains, such as *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA) (Atshan et al., 2023; Zaki et al., 2023). In one treatment of skin infections caused by *Staphylococcus aureus*, which is commonly known as a "superbug" due to its resistance to multiple antibiotics. In a study, a topical use of a phage cocktail was applied to treat *S. aureus* skin infections in mice, resulting in a significant decrease in bacterial load and faster healing compared to control groups (Kifelew et al., 2020) (Figure 2).

4.3. Combine use of phage therapy along with antibiotic

In some cases, phage therapy is combined with antibiotic treatment to cure the bacterial infection. Combining phage therapy and an antibiotic can cure or delay proliferation of bacterial infection such as a 62-year-old patient had a chronic hip prosthetic joint infection (PJI) which is caused by bacteria *Pseudomonas aeruginosa* was successfully treated with the help of a combination of personalized phage therapy along with antibiotic meropenem (Cesta et al., 2023) (Figure 2). Another infectious disease Pneumonic plague which is caused by *Yersinia pestis,* is presently cured with antibiotics but in some cases of MDR strains, combination of second-line antibiotic (ceftriaxone) with phage therapy has found to be retarded mortality and restricted bacterial expansion in the lungs (Vagima et al., 2022) (Figure 2). Other that these, a study has found that phage-antibiotic combinations have effective eradication of biofilms formed by *Staphylococcus aureus* strains which are difficult to eliminate which showed superior efficacy of phage-antibiotic combinations compared to using either of antibiotics or phage alone, showcasing its potential for fighting against biofilm infections (Kebriaei et al., 2023).

4.4. Current status of phage therapy research

Phage therapy is a swiftly evolving field, and there is continuous study targeted at improving its efficacy and safety. Phage therapy is also being now used in treatment of cancer where phages are utilized as delivery vehicle for various therapeutic genes and drugs (Petrov et al., 2022). One area of research is the development of phage cocktails, which are mixtures of phages that target multiple strains of bacteria. Phage cocktails have been shown to be more suitable and effective than individual phages in treating bacterial infections, as they can target a huge range of bacteria (Chan et al., 2013).

Another area of research is the development of tools for rapid identification of phages effective against specific bacterial strains. This is important for the timely treatment of infections, as it can take several days to isolate and characterize phages. One approach is use

Table 2. Advantages and challenges of phage therapy and antibiotic therapy

Advantages and Challenges	Phage Therapy	Antibiotic Therapy	References
Targeted therapy	Phages can be tailored to target specific bacterial strains, leaving other beneficial bacteria untouched	Broad-spectrum antibiotics can also kill beneficial bacteria, leading to dysbiosis	Suttle, 2007; Rohwer and Thurber, 2009; Golkar et al., 2014.
Multi-drug resistance	Phages can be effective against multi-drug resistant bacterial infections	Antibiotic resistance is a growing problem, and there are limited treatment options against infection of multi-drug resistant bacteria	Wright et al., 2009; Kutter et al., 2010; Alemayehu et al., 2012
Diversity of phages	Phages have a wide range of targets and can be isolated from various sources	Antibiotics have a limited range of targets and are typically derived from a small number of chemical classes	Wills et al., 2005; Abedon, 2015; Abedon, 2017.
Development of resistance	Development of phage resistance is the potential concern, but phages can be combined to reduce the likelihood of resistance	Development of antibiotic resistance is a major challenge, and once resistance develops, antibiotics become ineffective	Chan et al., 2013; Nilsson, 2014
Production	Phages can be easily produced and scaled up for mass production	Antibiotics can be difficult to produce and may have limited availability	Abedon et al., 2011; Lu and Koeris, 2011
Regulatory challenges	The regulatory framework for phage therapy is still evolving, and there are challenges associated with obtaining approval for the application of phages in human medicine	Antibiotics are well-established drugs with established regulatory frameworks	Fish et al., 2018; McCallin et al., 2019

of high-throughput sequencing techniques to identify and characterize phages in particular environmental samples. Other techniques like genome editing by CRISPR-Cas and synthetic biology in the field of phage research will create new avenues (Chen et al., 2019).

In addition, research is ongoing into the safety and regulatory aspects of phage therapy. Phages are regarded as safe for human use, as they are natural and non-toxic, but there is a need for standardized protocols for the production, purification, and quality control of phages for clinical use (Loc-Carrillo and Abedon, 2011; Lin et al., 2017).

5. Conclusion

In conclusion, phage therapy is a promising substitute to cure bacterial infections, particularly caused by MDR bacterial stains. The approach of phages therapy is multifaceted and can involve direct lysis of the bacterial cell, production of lysins, and modulation of the host immune response. However, application of phage therapy possesses several challenges, including the development of phage resistance and immune responses to the phage itself. Ongoing research is focused on optimizing its efficacy and safety, and phage therapy has the significant potential to become an important tool in the fight against antibiotic-resistant bacteria.

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Author contribution

SK designed the theme of the manuscript. SK, GKS, ST, KY, LY and OP conducted the literature search and wrote the manuscript. SK and GKS made tables. SK, GKS and LY made figures. SK and GKS critically reviewed and edited the final version of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Abedon ST, Kuhl SJ, Blasdel BG and Kutter EM. 2011. Phage treatment of human infections. Bacteriophage 1 (2): 66-85.

Abedon ST. 2015. Phage therapy of pulmonary infections. Bacteriophage 5(3): e1020260.

Abedon ST. 2017. Bacteriophage clinical use as antibacterial "drugs": utility and precedent. Microbiology Spectrum 5(4): BAD-0003-2016.

Ackermann HW. 2007. 5500 Phages examined in the electron microscope. Archives of Virology 152(2): 227-243.

Adebayo OS, Gabriel ARAO, Taiwo MO, and Kayode JS. 2017. Phage therapy: a potential alternative in the treatment of multi–drug resistant bacterial infections. Journal of Microbiology & Experimentation 5(7): 14–12.

Alemayehu D, Casey PG, McAuliffe O, Guinane CM, Martin JG, Shanahan F, Coffey A, Ross RP and Hill C. 2012. Bacteriophages φMR299-2 and φNH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells. mBio 3(2): e00029-12.

Arumugam SN, Manohar P, Sukumaran S, Sadagopan S, Loh B, Leptihn S and Nachimuthu R.2022. Antibacterial efficacy of lytic phages against multidrug-resistant *Pseudomonas aeruginosa* infections in bacteraemia mice models. BMC Microbiology 22: 187.

Atshan SS, Hamat RA, Aljaberi MA et al. 2023. Phage Therapy as an Alternative Treatment Modality for Resistant *Staphylococcus aureus* Infections. Antibiotics (Basel, Switzerland) 12(2): 286.

Bernhardt TG, Struck DK, Young R. 2001a. The lysis protein E of phi X174 is a specific inhibitor of the MraY-catalyzed step in peptidoglycan synthesis. The Journal of Biological Chemistry 276(9): 6093-6097.

Bernhardt TG, Wang IN, Struck DK, Young R. 2001b. A protein antibiotic in the phage Qbeta virion: diversity in lysis targets. Science 292 (5525): 2326-2329.

Bernhardt TG, Wang IN, Struck DK, Young R. 2002. Breaking free: "protein antibiotics" and phage lysis. Research in Microbiology 153(8): 493-501. doi:10.1016/s0923-2508(02)01330-x

Brüssow H and Kutter E. 2005. Phage ecology. Bacteriophages: Biology and Applications. CRC Press. Pp. 129-163.

Campbell NA, Reece JB. 2005. *Biology*. Pearson, Benjamin Cummings, San Francisco. Pp 338–339

Carlton RM. 1999. Phage therapy: past history and future prospects. Archivum immunologiae et therapiae experimentalis 47(5): 267-274.

Casjens S and Hendrix R. 2005. Bacteriophages and the bacterial genome. In: Higgins (Eds.), *The Bacterial Chromosome*, ASM Press, Washington, DC. Pp. 39-52.

Catalão MJ, Gil F, Moniz-Pereira J, São-José C, Pimentel M. 2013. Diversity in bacterial lysis systems: bacteriophages show the way. FEMS Microbiology Review 37(4): 554-571.

Cesta N, Pini M, Mulas T et al. 2023. Application of Phage Therapy in a Case of a Chronic Hip-Prosthetic Joint Infection due to *Pseudomonas aeruginosa*: An Italian Real-Life Experience and *In Vitro* Analysis. Open Forum Infectious Diseases 10(2): ofad051.

Chan BK, Abedon ST and Loc-Carrillo C. 2013. Phage cocktails and the future of phage therapy. Future Microbiology 8(6): 769-783.

Chang RYK, Chow MYT, Wang Y, Liu C, Hong Q, Morales S, McLachlan AJ, Kutter E, Li J and Chan HK. 2022. The effects of different doses of inhaled bacteriophage therapy for *Pseudomonas aeruginosa* pulmonary infections in mice. Clinical Microbiology and Infection 28 (7): 983-989.

Chang RYK, Wallin M, Lin Y, Leung SSY, Wang H, Morales S and Chan HK. 2018. Phage therapy for respiratory infections. Advanced Drug Delivery Reviews 133: 76-86.

Chen Y, Batra H, Dong J, Chen C, Rao VB and Tao P. 2019. Genetic Engineering of Bacteriophages Against Infectious Diseases. Frontiers in Microbiology 10: 954.

Clokie MR, Millard AD, Letarov AV, Heaphy S. 2011. Phages in nature. Bacteriophage 1(1): 31-45.

Dedrick RM, Guerrero-Bustamante CA and Garlena RA et al. 2019. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. Nature Medicine 25(5): 730-733.

Dion MB, Oechslin F, Moineau S. 2020. Phage diversity, genomics and phylogeny. Nature Review Microbiology 18(3): 125-138.

Durr HA and Leipzig ND. 2023. Advancements in bacteriophage therapies and delivery for bacterial infection. Materials Advances 4(5): 1249-1257.

Eriksson F, Tsagozis P, Lundberg K et al. 2009. Tumor-specific bacteriophages induce tumor destruction through activation of tumor-associated macrophages. Journal of Immunology 182(5): 3105-3111.

Fauconnier A. 2019. Phage Therapy Regulation: From Night to Dawn. Viruses 11(4): 352.

Fish R, Kutter E, Wheat G, Blasdel B, Kutateladze M and Kuhl S. 2018. Compassionate use of bacteriophage therapy for foot ulcer treatment as an effective step for moving toward clinical trials. Methods in Molecular Biology (Clifton, NJ) 1693: 159–170.

Golkar Z, Bagasra O and Pace DG. 2014. Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. Journal of Infection in Developing Countries 8(2): 129-136.

Górski A and Weber-Dabrowska B. 2005. The potential role of endogenous bacteriophages in controlling invading pathogens. Cellular and Molecular Life Sciences 62(5): 511-519.

Gutiérrez B, Domingo-Calap P. 2020. Phage Therapy in Gastrointestinal Diseases. Microorganisms 8(9): 1420.

Harper DR, Parracho HMRT, Walker J, Sharp R, Hughes G, Werthén M, Lehman S and Morales S. 2014. Bacteriophages and Biofilms. Antibiotics 3(3): 270–284.

Hibstu Z, Belew H, Akelew Y, Mengist HM. 2022. Phage Therapy: A Different Approach to Fight Bacterial Infections. Biologics: Targets and Therapy 16: 173-186.

Howard-Varona C, Hargreaves KR, Abedon ST, Sullivan MB. 2017. Lysogeny in nature: mechanisms, impact and ecology of temperate phages. ISME Journal 11(7): 1511-1520.

Jennes S, Merabishvili M, and Soentjens P et al. 2017. Use of bacteriophages in the treatment of colistin-only-sensitive *Pseudomonas aeruginosa* septicaemia in a patient with acute kidney injury-a case report. Critical Care 21(1): 129.

Khan A, Rao TS and Joshi HM. 2022. Phage therapy in the Covid-19 era: Advantages over antibiotics. Current Research in Microbial Sciences 3: 100115.

Kebriaei R, Lehman SM, Shah RM, et al. 2023. Optimization of Phage-Antibiotic Combinations against Staphylococcus aureus Biofilms. Microbiology Spectrum 11(3): e04918-22.

Kifelew LG, Warner MS, Morales S, Vaughan L, Woodman R, Fitridge R, Mitchell JG and Speck P. 2020. Efficacy of phage cocktail AB-SA01 therapy in diabetic mouse wound infections caused by multidrug-resistant *Staphylococcus aureus*. BMC Microbiology 20(1): 204.

Kutter E and Sulakvelidze A. 2004. Chapter 2. Bacteriophage Research: Early History. Biology and history. In *Bacteriophages: Biology and Applications*. CRC Press. Pp. 1-19.

Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S and Abedon ST. 2010. Phage therapy in clinical practice: treatment of human infections. Current Pharmaceutical Biotechnology 11(1): 69–86.

Laxminarayan R, Duse A, and Wattal C et al. 2013. Antibiotic resistance-the need for global solutions. Lancet Infectious Deases 13 (12): 1057-98.

Lin DM, Koskella B and Lin HC. 2017. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World Journal of Gastrointestinal Pharmacology and Therapeutics 8 (3): 162-173.

Loc-Carrillo C and Abedon ST. 2011. Pros and cons of phage therapy. Bacteriophage 1(2): 111-114.

Loenen, WA and Murray NE. 1986. Modification enhancement by the restriction alleviation protein (Ral) of bacteriophage lambda. Journal of Molecular Biology 190(1): 11–22.

Lu TK and Koeris MS. 2011. The next generation of bacteriophage therapy. Current Opinion in Microbiology 14(5): 524–531.

Marza JA, Soothill JS, Boydell P and Collyns TA. 2006. Multiplication of therapeutically administered bacteriophages in *Pseudomonas aeruginosa* infected patients. Burns 32(5): 644–646.

McCallin S, Sacher JC, Zheng J and Chan BK. 2019. Current State of Compassionate Phage Therapy. Viruses 11(4): 343.

Nagel T, Musila L, Muthoni M, Nikolich M, Nakavuma JL, Clokie MR. 2022. Phage banks as potential tools to rapidly and cost-effectively manage antimicrobial resistance in the developing world. Current Opinion in Virology 53: 101208.

Nilsson AS. 2014. Phage therapy — constraints and possibilities. Upsala journal of medical sciences 119(2): 192–198.

Pessina B, Guarnieri V, Scarallo L. 2023. Entering the era of phage therapy: A 'happy hour' for inflammatory bowel diseases. Allergy 78 (3): 889-891.

Petrov, G, Dymova, M, Richter, V. 2022. Bacteriophage-Mediated Cancer Gene Therapy. International Journal of Molecular Sciences 23: 14245.

Petrovic Fabijan A, Lin RCY, Ho J, Maddocks S, Ben Zakour NL, Iredell JR and Westmead Bacteriophage Therapy Team. 2020. Safety of bacteriophage therapy in severe *Staphylococcus aureus* infection. Nature Microbiology 5(3): 465–472.

Pirnay JP, De Vos D and Verbeken G et al. 2011. The phage therapy paradigm: prêt-à-porter or sur-mesure? Pharmaceutical Research 28(4): 934–937.

Rohwer F and Thurber RV. 2009. Viruses manipulate the marine environment. Nature 459(7244): 207–212.

Sarker SA, McCallin S, and Barretto C et al. 2012. Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh. Virology 434(2): 222–232.

Schmelcher M, Donovan DM and Loessner MJ. 2012. Bacteriophage endolysins as novel antimicrobials. Future Microbiology 7(10): 1147-1171.

Schooley RT, Biswas B, and Gill JJ et al. 2017. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* Infection. Antimicrobial Agents and Chemotherapy 61(10): e00954-17.

Sharma S, Chatterjee S, Datta S, Prasad R, Dubey D, Prasad RK and Vairale MG. 2016. Bacteriophages and its applications: an overview. Folia Microbiologica. 62(1): 17-55.

Souza EB, Pinto AR, Fongaro G. 2023. Bacteriophages as Potential Clinical Immune Modulators. Microorganisms 11(9): 2222.

Sulakvelidze A, Alavidze Z and Morris Jr JG. 2001. Bacteriophage therapy. Antimicrobial Agents and Chemotherapy 45(3): 649-659.

Summers WC. 2001. Bacteriophage therapy. Annual Review of Microbiology 55(1): 437-451.

Suttle CA. 2007. Marine viruses--major players in the global ecosystem. Nature reviews. Microbiology 5(10): 801–812.

Wills QF, Kerrigan C and Soothill JS. 2005. Experimental bacteriophage protection against *Staphylococcus aureus* abscesses in a rabbit model. Antimicrobial Agents and Chemotherapy 49(3): 1220–1221.

Vagima Y, Gur D, Aftalion M, et al. 2022. Phage therapy potentiates second-line antibiotic treatment against pneumonic plague. Viruses 14(4): 688.

Wommack KE and Colwell RR. 2000. Virioplankton: viruses in aquatic ecosystems. Microbiology and Molecular Biology Reviews 64(1): 69-114.

World Health Organization. 2014. Antimicrobial resistance: global report on surveillance. World Health Organization.

Wright A, Hawkins CH, Änggård EE and Harper DR. 2009. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. Clinical Otolaryngology 34(4): 349-357.

Wu N, Chen LK, Zhu T. 2022. Phage therapy for secondary bacterial infections with COVID-19. Current Opinion in Virology 52: 9-14.

Young R. 2014. Phage lysis: three steps, three choices, one outcome. Journal of Microbiology 52(3): 243-258.

Żaczek M, Weber-Dąbrowska B, Międzybrodzki R, Łusiak-Szelachowska M and Górski A. 2020. Phage Therapy in Poland - a Centennial Journey to the First Ethically Approved Treatment Facility in Europe. Frontiers in Microbiology 11: 1056.

Zaki BM, Hussein AH, Hakim TA, Fayez MS, El-Shibiny A. 2023. Phages for treatment of *Klebsiella pneumoniae* infections. Progress in Molecular Biology and Translational Science 200: 207-239.

Zinke M, Schröder GF and Lange A. 2022. Major tail proteins of bacteriophages of the order Caudovirales. The Journal of Biological Chemistry 298(1): 101472.