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Medicinal and immunological aspects of bacteriophage therapy to combat antibiotic resistance

Isra Noor[1], Muhammad Hassan Nasir[2], Aneeq Ur Rehman[3], Noof Javed[1], Warda Waheed[1], Areeba Waheed[1], Ishmal Jamil[1], Wajeeha Shafiq[4], Muhammad Haseeb[1], Divya Dhawal Bhandari[5], Hitesh Chopra[6]*, Ahmad Syibli Othman[3]*

[1]Department of Microbiology, University of Agriculture, Faisalabad 38000, Pakistan
[2]Faculty of Medicine, Universiti Sultan Zainal Abidin, Kuala Terengganu 20400, Malaysia
[3]Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Kuala Nerus 21300, Malaysia
[4]Department of Microbiology and Molecular Genetics, Bahauddin Zakariya University, Multan 60800, Pakistan
[5]University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160019, India
[6]Department of Biosciences, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Chennai 602105, Tamil Nadu, India

***Correspondence:** Hitesh Chopra, Department of Biosciences, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Chennai 602105, Tamil Nadu, India. chopraontheride@gmail.com; Ahmad Syibli Othman, Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Kuala Nerus 21300, Malaysia. syibliothman@unisza.edu.my
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Abstract

Bacteriophages are viruses that infect bacterial cells and use their machinery to reproduce. This unique characteristic holds immense promise for combating antibiotic-resistant bacterial infections, a growing global threat. There are two types: one of them is named temperate phages, which inject their genomic material into bacteria and integrate into the host's genome, while the second one is entitled as lytic phages that subdue the entire metabolism of the bacterium for the synthesis of its genome and proteins, including lytic proteins involved in breaking bacterial cell membrane and release of novel phages. In addition, phage therapy can be expressed through anti-biofilm activity and by triggering innate and adaptive immune cells responses. Moreover, no adverse effects of phage therapy have been reported. However, phage therapy is still grim for many and could influence some interpretations related to immune response, bacteriophage selections, and phage resistance in the future.

Keywords

Phage therapy, temperate phages, lytic phages, phage displayed vaccine, phage DNA vaccine

Introduction

Bacteriophages, also called phages, are pathogens of bacteria that can infect and destroy bacterial cells. These infections initiate after the interaction of phage particles with specific surface receptor proteins of

bacterial cells, preceding cellular reproduction. Nearly 90% of bacteriophages contain icosahedral heads that, following their integration of genetic material toward the bacterial cell, cause the cells they have infected to lyse, ending the infection cycle. This lysis could occur immediately (lytic cycle), or it can happen after a protracted period, depending on the phage's life cycle (lysogenic cycle) [1]. In the process of lysis caused by phage endolysins, the destruction of bacterial cells and their death occurs. Simultaneously, this releases new phages into the environment, giving them the chance to target bacteria resembling those just eliminated. Bacteriophages can be safely applied to the body because there is no receptor for them in higher organisms, they only target specific bacteria. This is similar to how antibacterial agents like antibiotics and antiseptics function [2]. The ability to avoid damaging the frequently beneficial natural microbiota coupled with mammalian bodies is crucial to selective toxicity, even if it has not always been stressed, especially historically [3, 4]. Phage treatment, as it is usually known, was first used to treat bacterial illnesses in the early 1900s when Frederick Twort and Felix d'Hérelle discovered phages [5]. Also, nowadays phage therapy is applied as a last option, when there is no other possibility, especially in immunocompromised persons [6]. Bacteriophage research has been intensified worldwide due to the demand for novel antimicrobials [7]. There is the Wroclaw, Poland, Phage Therapy Center, which uses phages to treat bacterial diseases, particularly persistent infections, that have been shown to prove unresponsive to antibiotic therapy [8].

This review aims to explain the role of phage therapy in dealing with the threat of antibiotic resistance. It initially focuses on the antibiotic crisis, the causes and consequences of antibiotic resistance, and then on the successful but still complex intricate use of phages as therapeutic agents. Lastly, the perspective of study on the challenges that phage therapy will bring to modern society, highlighting the three majors; education, accessibility, and economics. The study concentrating on more recent studies that looked into the phage to expand the range of efficacy of therapeutic phage combinations. Then, comparison between phage and antibiotic therapy demonstrates that phages are more convenient to employ as antibacterial medications [9]. Employing phages as antimicrobial approach(es) presented several challenges, especially given including lack of knowledge about the biology of phages, and longer experience in phage therapy [10]. Overall, there is no doubt about the ability of phages to treat antibiotic-resistant bacterial diseases, but on the other hand, the studies have less clear/predictable about the dynamics and possibility of successfully incorporating phage treatment into most Western research methods, regulations, and clinical drug applications.

The main critique of phage treatment is that inadequate human cases are reported regarding the efficacy of double-blinded phase III. Consequently, a doubt has been raised about whether phage therapy can treat all illnesses. Recent assessments explicitly examined the efficiency of phage therapy in individuals [8, 11, 12]. It is crucial to use phages that cannot infect bacteria lysogenically, does not encode bacterial virulence factor genes, and are incapable of transducing those genes. While administering phages directly into an animal's systemic circulation, considerable purification is necessary (for example, to get rid of the bulk of bacterial components, including endotoxins) [13, 14]. The "gold standard" like double efficacy assessment, has often not been reached by phage therapy. But rather than the outcome of the research being carried out and failing to show proof of effectiveness, the lack of funding for such endeavors is mainly responsible for the scarcity of such research. Phage therapy is an ancient technique that has the potential of both commercial usage in therapeutic purposes and the manner of bio-control goods. However, many phage products now on the market have not undergone enough in-depth testing, particularly concerning the medical treatment of individuals.

Antibiotic resistance

One of the most effective therapeutic approaches in the annals of medicine has long been antibiotic therapy. Antibiotics are compounds, mostly secondary metabolites of some bacteria and fungi, which target other bacteria [15]. Antibiotics prevented millions of deaths and contributed significantly to advancing several medical innovations, including cancer treatment and organ transplantation [16]. Antibiotics have genuinely

altered the world, but due to overuse and misuse of antibiotics by humans, society is facing an antibiotic crisis due to the emergence of multi-resistant pathogens. A so-called "post-antibiotic age" is soon approaching, and it would be devastating to lose the medical benefits of antibiotic prophylaxis [17].

Resistance: inherent or man-made?

The invention and usage of antibiotics by humans did not cause the naturally occurring phenomena of antibiotic resistance. Microbes and higher eukaryotes create a wide range of physiologically active compounds with antibacterial capabilities that have been reclaimed as contemporary antibiotics in various conditions [18]. However, the levels of these substances in these natural settings are frequently lower than clinically meaningful thresholds, indicating that resilience does not just develop to counteract their harmful effect. It has been suggested that rather than acting as rigid antimicrobial agents, antibiotics and their interactions with antibiotic resistance pathways function in nature as a means of communication amongst the individuals that make up a microbial community [19]. There is evidence that these chemicals alter community composition and elicit adaptive genotypic and phenotypic responses [19]. When looking at antibiotics from a biological standpoint, it is not surprising that the diversity of ancient bacteria contains genes that provide resistance to modern drugs, like uncontaminated arctic permafrost, indicating that antibiotic resistance persists even without human intervention [20–22].

Antimicrobial resistance is significantly made worse by human activities, notably clinical and industrial abuse of antibiotics. Antibiotics are employed in the production of crops and livestock to cure crop and fish infections, as well as, of course, to combat infectious diseases in people and animals [23]. In reality, others are usually blamed for misusing antibiotics, it has been stated that other nations' agricultural usage of antibiotics is considerably higher, citing estimates that the United States produces meat with up to 180 mg of a powerful antibiotic drug per kilogram [24]. Unexpectedly, billions of metric tonnes of antibiotics have leaked into wastewater streams and natural reserves as a result of antibiotic usage in agriculture. Farms located closer to cities, pharmaceutical waste being present, and inadequate water purification contribute to this issue by increasing the quantity of antibiotic exposure and its environmental persistence [25]. Continuous exposure of ambient microbial communities and pathogens to various antibiotics has accelerated the emergence and increased number of antibiotic resistance genes as well as their spread [25]. Gene flow, which is the interchange of genetic material among organisms by transformation, conjugation, or transduction, can be involved in development and spreading of antibiotic resistance. This process, in addition to spontaneous chromosomal mutations, strengthened due to the selective pressure of the drug, can result in resistance [26]. One of the primary sources of multidrug resistance for horizontal transmission is the gastrointestinal tract microbiota of antibiotic-treated animals and humans, where antibiotic-resistant bacteria have been preselected [27]. Furthermore, through mechanisms including biofilm formation, swarm adaptability, metabolic dormancy, and longevity, bacterial cells can develop temporary, non-genetically coded resilience [28].

A multitude of resistance mechanisms can be involved in interfering with each stage of antibiotic passage through the bacterial cell: to avoid the penetration of the drug, bacteria can change the structure of the outer membrane or cell wall, or they can increase the expression of the efflux pump to push the harmful chemicals-antibiotic out; they can also produce enzymes such as beta-lactamases that open the ring of the beta-lactam molecule and neutralize its lethal effect. Accordingly, antibiotic-induced synthesis of enzymes that alter the target of the antibiotic, hide it, or quantitatively change it [26, 29]. The bacterial mechanisms to provide antibiotic resistance have been shown in Figure 1.

Era of dry pipeline

The "golden age" of antibiotic development spanned from the 1940s through the 1980s, when more than 40 antibiotics were being developed and released for use in clinical settings. During this period, there was the discovery of a large number of new antibiotics, their subsequent use/overuse, and the modern emergence of resistance, but resistance to a particular antibiotic was rarely a cause for concern because newer compounds, often exhibiting stronger pharmacokinetic and pharmacodynamic attributes, were rapidly

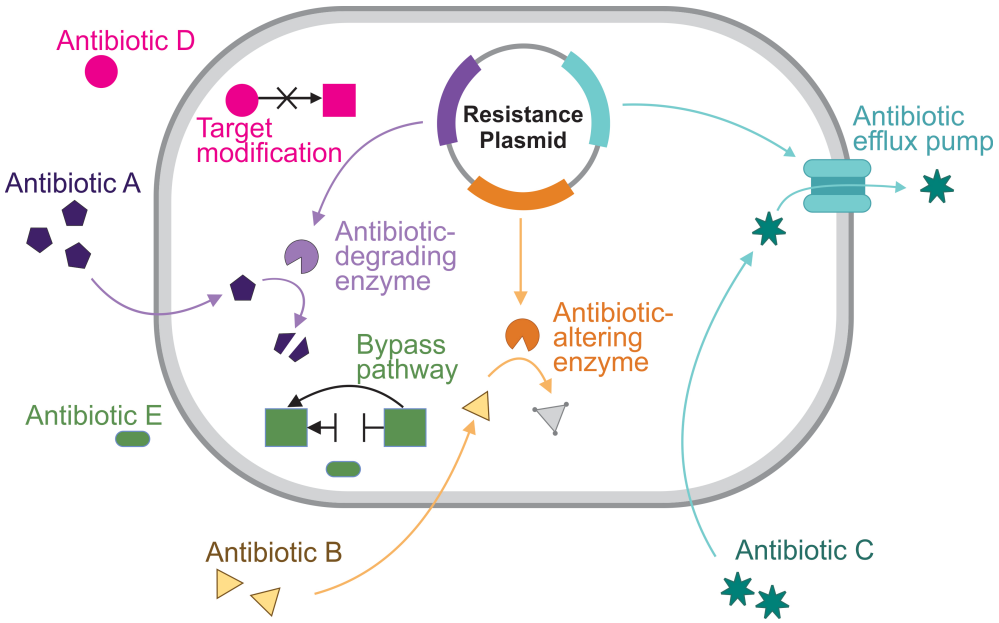

Figure 1. Bacterial mechanisms for the development of antibiotic resistance: antibiotic A, antibiotic B, antibiotic C, antibiotic D, and antibiotic E represent enzymatic inactivation of drug, remodulating envelope, activation of drug efflux pump, alteration of drug target, and inhibition of drug action, respectively

Note. Adapted from "Antibiotic resistance mechanisms", by BioRender.com (2024). Retrieved from: https://app.biorender.com/biorender-templates

developed [23, 30]. The implications of this counterproductive cycle, however, became increasingly apparent starting in the 1990s due to a continuous drop in the development and release of novel antibiotics. A situation known as a "dry pipeline" in the study and development of antibiotics refers to the fact that most newly produced antibiotics are modified or coupled variants of previously found chemicals. Innovative medications must prove not just their effectiveness but also their tolerability, an appropriate drug release profile, and cost-effectiveness, which is a difficult undertaking. About 5 of the 5,000 to 10,000 proposed antimicrobial compounds are thought to enter phase I research, of which only one is likely to receive regulatory approval for human use [30]. Pharmaceutical companies are reluctant to invest into antibiotic development when the chances/odds are against them because the drug development process is both costly and time-consuming. Additionally, the short-term use of antibiotics, the need for rigorous management programs, and their susceptibility to the development of resistance can significantly reduce a company's earnings [30]. The rapid emergence of antibiotic-resistant bacteria is even predicted to surpass recently launched medications, underscoring the need for innovative therapies [31]. Antibiotic resistance is an issue, and the dry pipeline makes it worse because it has slowed down our arsenal of treatment alternatives.

Consequences/crisis

The persistent development of antibiotic-resistance traits leads to the production of pathogens that are extensively drug-resistant, pan-drug-resistant (PDR), and multidrug-resistant (MDR) [32]. Researchers, doctors, and public health authorities have been interested in a particular bacterial species since they are mostly responsible for MDR infections, which are most commonly associated/linked to healthcare settings and are also the most severe. The acronym ESKAPE refers to the pathogens in this category, which includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., describes how its members might use a range of resistance mechanisms to prevent antibiotics' bactericidal activity [33]. Therefore, the World Health Organization (WHO) has classified the last four of these infections as a significant priority for introducing novel medicines, notably the carbapenem- and cephalosporin-resistant species [34].

MDR infections are more likely to be acquired by patients with underlying medical disorders, immunocompromised, and hospitalized patients (particularly in critical care units, surgical wards, or burns

units). But more and more publications are warning that "common" community-acquired illnesses might become resistant to antibiotic therapy in healthy people [35–37]. In the post-antibiotics era, even ordinary diseases and small wounds can be fatal [38]. Even a compilation of these metrics, which include rates of illness and mortality and monetary expenses, cannot fully capture the scope of the burden of antimicrobial resistance. Compared to patients with antibiotic-sensitive infections, people with MDR infections are more likely to experience treatment failure, have worse prognoses, have high death rates, have longer hospital stays, and increased complications risk or long-term consequences [23, 38, 39]. According to a recent analysis, by 2050, resistant strains would result in the yearly loss of 10 million lives, costing the global economy USD 100 trillion [40]. Regardless of age, social class, or place of residence, it poses a danger to world health that might impact everybody [41].

How to combat antibiotic resistance?

Antimicrobial resistance must be addressed by multifaceted, interdisciplinary, and global strategies. Establishing regulations for antibiotic use in both people and animals is a crucial element that must be considered [42]. Attention is needed since, in many nations, especially in underdeveloped nations, self-medication and easy availability of antibiotics without a prescription are dreadfully prevalent. The extensive use of antibiotics in livestock which has to be reduced is even more concerning. Medical professionals occasionally prescribe antibiotics for conditions that are not appropriate for them. Examples include treating viral or fungal infections with antibiotics, needlessly extending antibiotic courses, or using antimicrobials with a wider spectrum than is typically necessary for a given infection. By supplying the industry with administrative and financial stimulus, the problem of the dry pipeline might be reduced [43] or by "reviving" outdated antibiotics. This is the case with chloramphenicol and polymyxins, which were discontinued because of safety concerns (nephrotoxicity/neurotoxicity and uncommon yet potentially lethal hematological adverse effects, respectively), but are currently being reinstated [44, 45]. The most important thing is to promote research into novel therapeutic options and to reinvigorate interest in underutilized ones, like phage therapy. The detailed comparison of phage and antibiotic therapy has been portrayed in Table 1.

Discovery of bacteriophage therapy

The history of discovery bacterial viruses is more than 100 years old. Today it is recognized that the discovery of the bacteriophage belonged jointly to two microbiologists, Twort (1915) and d'Hérelle (1917), who independently discovered bacteriophages. Suppose the priority of the description of the lytic principle unquestionably belongs to the Englishman [5]. In that case, there is no doubt that Twort had made an imprecise interpretation and, above all, had not pursued his research and still least considered a therapeutic use [5]. Very early on, he anticipates the relationship between a phenomenon observed in the laboratory and the phenomenon of healing clinically. For him, the appearance of clear/transparent plaques, observed in the petri dishes on which the bacteria responsible for bacillary dysentery grew, seemed to announce a possible cure. The pathogenesis and pathology of bacillary dysentery are dominated by two factors acting in the opposite direction: the dysenteric bacillus, a pathogenic agent, and the bacteriophage filtering microbe, agent of immunity, affirmed, "It is logical to propose as a treatment for bacillary dysentery the administration, as soon as the first symptoms appear, of active cultures of the bacteriophage microbe" [62]. As a biologist at the Pasteur Institute in Paris, he had to take an interest in an epizootic of avian pox epidemic in France [63]. He took this opportunity to generalize his conclusions about the natural history of healing by bacteriophage. He further affirmed from that moment that immunity is contagious in the same way as the disease itself. It also follows from the facts that the ingestion of a culture of the bacteriophage microbe from a strain endowed with an exalted virulence for the pathogenic bacillus must be of such a nature as to confer immunity. From then on, d'Hérelle explained the natural history of the epidemic and its extinction by the diffusion of a bacteriophage. On this same note, he mentions an ongoing study of the experimental control of the epizootic. At the same time, d'Hérelle isolated bacteriophage active against different species of bacilli (in addition to the previous ones, *Escherichia coli*, *Proteus*, and several *Salmonella*) [64]. He demonstrated with a lot of assurance that it was a "germ" and not a "diastasis". The

Table 1. Comparison of phage therapy with antibiotic therapy

Property	Phage therapy	Antibiotic therapy
Specificity	Phages have a high degree of species and strain specificity as they only disrupt target bacteria. Therefore, ecologically important bacteria (e.g., intestinal microbiota) remain safe [46].	Antibiotics often kill a broad spectrum of both gram-positive and gram-negative bacteria, including beneficial bacteria, which is increasingly viewed as undesirable for normal microbiota [47].
Mechanism of action	During the lytic infection cycle, phage attaches to the bacterial cell's receptors, then delivers its genomic content inside the cell and undergoes replication through bacterial transcription, translation, and assembling process. After forming new phage particles, they leave the cytoplasfm through the lysis of bacteria. And this procedure is repeated as the escaped phages infect other bacterial cells [48].	Antibiotics act in the following ways: • Inhibit the synthesis of cell wall • Breakdown of cell membrane structure and function of the bacterial cell • Inhibit the function and structure of nucleic acids • Inhibition of synthesis of protein • Disturb key metabolic pathways of the bacterial cell [49].
Biofilm degradation	There has been great interest in using phage therapy to eliminate biofilms. This is caused by phages' capacity to produce enzymes (depolymerases) that break down a biofilm's extracellular polymer matrix. Notably, biofilm-forming bacteria do not shield cells from bacteriophage destruction by producing extracellular polysaccharide-based matrices [50].	Multiple tolerance mechanisms in biofilms forming bacteria resistant to antibiotic treatment (therapy). Continuous administration of antibiotics results in the persistence of biofilm infections, which increases the risk of the emergence of antibiotic resistance (genetic resistance) [51].
Immune response	Phages may cause innate and adaptive immune cells to respond, which could affect the efficacy of phage therapy. When pathogen recognition receptors (PRR) identify DNA and RNA derived by phages, innate immune cells can be activated. Moreover, phages can induce Antibody production as they have immunogenic proteins [52].	Antibiotics do not directly affect innate immune response but involve releasing pathogen-associated molecular patterns (PAMPs) in response to compromised bacterial cell walls. They do not induce antibody production [53].
Side effects	No severe adverse effects have been reported against phage therapy, making it an attractive treatment against bacterial infections [54].	Antibiotics have numerous side effects, including allergies, intestinal disorders, and disturbance in the nervous system, and also promote various secondary infections (yeast infections) [55].
Development of resistance	The following strategies allow bacteria to develop resistance to phages: • Preventing the phage from attachment to its surface by mutations in receptor protein (Y) is known as phage adsorption inhibition [56]. • By inhibiting the injection of phage genome into the cell, known as injection blocking. • After phage genome injection into a host, bacterial endonucleases can recognize and eliminate foreign DNA, which results in phage inhibition, known as restriction-modification. • The virus is prevented from spreading when phage-infected cells eventually die before completing the lytic cycle, known as abortive infection. • Acquired resistance can also develop by selecting non-susceptible strains based on the clustered regularly interspaced short palindromic repeats (CRISPR) system [57].	Resistance to antibiotics develops in the following ways: • Efflux pumps, which excrete the antibiotic from the cell. • Antibiotics can deactivate through enzymes. • Bacteria can produce another protein to bypass the inhibited one, target bypass. • Modification in the antibiotic target sites. • Resistance also happens through reduced uptake of antibiotics. • Quorum sensing allows bacteria to transfer their antibiotic-resistance genes to other bacteria [58].
Potential to overcome resistance	Bacterial strains that get resistant to phages are lower in fitness, so they could not survive more. Phages may also change; they can evolve to compete with bacteria that are resistant to them. Moreover, the development of Phage resistance may be completely avoided if phages are utilized in cocktails (consisting of different types of phages) [59].	A regimen of two to three antibiotics can be used against resistant infections but can lead bacterial strains to MDR strains. Resistance to antibiotics may spread to other bacteria, and new antibiotics against resistant bacteria may take several years to develop [60].
Discovery	New phage isolation and selection are less time- and money-consuming processes [54].	An effective antibiotic medicine development often costs millions of dollars and takes several years to produce, in addition to assessing potential toxicity [61].

main criterion was demonstrating the phenomenon's natural viral aspect, the appearance of clear patches on the petri dishes. But then he thought it was just one unique microbe while specifying that he had never isolated two identical bacteriophages. He named it bacteriophage intestinal and showed that this microbe,

whose expression is variable from one isolate to another (as spectrum and virulence), is able to acquire a specificity in contact with such bacterial species. This assertion is inaccurate as per numerous studies. Indeed, there are thousands of different bacteriophages per their morphology and their specificity, and that each bacterial species corresponds to at least one phage. Be that as it may, in two years, d'Hérelle has described the main characteristics of this new entity, developed the means of isolating it, purifying it, making it the principal agent healing of certain diseases, and explaining the history natural to certain epidemics.

d'Hérelle recounted his first attempts at "phage therapy" in children at the Necker-Enfants Malades hospital during the summer of 1919, not without first having absorbed and caused to be absorbed "his" bacteriophage to his entourage to check its safety. Five children with bacillary dysentery were successfully treated. From then on, many other works were undertaken [11]. In 1915, the bacteriophage was discovered by Twort and d'Hérelle firstly used phage for therapy in 1926. Morison successfully developed phage therapy for cholera epidemic in 1932, however antibiotics overshadow the phage therapy in 1940s. In 1990s, the biotech industry initiated to explore phage therapy after the several successful phage therapy experiments conducted by Smith and Huggins in 1980s. Phage therapy was adopted to successfully cure mice from vancomycin-resistant *Enterococcus* and methicillin-resistant *Staphylococcus aureus* in early 2000s [65]. The Japanese, while contesting the very nature of the bacteriophage, by intravenous injection of a "bacteriolysis of the Shiga bacillus", will eliminate the living bacillus from the bile of rabbits [66].

From the beginning of the year 1920, the author tried to demonstrate the chemical nature of bacteriolysis, which he called ferment [67]. While scientists will begin works that already hinted at the ubiquity of the bacteriophage, they preferred to study the "d'Hérelle phenomenon" or the "alleged bacteriophage" (so-called bacteriophage) by challenging the viral theory of d'Hérelle [68]. But, at the beginning of 1920, d'Hérelle had embarked on Indo-China. He had met Yersin, passing through Paris, which had offered to study the possibilities of treating rinderpest (Barbone). During his stay in the Far East, d'Hérelle generalized in animals the role of bacteriophage as a healing agent, not only in buffalo Bluebeard but plague in rats and flacherie in the silkworm: "I had not had, until now, the opportunity to seek the bacteriophage microbe only in diseases presenting intestinal manifestations: bacillary dysentery, fevers enteric, avian typhoid; in all these illnesses, I had succeeded in isolating a bacteriophage microbe active against the pathogenic bacteria. It was interesting to check whether the fact remained limited to intestinal diseases or if it was a general defense phenomenon" [69].

Phage therapy as an alternate treatment

One of the most important public health concerns is bacterial antibiotic resistance. Drug resistance in bacteria has become a major threat to human life. It has been estimated that nearly 48,000 people die in the USA and Europe only due to multidrug resistance pathogens. These MDR bacteria are also referred to as "superbugs". According to Lord Jim O'Neill, the prime minister of UK, antimicrobial resistance in bacteria could cause the death of 10 million people worldwide and an economic loss of 100 trillion USD by 2050. Today bacteriophages have been proposed as an alternative to antimicrobials for curing infections caused by superbugs. The use of bacteriophages to treat bacterial infections is known as "phage therapy or PT" [70–72].

Types of bacteriophages

Based on their modes of replication inside the bacterial host, there are two types of phages (i.e., the temperate phages and the lytic phages). Temperate phages bind with specific receptors on the bacterial cell, inject their genetic material into the bacteria, and then integrate it into the host's genome. Another type of phage is the lytic phages that upon entry subdue the bacterial biosynthetic machinery for synthesis of the genome and viral proteins including endolysins which degrade the bacterial cell wall, allowing the release of newly assembled phages [59, 70, 73].

Phage therapy mechanism

The biologics behind the phage therapy are also based on the bacteriophages' replication mechanism. In the case of the lytic phage, the cycle starts with attachment to specific receptor proteins on the bacterial cell. In both gram-negative and gram-positive bacteria, these receptor proteins can be present on cell wall (such as the teichoic acid, peptidoglycan in the case of gram-positive bacteria), or can also be located on the capsules or even the pili and flagella [59, 74]. Once interconnection is established between the bacteriophage and the bacterial receptor, the virus can infect the host by injection of viral genetic material into the bacterial host. When the viral genetic material enters the bacterial cell, it subdues the host's self-producing machinery. The next step is the production/multiplication of novel phages, followed by their release thanks to activity of lytic proteins-endolysins. The result is bacterial death and newly released phages are ready to infect other bacteria carrying the same receptors as the previous host. The lytic phages, which mostly infect human pathogens, belong to the orders of Microviridae ("the tail-less phages") and Caudovirales ("the tailed phages"), and these have single-stranded DNA or double-stranded DNA as the genomic material, respectively. Other than the lytic phages, which have great therapeutic significance in treating bacterial infection, the temperate phages cycle begins with attachment to the cellular receptors of bacteria. The subsequent steps differ because, in the case of temperate phages, the incorporation of phage genomic material takes place into the bacterial genome. So the temperate phage replicates with the bacteria itself, and under stress conditions, it may also cause the lysis of bacterial cells [59, 70, 73].

It must be noted that lytic phages are highly preferred over lysogenic phages because the latter can transfer virulent genes from one bacterium to another. However, in some cases, the lysogenic phages were considered for the therapy, such as for treating infection caused by *Clostridium difficile* for which no strictly lytic phage has been yet obtained. Also, temperate phages can be justified in emergencies with time constraints and when lytic phages are unavailable [59, 70, 73]. The mechanism of phage therapy has been sequentially illustrated in Figure 2.

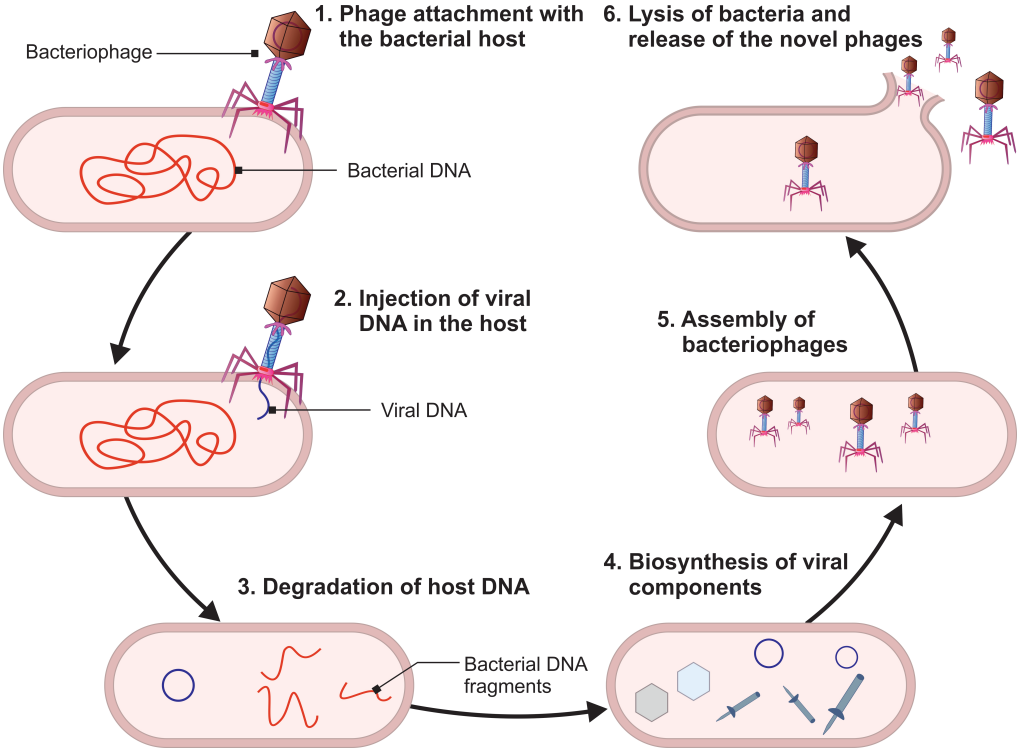

Figure 2. The mechanism of phage therapy. (1) Adherence of bacteriophage on the bacterial host; (2) injection of bacteriophage viral genome to bacterial host; (3) degradation of host bacterial DNA; (4) biosynthesis of viral components; (5) assembly of bacteriophage; and (6) lysis of bacterial cell membrane and extrude novel bacteriophages

Unique pharmacology of phage therapy

The pharmacodynamics of phage therapy is linked with its pharmacokinetics. Phages must be applied at the side where there is a bacterial infection. When phages are given intravenously, very few of them can reach the site of infection, and they also clear from the blood very quickly. The success of phage therapy depends upon the initial dose and the timing of the administration of phages. Therefore, it is better to use more virulent phages in therapy. A virulent phage has a big burst size which is very beneficial in suppressing the growth of bacteria [72]. There are two categories of phage therapy. One is using a single phage specific for the bacteria causing the infection, and this type is also referred to as "mono-phage therapy". The other type of phage therapy is called "polyphage therapy" as it employs a combination of phages (i.e., a phage cocktail). Bacteria have developed mechanisms to escape from the phages that kill them, such as using outer membrane vesicles as decoys, prevention of phage adsorption, cleavage of phage genetic material, preventing the phage assembly, blocking the entry of phage DNA, bacterial suicide, and many other newly discovered mechanisms. To combat the resistance, the use of polyphage therapy is recommended. To optimize the phage cocktail, many procedures have been introduced that allow the selection of more lytic phages [75, 76].

Advantages of phage therapy

There are some advantages of using phage therapy to combat bacterial infection, which is given as follows:

a. Phages are capable of increasing their numbers when the host is present. They estimate their dose themselves and this phenomenon is called auto-dosing. As a result of this phenomenon, there is no need for the repeated inoculation of phages at the site of infection [77].

b. Phage therapy costs are low as phages are cheap to isolate and propagate in high numbers [70].

c. It is safe as phages are host specific. They do not harm the human microbiome, the human cells, or the commensal flora of the environment [59, 70, 77].

d. Phages stay in the body till there is the infecting bacteria. Once the infection resolves, phages are automatically eliminated [59, 70, 77].

e. Phages also have anti-biofilm activity [77].

f. Phages mutate alongside the bacteria and continue to kill bacteria [59, 77].

g. Phages can also be genetically modified to give them characteristics such as increased host range, increased amplification, and the ability to target intracellular pathogens [70].

Standardization of phage therapy

Phage therapy can be standardized by strictly using the lytic phages, confirming the antimicrobial activity of phage against the bacteria, identifying the bacterial receptors that allow the elimination of resistance, and using combination therapies to combat the resistance [59]. Another approach is to use phage lytic proteins instead of whole viruses or a combination of phages with antimicrobials to combat the resistance [71, 78].

Future implications of phage therapy

The bacterial ability to develop resistance against antibiotics has been a concern since the development of antibiotics [79]. The overuse and misuse of antibiotics have led to an alarming situation worldwide, as no new antibiotics have been discovered in decades [80]. When a patient has a severe infection, the medical staff must choose various antibiotics to treat it. However, today there are strains of bacteria that are resistant to these newly developed drugs; as a result, these treatments are becoming ineffective. One possible solution might be phage therapy; however, this technique is still in its infancy [81]. The use of bacteriophages for medical treatment has increased in recent years. Phages are widespread viruses and are more abundant than any other organism on the earth [82]. Given that antibiotics are becoming less effective

against infections because of the spread of antibiotic-resistant bacteria, it is imperative to investigate the use of alternative therapies.

Clinical phage therapy

Clinical phage therapy uses bacteriophages to treat and prevent infections in humans. It is also practiced as a means of modifying the microbiome. In a few countries, clinical phage therapy is permitted for routine use. However, the corresponding data from these efforts remain limited [83]. Only a handful of clinical trials have been approved in this area, and few are currently completed. The PhagoBurn trial, conducted in 2013 under both good manufacturing practice (GMP) and good clinical practice (GCP), is the largest clinical trial on phage therapy in Europe. The results of a trial involving patients with *Pseudomonas aeruginosa* wound infections were compared to those of routine care to evaluate the effectiveness and acceptability of phage therapy and traditional methods [84]. Each treatment was topically administered for seven days. One group received phages, and the other received a managed treatment. The phage remedy organization had a quicker recovery than the control institution (well-known treatment). No adverse consequences have been observed in the phage-handled institution. The confined efficacy of the phage cocktail became because of a significant drop in phages after GMP manufacturing, leading participants to hold a much lower awareness of phages than predicted at the beginning. Extra importantly, susceptibility checking out was no longer completed before treatment, and bacteria became immune to low doses of phages after treatment failed [84].

Vaccination

Phages have been used to carry vaccines containing antigens and other substances. Antigens expressed on their surfaces can be delivered directly through phage particles [85]. Phages that induce both humoral and cell-mediated immunity can act as effective adjuvants or immune-stimulating agents. However, DNA vaccines are created by incorporating the sequence vital for vaccine antigen synthesis into the phage genome, which then acts as a vehicle for delivering a DNA vaccine. Phage display vaccines do not need adjuvants because bacteriophages are intrinsically immunogenic and can act as natural adjuvants, which makes them more effective than other types of vaccines [77].

Biological control

The chemicals used to control the microorganisms are toxic to the environment and dangerous to living organisms. Phages are considered one of the most plentiful biological beings on the planet. Phages can be used to control the spread of disease-causing agents without any harmful effects on other living organisms. Bacteriophages are a form of biological defense used in the agriculture and food industries to protect them from contamination with pathogenic and spoilage bacteria. Since bacteriophages are specific with narrow spectra, they can be used to eliminate specific bacteria or even certain types/strains of bacteria [86].

As human populations grow, so do the numbers of resistant bacteria, which has created a demand for natural biocontrol agents. More resistant bacterial species are constantly rising and the food industry may take a hit. The discovery of some natural biocontrol agents has challenged the food industry, which does not drastically affect human health and food quality [87]. Bacteriophages have been shown to be effective in protecting food from bacterial pathogens. As a result, phages are now being used at all food processing steps. *Salmonella* and *Campylobacter* are the two main pathogens that cause diseases in the poultry industry. The researchers show promising results for using phages against these organisms [88].

Biofilms

A biofilm is a community of microbes living on or in another material, such as a multicellular organism or on the surface of a solid object. The cells in biofilms are surrounded by an extracellular matrix (EPS) that is formed by the microorganisms themselves [89]. Bacteria that reside inside biofilms show a high degree of resistance to antibiotics and disinfectants. Bacteria are protected with a matrix made of lipids, nucleic acids, polysaccharides, and proteins [90]. The bacteriophage can disrupt this layer, compromising the bacteria's protection. Phages secrete a polysaccharide-degrading enzyme termed depolymerases, which break down

matrix and open up new entry points for the phage to interact with its receptor on the host cell [91]. Biofilms, the slime-like layer that *Clostridium difficile* forms around itself to protect itself from antibiotics, can impair the antimicrobial activity of antibiotics and contribute to their virulence. A cocktail of *Clostridium difficile* phage has been shown to reduce biofilms, preventing colonization when used alone or in a mixture with vancomycin [92].

Degenerative diseases

In recent years, regenerative medicine has gained popularity with the creation of new therapies based on the idea that we can reprogram cells to form new tissues and organs. The motivation is to use several *in vitro* and *in vivo* techniques that take advantage of the body's natural healing processes to treat patients with chronic illnesses like diabetes, osteoarthritis, cardiovascular and central nervous system (CNS) degenerative disorders, and crippling injuries [93].

It is known that bacteriophages may penetrate the blood-brain barrier to reach brain cells, where they can then use their antibacterial properties to provide immunity [94]. Scientists have controlled this characteristic of phages to develop a therapy for nervous system illnesses. A study tested anti-Shiga phage medication and found that it prevents bacterial meningitis in mice [77]. Plaque binding potential of bacteriophages has been used to develop treatments for Alzheimer's and Parkinson's illnesses. In general, the plaque-binding potential of bacteriophages has been shown to be directly related to the effectiveness of immunizations in treating Alzheimer's and Parkinson's diseases. Bacteriophage preparations can be used as an effective way to lower the potential of neurodegenerative diseases caused by elevated pro-inflammatory cytokines and amyloid-beta protein ingestion [95].

Future challenges and bacteriophages

The employment of bacteriophages to treat bacterial infections in humans is rare and has only been studied in academic settings. Some phages have been treated in mice, and the results suggest that bacteriophage therapy for bacterial infections may be effective. Other reports provide little supporting evidence for clinical efficacy and safety. The development of bacteriophage therapy is still in the early stages, and the bacteriophage preparation process is difficult. Following are some of the challenges of bacteriophage therapy.

Immune response

The mechanisms used to distinguish between bacteriophages and their products may recognize a non-self-antigenic molecule or "mask" that results from phage production or degradation. Furthermore, the response induced by bacteriophages is likely beneficial in reducing the potential impact of phage administration [96]. When the phage strains were injected, the immune system response was observed in animal and human experiments despite the prior exposure and course of administration. In animals, research shows that the phagocytic cells operated upon the bacteriophages within a few minutes after their insertion [97].

Bacteriophage selection

Phage selection was often underestimated and led to wrong phage choices for treatment. Phages, like other proteins, have distinct shapes that enable them to bind specific targets on their prey. These binding sites can differ from phage to phage and even within a single phage species, making selecting and identifying good candidates difficult [98]. Despite the limited success of phage therapy, there seems to be a lack of studies on this subject. Although phage therapy is still being tested and not yet fully understood, researchers have also pointed out that several parameters need to be controlled. A bacteriophage must be specifically active against a given bacterial strain to be considered a potential therapeutic agent. It can be demonstrated by incubating the bacteriophage with the target bacteria and neutralizing it. For example, researchers found that it is considerably simpler if *Pseudomonas aeruginosa* instead of *Streptococcus aureus* is the aim of the bacteriophage for infection control or diagnosis [99].

Phage resistance

The topic of phage-resistance emergence is a critical component in comprehending the ecological consequence of phages on the bacterial population when addressing the connections between bacteriophages and their hosts [100]. The development of bacterial resistance to bacteriophages is possibly plausible since bacteria possess or may evolve in many ways to avoid viral infections. It may be possible when bacteria change their recognition molecules, switch off the signaling pathways involved in phage infection, or they can probably modify their metabolism and acquire additional resources to make them resistant to phage attacks [96]. The protein modification of membrane for the alteration or receptor loss has been observed in *Vibrio cholerae* [101], *Streptococcus aureus*, *Bordetella bronchiseptica*, and *Escherichia coli* [96]. The bacteria provide adaptive immunity against the phages with the help of the CRISPRs and CRISPR-associated genes. However, introducing a cocktail of phages and combined therapy (antibiotics + phages) can be very influential in reducing the development of resistance to phages among bacteria. Given that all the findings indicate that the virus's capacity to promote bacterial resistance, the quantity required to prevent bacterial resistance development is an essential consideration in selecting a therapeutic bacteriophage [102].

Conclusions

In conclusion, it is imperative to take immediate action to tackle the escalating issue of antibiotic resistance effectively. The inclusion of phage therapy in a global strategic approach is imperative for combating it. To address the growing concern of antibiotic-resistant bacterial infections, it is imperative to promptly initiate deep evaluation and implementation of phage therapy as a viable treatment option. The inherent capacity of phages to undergo modifications and adjustments confers a significant advantage. Although certain aspects of optimal phage therapy usage remain unknown, the field is progressing rapidly. Ultimately, despite the potential challenges in achieving widespread availability of phage therapy, its implementation could bring social, commercial, and economic benefits that extend beyond improving patient health outcomes.

Abbreviations

CRISPR: clustered regularly interspaced short palindromic repeats

MDR: multidrug-resistant

Declarations

Author contributions

IN, MHN, and ASO: Conceptualization, Writing—original draft, Writing—review & editing. AUR, NJ, WW, AW, IJ, WS, MH, DDB, and HC: Writing—original draft, Writing—review & editing.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

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Not applicable.

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