Antimicrobial-resistant infections and bacteriophages as an alternative treatment

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Abstract

The emergence and dissemination of highly resistant microorganisms is one of the major issues faced by public health today. Infections brought on by bacteria which are resistant to all available antimicrobials in the therapeutic arsenal have already started to appear. In stark contrast to the demand, there is a dearth of new innovative techniques to treat microbial infections that pose a serious concern due to resistant microorganisms. Numerous advanced techniques are being researched, with bacteriophage therapy as one of the possibilities. Bacteriophages represent an alternative option to combat these extensive drug-resistant microbes, because they are narrow-spectrum and can be employed to specifically kill target bacteria without upsetting the entire community structure through off-target impacts. As the number of phage-based research facilities increases globally, the encouragement of well-designed clinical trial growth, standardization of phage cocktail manufacture and storage, and advancement of international collaboration will become more crucial. The main aim of this review is to discuss the history, advantages, limitations, and present state of bacteriophage research and its applications to fight against antibiotic resistance, with an emphasis on ongoing clinical trials and animal models of phage therapy administration. The purpose is to discuss bacteriophages as an alternative option

to treat infectious pathogens that have developed resistance to all or most of the available antimicrobial drugs.

1. Introduction

Antimicrobial resistance (AMR) is the term used to describe the ability of microorganisms, such as bacteria, fungi, viruses and parasites, to survive and proliferate in the presence of medications intended to eradicate them (Salam *et al*., 2023). Infections caused by such microbes are difficult to treat and are associated with a high rate of morbidity and mortality worldwide (Alvi *et al*., 2021). AMR has become one of the biggest worldwide issues of the 21st century because of the speed at which the rate of AMR infections is increasing and the dearth of new antimicrobial drugs being developed to address this problem (Tang *et al*., 2023). Multi-drug resistant (MDR) microorganisms are currently causing more than 700,000 annual deaths, and by 2050, this figure is expected to reach about 10 million, surpassing the cancer mortality rate (Peng *et al*., 2022). Due to the practical difficulty of treating diseases caused by resistant pathogens, current antibiotics are becoming useless against these microorganisms (Ma *et al*., 2020).

The 'ESKAPE' pathogens, including *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp. are identified by the Infectious Diseases Society of America as the most critical AMR species, and they pose a major global health threat (Namonyo *et al*., 2022). The advent of resistant clinical strains of microbes has put us in the hazardous position of entering a "post-antibiotic era" of untreatable diseases and outbreaks around the world (Kaur *et al*., 2021). Antibiotic usage, in particular, puts microorganisms under selective pressure, leading to the emergence of drug-resistant microbes. As a result, a variety of drug-resistant microbes are attributed to various infections (Xiang *et al*., 2020). In essence, antibiotics are no longer a suitable candidate for

fighting AMR due to which, researchers are looking for novel alternative approaches to combat resistant variants of microbial pathogens (Easwaran *et al*., 2021).

1.1. Mechanism of antibiotic resistance

An antibiotic is a synthetic or natural chemical that either destroys or inhibits the development of microorganisms (Lewis, 2020)**.** These are the "miracle drugs" for treating bacterial illnesses, however, their increased accessibility has led to their broad and routine usage worldwide (Manohar *et al*., 2018). The development of antibiotic resistance in bacteria is an evolutionary process. From a therapeutic perspective, when an antibiotic is first introduced, all targeted pathogens are still susceptible to it. However, with continuous use, bacteria become resistant to the antibiotic (Salam *et al*., 2023). AMR in bacteria has grown to be a serious worldwide issue. Increasing death rates and healthcare expenses are a result of growing antibiotic resistance (Wang *et al*., 2018).

AMR, according to the World Health Organization, is a natural process that happens when bacteria become resistant to antibiotics that they were once sensitive to and that were effective in treating infections caused by these bacteria (WHO, 2020). Antimicrobial drugs induce drug resistance in microorganisms by altering their metabolic pathways or producing inactivating compounds that enable them to resist antimicrobials (Xiang *et al*., 2020). Bacteria develop resistance mechanisms that were discovered, such as i. enzyme inactivation, ii. a decrease in cell permeability, iii. modification of the target active site, and iv. increased antibiotic efflux as a result of overexpression of efflux pumps (Abo-Aeid *et al*., 2021).

Drug inactivation by bacteria produces enzymes that alter or inactivate the antibiotics. One such example is the ability of β-lactamases to inactivate the β-lactam ring structure, which is necessary for the action of antibiotics like penicillin, monobactams, cephalosporins, and

carbapenems (Jing-Sheng *et al*., 2023). Bacteria can become resistant to antibiotics by decreasing the susceptibility and affinity of the drugs toward the active site of the penicillin-binding protein (PBP), either by adding exogenous DNA or changing the PBP gene. Additional measures to lower antibiotic concentrations include decreasing membrane permeability and over-expressing the efflux pump, as shown in **Figure 1** (Breijyeh *et al*., 2020). Furthermore, some phenotypic traits, such as quorum sensing and biofilm development, may be present in specific bacterial strains, making them more resistant to the effects of antibiotics (Abo-Zeid *et al*., 2021).

It is impossible to pinpoint a single mechanisms for the quick spread of AMR among bacterial populations. Complex procedures are frequently the main cause (Mancuso *et al*., 2021). Based on this, the antibiotic resistance mechanism are classified into four groups:

i. Intrinsic resistance, in which bacteria have the ability to modify their structures or parts (Reygaert, 2018).
ii. Acquired resistance, where resistant bacteria can exchange DNA and new resistant genes with one another (Arsene *et al*., 2022).
iii. Genetic alterations in the DNA can modify how proteins are produced, resulting in new components and receptors that the antibiotic cannot identify (Palma *et al*., 2020).
iv. DNA transmission by conjugation, transduction, or transformation which is a horizontal gene transfer mechanism between bacteria (Lima *et al*., 2020).

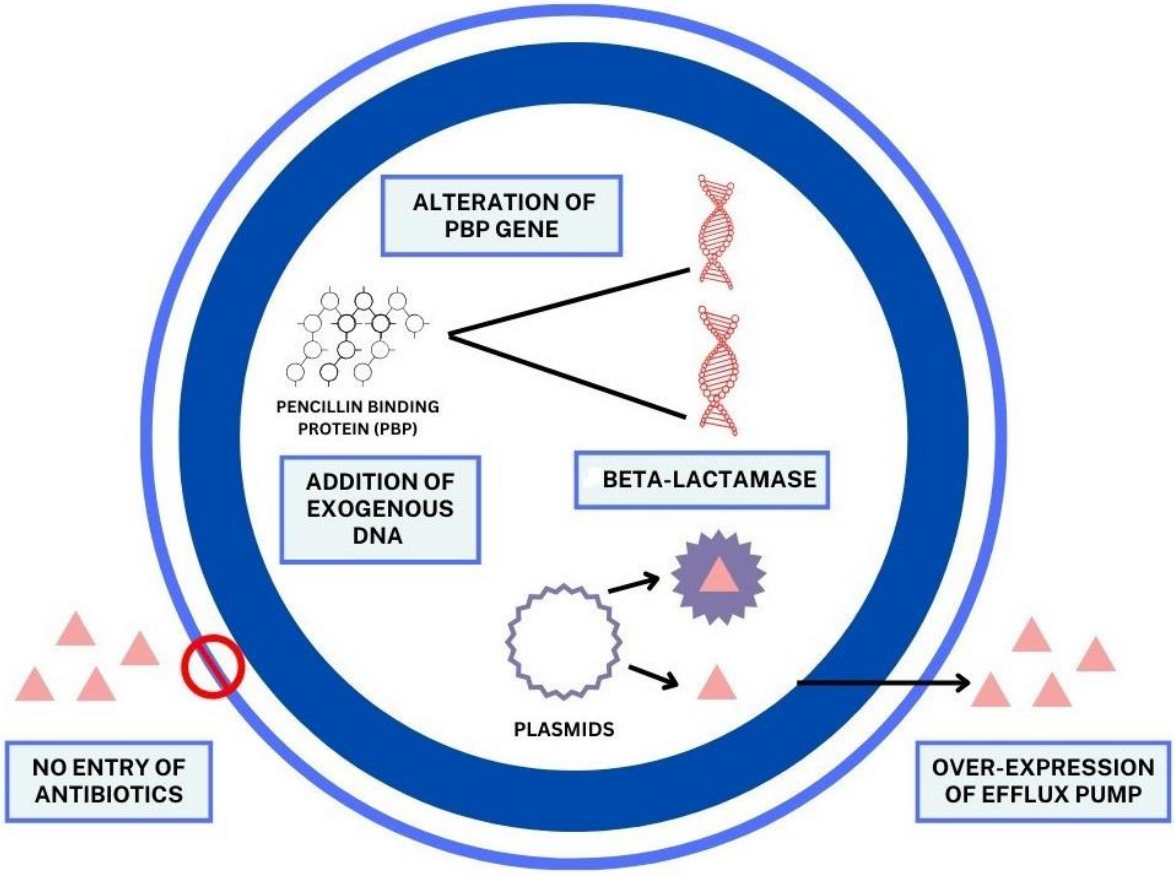

Figure 1: A summary of various mechanisms of antibiotic resistance.

AMR traits are distributed vertically to daughter cells of the same species and horizontally by mobile genetic elements (transposons and plasmids) to the same as well as different species (Zohra *et al*., 2021). Microbes that are multidrug-resistant (MDR), extensively drug resistant (XDR), or pandrug resistant (PDR) are expanding widely across the globe (Rima *et al*., 2021). AMR is spreading like wildfire now and is considered one of the top priority research areas by international organizations (Zohra *et al*., 2021). The global burden of microbial diseases, the failure of effective antimicrobials due to MDR strains, and the high cost of existing diagnostic facilities together warrant the development of improved, specific, and low-cost infection treatment methods (Manohar *et al*., 2018). Since the nature of an antibiotic's structure and its

affinity for various bacterial structures play a major role in its mechanism of action, understanding the mechanism of action of these drugs is "the condition sine qua non" for comprehending the emergence of resistance to them (Kapoor *et al*., 2017).

Antimicrobial resistance emerges and spreads due to many factors other than the duration of antibiotic therapy, for which there is insufficient empirical data and knowledge. According to the urgency of the need, the WHO released a list of microorganisms in 2017 that require immediate attention with new antibiotics, with categories of priority as critical, high, or medium, as shown in **Figure 2** (Breijyeh *et al*., 2020). These pathogens can be dangerous for patients in nursing homes, hospitals, and other facilities, as well as for those whose illnesses require the medical devices like blood catheters and ventilators (Santajit & Indrawattana, 2016).

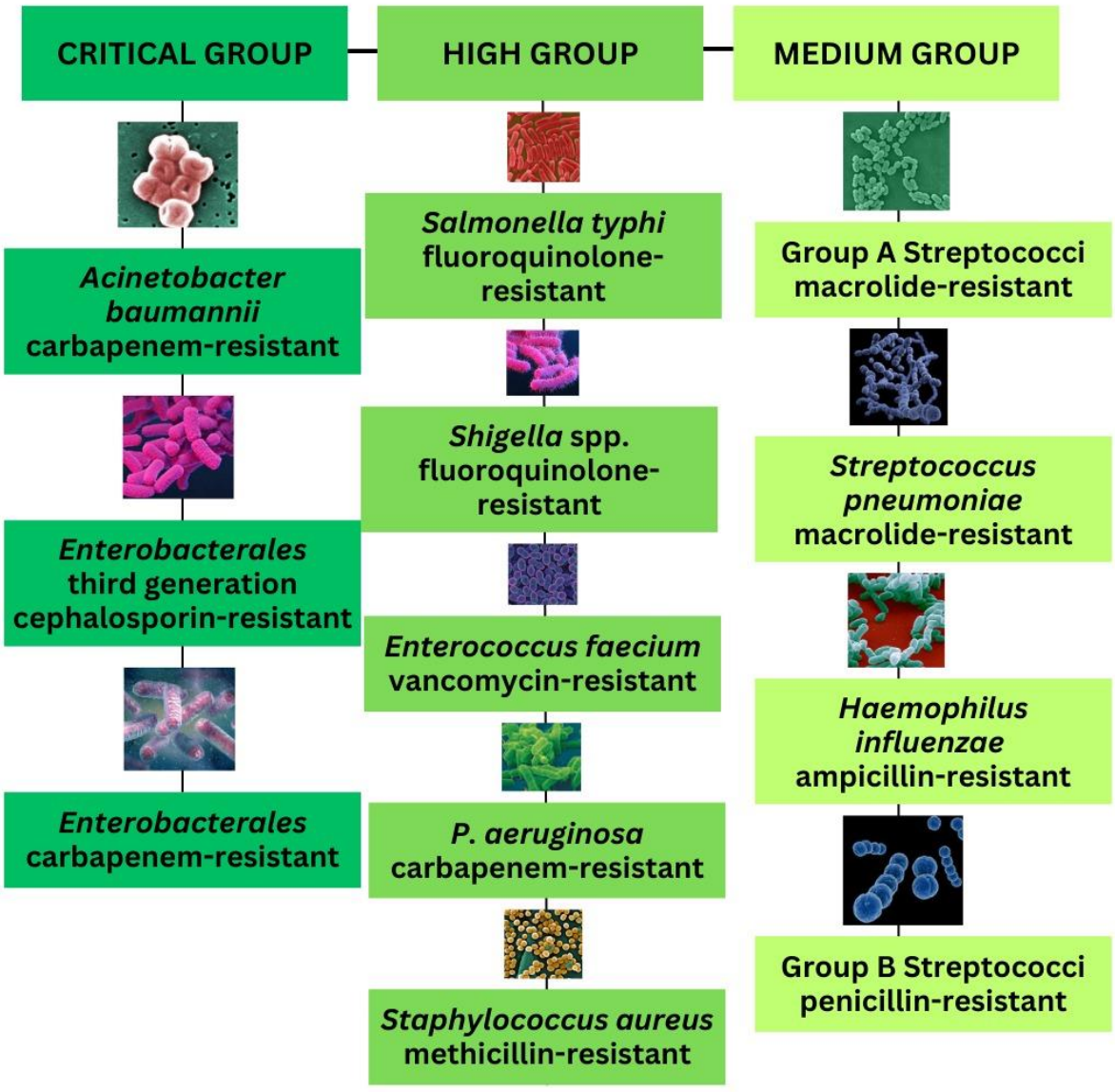

Figure 2: WHO has categorized pathogens into three priority groups based on their AMR profile.

1.2. Strategic data to guide the AMR response

The Global Antimicrobial Resistance and Use Surveillance System (GLASS) was introduced by WHO in 2015 to bridge knowledge gaps and provide guidance for policies at all levels (Tang, 2023). GLASS offers a standardized method for nations, territories, and places to gather,

analyze, interpret, and share data. Additionally, it keeps an eye on the state of both new and old national surveillance systems, with a focus on the representativeness and caliber of data gathering. Surveillance networks that enable GLASS enrollment and offer technical assistance to countries have been formed in some WHO areas (WHO, 2023). The main objectives of GLASS are to support worldwide AMR surveillance and identify AMR's causes. This would entail offering counsel and direction to assist countries in putting corrective measures into place as needed (WHO, 2021).

1.3. Antibiotic stewardship programs and AWaRe Classification

Antibiotic stewardship programs are designed to maximize their usage while balancing their effectiveness with the potential risks they pose to both current and future patients (Hulscher & Prints, 2017). Overuse of antibiotics is one of the main causes of antimicrobial resistance; therefore, strong antimicrobial stewardship systems are required to guarantee the appropriate use of antibiotics (Sneddon *et al*., 2024). Reducing the duration of treatment is a fundamental component of ASPs and a popular method used to reduce antibiotic usage (Mo *et al*., 2023). The essential strategies of ASPs are pre-authorization, formulary restriction, and prospective audits with intervention and feedback. Additional strategies include dose optimization, timely de-escalation/streamlining, education, parenteral to oral switch, combination therapy, antimicrobial order forms, decision support and computer surveillance, guidelines, and automatic stop orders (Alghamdi *et al*., 2018).

Antimicrobial stewardship is a concerted effort to decrease the emergence and spread of microbial resistance by educating and convincing medical practitioners to prescribe antimicrobial medicines according to the right choice, dosage, and duration for better patient outcomes (Salam *et al*., 2023). WHO developed the AWaRe (Access, Watch, and Reserve) classification system

for antibiotics in order to decrease improper antibiotic use and increase access to appropriate treatment (Ajulo & Awosile, 2024). For the most prevalent clinical infections, the WHO AWaRe antibiotic book offers succinct, evidence-based recommendations on the antibiotic to use, dosage, mode of administration, and length of treatment in both primary care and hospital settings (WHO, 2019).

2. Bacteriophages as an alternative to antibiotics

In the current situation, bacteria resist antibiotics used as a last resort, such as the carbapenem group, and colistin thereby, severely restricting the clinical treatment choices available for infections (Aslan & Akova, 2022). Numerous researchers are working hard to explore the best alternatives for antibiotics from natural organic resources to tackle AMR (Gupta & Sharma, 2022). Since the rate of new medication discovery is far slower than the rate at which MDR and XDR bacteria evolve, there is an urgent need for innovative therapeutic choices (Tarin-Pello *et al*., 2022). One of the burgeoning therapeutic alternatives for the treatment of resistant bacterial infections is bacteriophages (viruses that eat and kill bacteria) (Strathdee *et al*., 2023). Phage therapy was identified more than a century ago as a naturally occurring option that was effective in treating bacterial illnesses because of its low intrinsic toxicity, self-replicating nature, and ability to co-evolve with the host (Liang *et al*., 2023). Phage therapy offers several features that make it an extremely potential substitute, as elucidated below.

2.1. Bacteriophages and their history

Bacteriophages, or simply phages, are viruses that particularly target and infect specific bacterial hosts (Ye *et al*., 2024). They were first discovered in England by Frederick Twort in 1915 and then by Felix d Herelle in 1917 in France (Letarov, 2020). Felix d Herelle is considered the father of bacteriophage therapy. However, phage therapy was disregarded despite encouraging

outcomes in the management of human pathogenesis due to increasing antibiotic efficacy (Gontijo *et al*., 2021). Nonetheless, the advent of MDR pathogens has given attention to phages as the antimicrobial agents of the twenty-first century. Several studies have assessed the use of bacteriophages to manage pathogens, including phage treatment for animals, encapsulated phages for enhanced phage therapy, and phages as food sanitizers (Costa *et al*., 2023). Bacteriophages are one of the most abundant entities present naturally in soil, freshwater, seawater, and sewage (Aishat *et al*., 2021). They can infect and kill microbes without endangering humans. They exhibit a variety of sizes and shapes, ranging in length between 20 and 240 nm (Cuntín-Abal *et al*., 2024).

Phages are obligate parasites composed of a nucleic acid enclosed by a three-dimensional structure: the head, tail, and spiral contact sheath (Elois *et al*., 2023). The structure of phage capsids is encoded in a circular ssDNA that can be re-engineered using standard DNA editing procedures (Petrenko, 2018). They can have two types of tails: tail fibers, which are longer and attach to their receptor sites without enzymatic activity; and tail spikes, which consist of compact proteins with some enzymatic activity (Leprince & Mahillon, 2023). dsDNA makes up the majority of phage genomes, but they can also have ssDNA, dsRNA, and ssRNA genomes (Nguyen *et al*., 2023).

2.2. Life cycle and mechanism of action

Bacteriophages can be classified according to their life cycles of reproduction when they infect a bacterium, which may be lytic, lysogenic, or chronic (Chevallereau *et al*., 2021). Each of these reproduction cycles can be further divided into five different phases: adsorption, injection of nucleic acid, replication of genetic material and genome expression of phages, virion assembly, and transmission (Grabowski *et al*., 2021). In the lytic cycle, a phage attaches itself to host

bacteria, injects its DNA, multiplies by capturing the host's molecular machinery, and ultimately accomplishes lysis of host bacteria while simultaneously releasing its progeny (Kakasis & Panitsa, 2018). The majority of lytic phages use two different types of proteins: the holins and the lysins. The holins are used for puncturing the cytoplasmic membrane of bacteria and serve as a synergistic agent with the endolysins, which breakdown the bacterial cell wall (Samir, 2024). Endolysins are peptidoglycan hydrolases that are translated during the lytic cycle of the bacteriophages (Jansson *et al*., 2024).

On the other hand, under the lysogenic reproduction cycle, phages directly inject their DNA into the host bacteria, transferring genetic material to each host's progeny as the bacterium replicates. This integrated viral genome is referred to as "prophage" which is distinguished from "provirus" which is a virus that has been integrated into eukaryotic genome (Hitchcock *et al*., 2023). These phages, known as temperate phages, can stay in the dormant lysogenic cycle which involves replicating viral DNA along with bacterial chromosomes until the viral genes are activated by suitable conditions (UV light, oxidative stress, temperature, etc.). This activation encourages prophage induction resulting in the lysis of host bacteria as the phage DNA departs the chromosome and multiplies widely (Abdulrahman *et al*., 2020). The main differences between the lytic and the lysogenic life cycles can be seen in **Figure 3**.

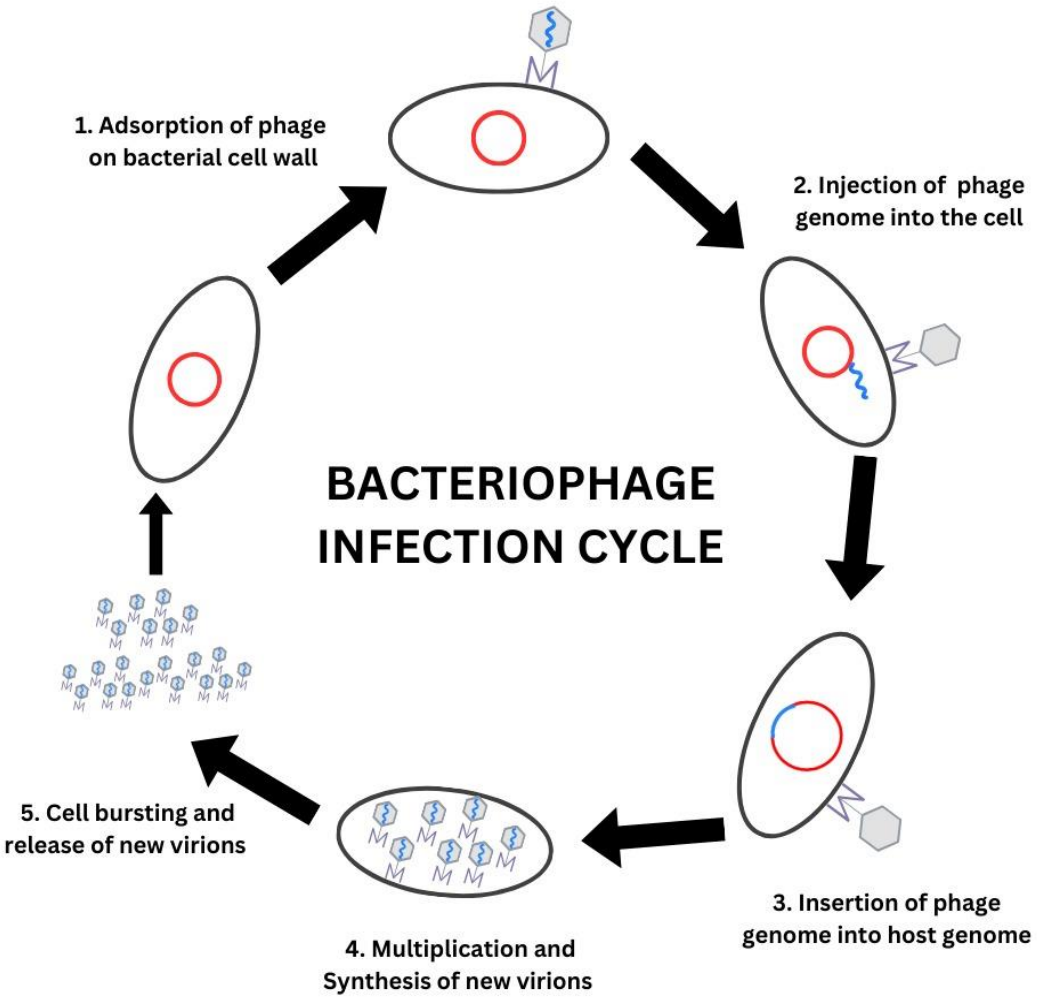

Figure 3. Life cycle of bacteriophages

Phages associated with chronic cycles belong to the Inoviridae, which consists of species of filamentous ssDNA phages. Furthermore, they have been found in almost every biome, including terrestrial, aquatic, and host-associated states (Roux *et al*., 2019). Because of their physical properties, chronic life cycle phages linked to the eukaryotic hosts may have a significant effect on host health. For example, Pf phage filamentous virions encourage the formation of strong

biofilm by their bacterial hosts (Chevallereau *et al*., 2021). They may carry the virulence factors and toxins that raise the pathogenicity of their microbial hosts in their relationship with the immune system of mammals (Sweere *et al*., 2019). Overall, these findings indicate that a significant portion of phages in the biosphere is generated via chronic life cycles; nevertheless, it is yet unknown whether these cycles are connected to certain ecological situations or settings (Chevallereau *et al*., 2021).

Phages are classified according to their different morphotypes, which comprise tailed, filamentous, and icosahedral phages. These types were further differentiated based on the organization of genetic material, such as ssDNA or ssRNA (Connell *et al*., 2021). The family Myoviridae possesses an icosahedral capsid connected to a lengthy contractile tail joined to a contractile sheath. The Siphoviridae family is distinguished by a longer, non-contractile tail (Cuntín-Abal *et al*., 2024). The family Podoviridae consists of short tails. Although, Cystoviridae and Tectiviridae lack tails, they do not have distinct outer groups that allow them to interface with the cell wall of the bacteria (Costa *et al*., 2023).

3. Therapeutic potential of bacteriophages

The therapeutic efficacy of phages involves the administration of a purified cocktail directly to the patient, stimulating the natural predator-prey relationship between the bacteriophage and its host bacteria (Raza *et al*., 2021). Lytic phages are mostly used in clinical research for the purpose of potential therapeutic applications, replicate exponentially inside the target bacteria only after an initial infection, and are important for therapy because of their reduced transduction potential (Chanishvili & Aminov, 2019). Lysogenic phages are thought to have a higher propensity to transmit virulent genes from AMR-producing host bacteria to less virulent bacteria (Hitchcock *et al*., 2023). This can happen through lysogenic conversion, which is the process of

transferring non-essential prophage genes into the bacteria changing the bacterial phenotype, and promoting the development of resistance mechanisms (Citorik, 2018). Thus, there is a considerable chance that lysogenic phages will exacerbate antibiotic resistance rather than lessen it (Saha & Mukherjee, 2019).

The potential advantages of utilizing phages as bio-recognition components validate the idea of using them instead of antibiotics (Hussain *et al*., 2021). Unlike traditional antimicrobials, which target one or more cell components like the cell wall, cytoplasmic membrane, metabolic activities, or nucleic acids, phages have their specific machines and their specificity is far higher for the organism they attack (Arsene *et al*., 2022). Bacteriophages have the ability to breakdown the extracellular matrix of biofilms enzymatically, preventing biofilm dissemination. Numerous preclinical animal studies have validated the use of phage therapy in clinical illnesses involving biofilm formation (Doub, 2020). Phage virions may also be used as stabilizers in the antibacterial and antibiotic production synthesis of metallic nanoparticles (Paczesny & Bielec, 2020). It has been demonstrated that bacteriophages are useful in treating some hospital-acquired infections, including those caused by *Acinetobacter spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Popova *et al*., 2012). Furthermore, bacteriophages also limit allergic reactions because they can only reproduce at the site of infection, whereas antibiotics can travel throughout the body and disregard the infection site. These are more eco-friendly, based mostly on natural selection, and capable of rapidly identifying bacteria compared with the new antibiotics, which may need several years and expensive clinical trials to develop (Raza *et al*., 2021). Phage diversity and abundance may render them a more discerning treatment than antibiotics for a range of applications, such as decontamination and antibacterial therapy, diagnosis of disease,

and control. Additionally, medical laboratories have employed phage typing to determine the species and subtypes of bacteria, including *Bacillus* and *Salmonella* (Nikolich & Filippov, 2020).

Phage therapy has several advantages:

i. It is efficient against pathogenic microorganisms that are resistant to multiple drugs (Kaur *et al*., 2018).

ii. It eradicates dangerous bacteria by specific targeting and does not negatively impact the typical microbial flora (Ghajavand *et al*., 2021).

iii. Mutation frequency of phages is substantially higher than that of bacteria; hence, they can quickly adapt to newly emerging phage-resistant bacterial mutants (Raza *et al*., 2021).

iv. Phage treatment is less expensive than conventional antibiotics (Ullah *et al*., 2020).

v. Compared to antimicrobial medications, phage therapy is incredibly rarely associated with adverse events (Kaur *et al*., 2018).

Phage therapy, therefore, is the insertion of phage "cocktails," i.e., mixtures of various bacteriophages, into infected patients. It can not only eliminate the microbial cells directly, but it can also have the ability to stimulate the immune system of humans to fight bacterial illness (Paczesny & Bielec, 2020). In view of the present understanding of phage biology and their increasing uses as therapeutic agents, phage treatment is thought to be significantly advanced to demonstrate its full potential (Raza *et al*., 2021).

Phage therapy has potential limitations, which include:

i. The diversity of microbial infections cannot be cured by a single phage type due to its high specificity and limited host range (Hyman, 2019).

ii. Even in certain times, treating a single bacterial infection requires the utilization of phage cocktails (Abedon *et al*., 2021).

iii. By modifying their phage receptor, bacteria can develop resistance against phages (Nikolich & Filippov, 2020).

iv. The human immunological reactions to phages are still unclear and may cause an undesirable reaction (Raza *et al*., 2022).

However, the narrow host range of the bacteriophage causes the bacterial pathogens to develop resistance to it. To address this problem, phage cocktails are utilized that offer a wider antibacterial spectrum (Aishat *et al*., 2021). The effectiveness of phages has been examined using a variety of animal models; in vivo research on phage therapy that has been published to date is summarized in **Table 1**.

Table 1. A summary of the therapeutic potential of bacteriophages (*in vivo* experiments).

Experimental Model	Phage	Host Bacteria	Dose & route of administration	Outcomes	Reference
Galleria mellonella wax moth larvae model	Escherichia phage (ECP311) Klebsiella phage (KPP235) Enterobacter phage (ELP140)	*Escherichia coli* *Klebsiella pneumoniae* *Enterobacter cloacae*	Group 1: Bacterial dose: 10^8 CFU/ml, treated with single dose of phage (10^4 PFU/ml). Group 2: Bacterial dose: 10^8 CFU/ml, treated with multiple doses of phages (10^4 PFU/ml) at 0^{th} h, 6^{th} h, 12^{th} h and 24^{th} h. Route of administration: intrahemocoelic	When treated with single phage dose: 90% survival rate. When treated with multiple doses of phages: 100% survival rate at 6 h interval.	Manohar *et al*., 2018
Galleria mellonella larvae model	Phage VL1	*Pseudomonas aeruginosa*	Bacterial dose: 10 CFU/10 µL of bacteria, Phage dose: 10^4 or 10^5 PFU/10ul Route of administration: Injection in to last left-side pro-leg of the larvae	70% survival rate observed at an MOI of 10^4	Lerdsittikul *et al*., 2022
Mouse	RLP	*Pseudomonas*	Bacterial dose:	Phage-treated group: 92% survival	Alvi *et al*.,

bacteremia		*aeruginosa*	10^7CFU/ml Phage dose: 10^9 PFU/mice Intraperitoneal route	Untreated group: 7.4% survival rate	2020
Mouse bacteremia	Phage BL02	*Klebsiella pneumoniae*	Dose: 2x10^8 PFU/mouse Intraperitoneal route	100% survival rate	Liang *et al*., 2023
Mouse pneumonia	*A. baumannii* phage Bϕ-R2096	*Acinetobacter baumannii*	Single phage dose Intranasal route	>2 $\log^{10}$ CFU reduction in CFU on day 1. By day 3, bacteria were cleared from mice's lungs. Bacteremic group: Expired by day 5. The treated group's survival rate: a) MOI = 10 (100%), b) MOI = 1 (60%), c) MOI = 0.1 (30%)	Jeon J *et al*., 2019
Mouse pneumonia	PBAB08 or PBAB25	*Acinetobacter baumannii*	Dose: 10^8 PFU/mouse Intranasal route	2 $\log^{10}$ CFU reduction between treated and untreated group on days 3 and 4. Treated group – 35% survival, untreated group – 15% survival	Cha *et al*., 2018
Mouse pneumonia	vB_KpnM-Teh.1	*Klebsiella pneumoniae*	Dose: 10^9 (MOI=10) or 10^{10} (MOI=100) PFU/mouse Intraperitoneal route	MOI = 10, resulted in 5 $\log^{10}$ reduction in CFU 3 days post infection. MOI = 100, resulted in 7 $\log^{10}$ reduction	Soleimani., 2020
Mouse pneumonia	Bϕ-R656 and Bϕ-R1836	*Pseudomonas aeruginosa*	Dose: 10^7 PFU/mouse (MOI = 10) Intraperitoneal route	Phage-treated groups: 66% and 83% survival on day 12, compared with 0% survival by day 3 in un treated group. 1–2 $\log^{10}$ reduction in CFU/g in lungs with both phages on day 1, 6 $\log^{10}$ reduction from both phages on day 5, with some mice undergoing complete clearance of bacteria	Jeon *et al*., 2019
Mouse pneumonia	PEV20	*Pseudomonas aeruginosa*	10^7 PFU/mouse, single dose at 2h post infection Inhalation route	5 $\log^{10}$ reduction in CFU/g in lungs at 24h	Chang *et al*., 2018
Mouse bacteremia	AB3P1	*Acinetobacter baumannii*	10^8 PFU/ml Intraperitoneal route	Treated group: 100% survival at 6 weeks, untreated group: 0% survival	Jasim *et al*., 2018
Mouse bacteremia	vB_KpnP_IME321	*Klebsiella pneumoniae*	Prophylactic treatment: depolymerase 50μg/mouse. Intraperitoneal, single dose 6h prior to infection. Therapeutic	100% survival in mice treated with Dp42 in both prophylactic and therapeutic treatment. When infected with Dp42 pretreated *K. pneumoniae*, mice showed 80% survival compared with 0% of untreated mice. CFU/g in	Wang *et al*., 2019

			treatment: Depolymerase 50µg/mouse. Intraperitoneal, single dose 30 min after infection	liver and lungs were 3 $\log^{10}$ lower than in untreated group, and were 5 $\log^{10}$ lower in spleen.	
Mouse bacteremia	*E. coli* IHE3034	*Escherichia coli*	10^8 PFU/mouse, single dose at 10 min, 1h or 2h after infection Intravenous	95-100% survival if administered within 1h, 33% survival if administered within 2 hours. No untreated mice survived.	Schneider *et al*., 2018
Mouse bacteremia	Monovalent and polyvalent phages	*Escherichia coli* *Klebsiella pneumoniae* *Pseudomonas aeruginosa*	10^9 PFU/mouse at 5h, 14h and 18h after infection Inraperitoneal	100% survival in all pathogen-infected mice after 5 days, compared with 0% in control. Single phage only rescued 60% of mice. In *E. coli* and *K. pneumoniae* co-infection, different phage cocktails showed different survival rates, 100%, 40%, and 0% for each cocktail	Kaabi & Musafer, 2019
Mouse bacteremia	Anti-pseudomonal øPEV20	*Pseudomonas aeruginosa*	10^4 PFU/mouse, single dose at 1h after infection Intravenous	8 $\log^{10}$ reduction in CFU after 2.5h, compared with non-treated mice. Concentrations lower than 10^4 were ineffective in treatment of the infection.	Lin *et al*., 2020

Phage therapy has demonstrated advancement in recent years, as proved by multiple phage efficacy trials, yet the treatment lacks regulatory approval for usage in humans (Kaur *et al*., 2021). Despite encouraging results in certain regions of the world, phage therapy needs more advanced experimental trials to prove its safety and effectiveness. The phage collection, isolation, purification, preparation, storage, and pharmacological processes ought to be thoroughly examined and handled separately (Kakasis and Panitsa, 2019). To assess the quality of phage therapy, it is essential to isolate and characterize more appropriate phages and evaluate their safety and efficacy (Vukotic *et al*., 2020).

Conclusion

In the fight against multidrug resistance, bacteriophages are expected to be among the most potent weapons, with the help of cutting-edge scientific research and rigorous scientific

applications. In order to tackle this global dilemma, phage therapy presents a strong supplement to the creation of novel antimicrobial drugs. Clinical studies of phage therapy may be designed as supplementary therapy or monotherapy, depending on the type of infection, the target bacteria's level of resistance, and the effectiveness of currently available antimicrobials. The creation of novel bacteriophage treatments and the planning and mitigation of clinical trial risk can both be greatly aided by the use of animal model systems. Although the application of phage therapy in humans has thus far been restricted, these therapies have shown that phages can be utilized as a proof-of-concept to treat life-threatening bacterial illnesses. In order to further understand the effectiveness of bacteriophage therapy in a clinical setting, attention must be directed towards pre-clinical pharmacokinetics and pharmacodynamic optimization of bacteriophages against MDR microbial infections, using quantifiable endpoints. Further advancements in this exciting technique to tackle illnesses will also be necessary for the creation of highly predictive animal model systems, well-planned clinical studies, and trustworthy sources of bacteriophage production.

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