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Article

Bacteriophages: Biology, Classification, Life Cycles, and Their Potential Role in Combating Antimicrobial Resistance

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Abstract: Bacteriophages (phages) are viruses that infect bacteria and rank among the most abundant biological entities on Earth. Structurally, they consist of a nucleic acid genome enclosed in a protein capsid, with some possessing tail structures for attachment to hosts. They are classified into two main types based on their life cycles: lytic phages, which rapidly replicate and destroy bacterial cells, and lysogenic phages, which integrate into the host genome and remain dormant.

Phages have attracted growing attention as a promising alternative to antibiotics amid rising antimicrobial resistance (AMR), offering targeted elimination of bacteria with minimal impact on beneficial microbiota. They are also widely utilized in molecular biology, food safety, and biotechnology. However, challenges such as narrow host range, immune responses, regulatory barriers, and the risk of horizontal gene transfer continue to hinder their broader clinical application, though advances in genetic engineering and phage cocktail development are helping to address these hurdles.

This study provides a comprehensive overview of phage biology, classification, mechanisms of action, and life cycles, with particular focus on their role in combating AMR. Overall, bacteriophages represent a versatile and powerful biological tool, and continued research and innovation are essential to fully unlocking their potential in addressing global infectious disease challenges.

In conclusion, bacteriophages represent a powerful and versatile biological tool with significant potential in medicine, research, and industry. Continued exploration and innovation are essential to fully harness their capabilities in combating bacterial infections and addressing global health challenges.

Keywords:phage, bacteriophage, antimicrobial resistance, phage therapy, double-layer agar, plaque assay.

1. Introduction

Viruses are regarded as intracellular parasites that require a specific host cell for replication. A type of virus that targets and replicates only within bacterial cells is called a bacteriophage [1]. Bacteriophages (phages) are viruses that specifically infect bacteria. They can be found in almost

all ecosystems. It is estimated that approximately 1031 phages exist globally (108 phage species predicted), making them the most prominent biological system on earth [2],[3],[4],[5],[6]. Bacteriophage is derived from "bacteria" and the Greek word phagein, signifying "to devor." The term "Prokaryote viruses" is preferable because it also includes viruses, mostly from hyperthermophiles, that do not resemble conventional bacteriophages. They are the most abundant organisms in the biosphere [7]. Bacteriophages were the first type of viruses to be discovered. Many foundational discoveries in molecular biology—the proof that DNA was the molecule transmitting genetic information, the basic mechanisms of gene regulation, and the genetic code —were made using bacteriophages [8].

These viruses can be double-stranded DNA (most common), single-stranded DNA, single-stranded RNA, and double-stranded RNA (least common). Most virions (96%) are tailed; other types are cubic, filamentous, or pleomorphic. These virions attach to specific bacteria and achieve killing by enzymes, endolysins, and holins, without affecting the commensal microflora because of host specificity [9]

2. Historical background

Phages were first described in 1915 by Frederick Twort and in 1917 by Felix d'Herelle. Both men discovered phage when the bacteria they were working with lysed. The agent responsible for the lysis was transferable from culture to culture, invisible under light microscopy, and passed through the smallest filter available to them. d'Herelle coined the term "bacteriophage", signifying an entity that eats bacteria [10].

In 1896, Ernest Hanbury Hankin, a British bacteriologist working as the Chemical Examiner and Bacteriologist to the Government of the United Provinces and of the Central Provinces of India, demonstrated that the waters from the Indian rivers Ganga and Yamuna contained a biological principle that destroyed cultures of cholera-inducing bacteria. This substance could pass through Millipore filters, which are known to retain larger microorganisms such as bacteria. He published his work in French in the *Annals of the Pasteur Institute* [11]. In 1915, while he was studying the growth of vaccinia virus on cell-free agar media, Frederick Twort, a British microbiologist, noted that "pure" cultures of bacteria may be associated with a filter-passing, transparent material that may entirely break down bacteria in a culture into granules [12]. This "filterable agent" was demonstrated in cultures of micrococci isolated from vaccinia: material from some colonies that could not be subcultured was able to infect a fresh growth of micrococcus, and this condition could be transmitted to fresh cultures of the microorganism for an almost indefinite number of generations. This transparent material, which was found to fail to grow in the absence of bacteria, was described by Twort as a ferment secreted by the microorganism for a purpose that was unclear at the time.

Two years after this report, Félix d'Herelle independently described a similar experimental finding while studying patients with bacillary dysentery or recovering from it. He isolated a so-called "anti-Shiga microbe" from the stools of recovering Shigella patients by filtering stools incubated for 18 h. This active filtrate, when added to either a culture or an emulsion of the Shiga bacilli, caused arrest of the culture, death, and finally lysis of the bacilli [13]. D'Herelle described his discovery as a "veritable" microbe of immunity and an obligate bacteriophage. He also demonstrated the activity of this anti-Shiga microbe by inoculating laboratory animals with it as a treatment for shigellosis, thereby seeming to confirm the clinical significance of his finding by satisfying at least some of Koch's postulates.

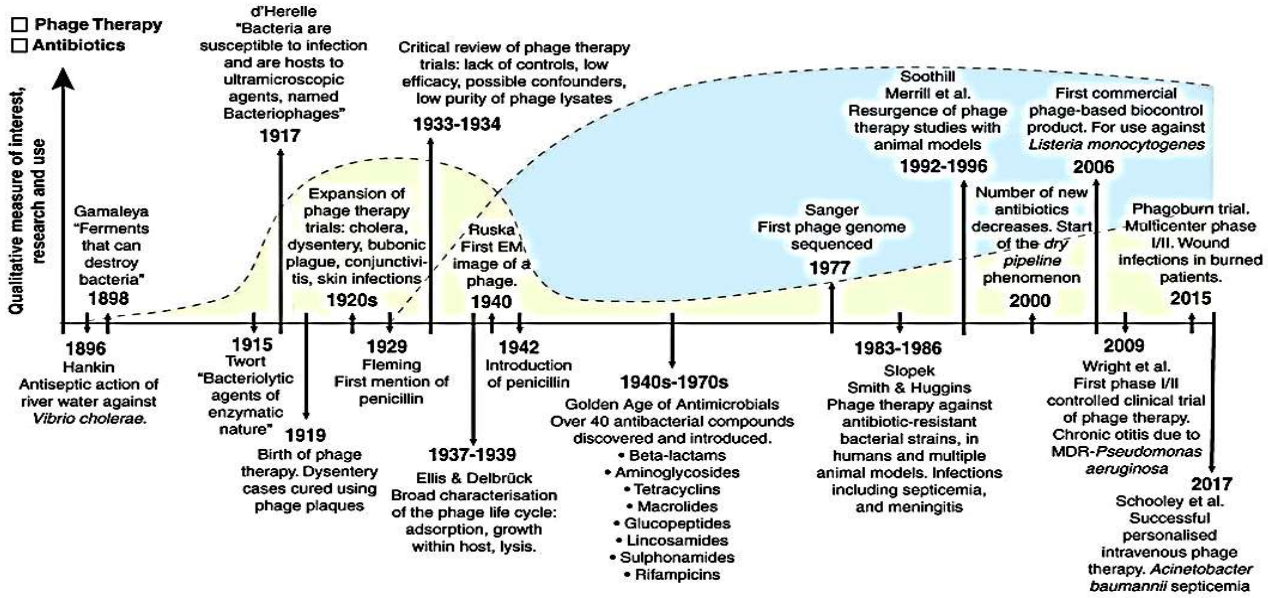

Figure 1: Timeline of some of the important events that occur during the studies of phage therapy and antibiotics. The curves in the figure represent a qualitative measure of the overall interest, research, and use of phage therapy and antibiotics, showing how the introduction of antibiotics and the critical review of the early phage therapy studies coincided to bring phage therapy research and development to an almost complete standstill around the 1940s [14].

3. Methodology

The present review, Overview of Bacteriophages, was conducted through a comprehensive literature search by independent reviewers using electronic scientific databases. Relevant studies published in PubMed, Google Scholar, and Web of Science were systematically examined. In addition, selected unpublished online resources related to the topic were also reviewed.

The search strategy included the following keywords*: bacteriophages, phage therapy, alternatives to antibiotics, bacteriophage isolation, double-layer agar technique, plaque assay, phage typing.*

4. Result and Discussion

Bacteriophage classification

To approve and coordinate the taxonomic coding and nomenclature of viruses, the International Committee on Taxonomy of Viruses (ICTV) was established in 1973. Notably, classification is crucial for the following reasons: finding novel bacteriophages, determining how phages are related to one another, and maintaining bacteriophage databases and collections. Additionally, it can be used to identify either hazardous bacteriophages in the biotechnology and fermentation industries for control and eradication purposes or beneficial bacteriophages with medicinal and industrial applications [14][15]. Phages are classified into families based on their morphology, nucleic acid composition, capsid structure, and host range. The International Committee on Taxonomy of Viruses (ICTV), more specifically, the Bacterial and Archaeal Viruses Subcommittee (BAVS), is responsible for phage taxa. Phages are divided into different morphotypes. *Caudiviricetes* comprises dsDNA-tailed bacterial and archaeal phages. Non-tailed phages are distributed into different morphotypes, which include the *Corticoviridae* (circular dsDNA), *Tectiviridae* (linear dsDNA), *Plasmaviridae* (circular supercoiled dsDNA), *Cystoviridae* (tri-segmented dsRNA), *Leviviridae* (ssRNA), *Tubulavirales* (ssDNA phages), and *Microviridae* (ssDNA phages). The vast majority of phages identified to date belong to the *Caudoviricetes class*, which is currently divided into 7 orders, 44 families, and 44 subfamilies [16]. It contains phages with varying tail lengths, icosahedral capsids, and double-stranded DNA genomes [17],[18]. These numbers, however, are constantly changing due to the rapid increase in the amount of sequenced data available. The three major families that make up the Caudovirales account for 60% of the described phages: Siphoviridae, Myoviridae, and

Podoviridae [15],[19],[20]. However, only three to four percent of the phages studied (polyhedral, filamentous, and pleomorphic) belong to the ten families examined, and some of these families are extremely small [21].

Biological Features and Structure of Bacteriophages

Generally, phages possess DNA or RNA genomes stored within highly symmetric protein capsids for protection. These capsids can be icosahedral, helical, or other shapes. They may be covered with an outer lipid membrane (e.g., Cystoviridae) or have a lipid membrane covering the capsid (e.g., Tectiviridae, Corticoviridae). In addition to genome protection, phages require apparatuses for host cell recognition. Simple phages like Leviviridae have a single minor capsid protein for this purpose, while more complex phages like Inoviridae and Tectiviridae dedicate multiple proteins to host cell recognition. Caudovirales possess highly efficient tail protein complexes for DNA transfer. Moreover, complex phage particles may contain proteins for environmental sensing, binding to suitable matrices, and other functions [22].

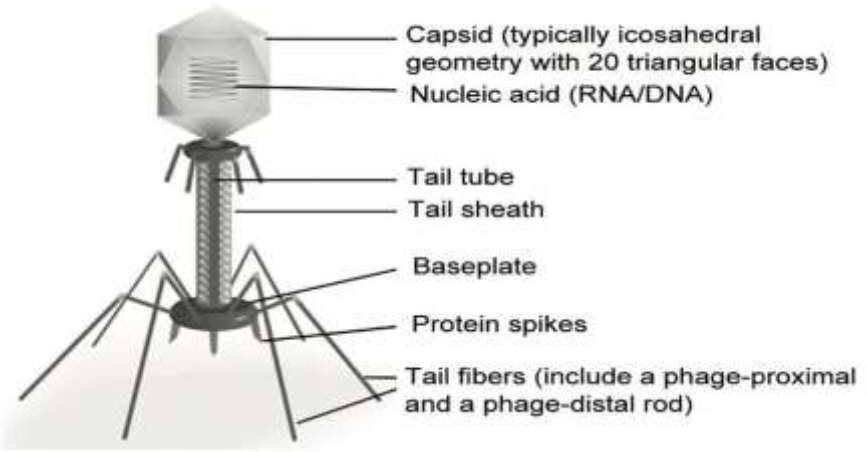

Figure 2:major parts of the bacteriophage structure [9]

Bacteriophage life cycles.

Phages can be classified into two types based on their different modes of action on host bacteria [15],[23]. The first type is the virulent (lytic) phage, which replicates and proliferates inside the host bacterial cell. Eventually, it lyses the bacteria, releasing progeny phages [24]. The virulent phage attaches to specific receptors on the bacterial surface and injects its genetic material into the host for replication. The resulting progeny phages cause bacterial lysis via holins and endolysins, thereby terminating the infection. These progeny phages are then released into the surrounding environment to initiate killing again. Under normal circumstances, the second type of phage does not produce progeny phages or cause bacterial lysis. Instead, it integrates its genome into the host bacterial chromosome and transfers it to the progeny bacterium's genome as the bacterium replicates and divides. This type of phage is referred to as a temperate phage or a lysogenic phage [25],[26]). In the lysogenic cycle, the phage genome (known as a prophage) replicates with the host DNA, either integrated into the host chromosome or in a free, plasmid-like state, forming a long-term, stable coexistence with the host [27]. Subsequently, when the bacterium replicates and divides, the phage genome is transferred to the progeny bacteria's genomes, a process accompanied by vertical genetic transfer [27].

Upon binding to their specific receptor, phages form a pore in the bacterial cell wall and inject their DNA into the cell, while the viral capsid remains outside the bacterium. This is followed by the expression of phage early genes, which, in the case of lytic phages, redirect the bacterial biosynthetic machinery toward the synthesis of viral nucleic acids and proteins. Assembly and packing of phages is then observed before bacterial cell lysis and release of phage progeny occur. Phages' late enzymes, such as lysins, holins, and murein synthesis inhibitors, are then employed during the virion burst in the extracellular environment. The number of viral particles released, or burst size, varies greatly depending on the phage, the state of the bacterial host, and other environmental factors, such as the nutritive components surrounding the host [28].

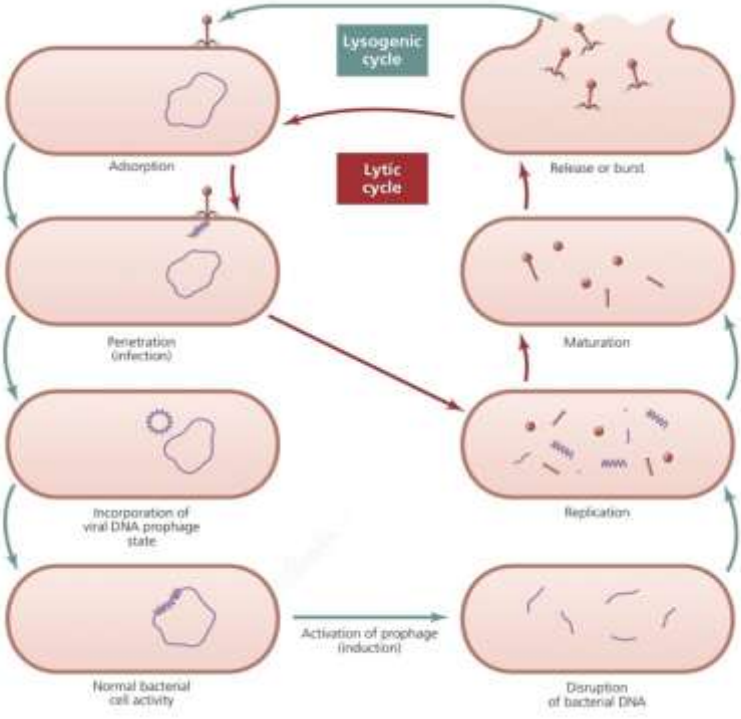

Figure 3: life cycles of bacteriophage [29].

Mechanisms of Phage Action Against Bacteria

Phage therapy typically employs virulent phages to lyse bacteria and treat pathogenic bacterial infections. The lysis of bacteria by virulent phages can be divided into two processes based on their genome types: single-stranded genome phages encode lysogenic effector molecules capable of inhibiting bacterial peptidoglycan biosynthesis, while double-stranded DNA phages synthesize two proteins—holin and endolysins—that disrupt the intracellular membrane or cell wall of the host bacteria [30,31]. When the phage lytic cycle is completed, and progeny phages mature within the bacterial cell, holin forms pores in the bacterial inner membrane. Subsequently, endolysins pass through the inner membrane and act on the bacterial cell wall's peptidoglycan, causing peptidoglycan linkages to break and leading to osmotic lysis of the bacteria. The growth and lysis of bacteria by virulent phages can be divided into four stages, as depicted in Figure 1: adsorption, penetration, biosynthesis, maturation, and release [32]**.** During adsorption, phages specifically attach to receptors on the bacterial surface through phage-encoded polysaccharide depolymerases that recognize components such as capsules, lipopolysaccharides (LPS), and biofilm extracellular matrices [33]. This interaction is highly specific and represents the essential first step of infection. Although phages may adsorb to both living and dead bacterial cells, successful infection requires a viable host because phage nucleic acid cannot be injected into dead bacteria.

Double-layer agar technique

The ability of a phage to lyse its host bacterium, producing a plaque within a bacterial lawn, led to the discovery of phages in 1915 by Frederick W. Twort and is the basis of the classic plaque assay, the double-layer agar (DLA) technique, which has been used ever since [12][34],[35] to identify and enumerate phages and isolate mutants. In recent years, interest in phages has increased not only for their potential as alternatives to antibiotics (phage therapy) but also for their applications in many other fields (phage display, immunology, microbial genetics, diagnostics, vaccine development, biosensors, etc.). Isolation, detection, and/or enumeration of phages are essential across these applications, and growing interest in phages has broadened the use of the DLA technique. As a consequence, the efficiency of this method has several implications across different areas of biology [36],[37],[38]. While many phages form plaques that are sufficiently large and well-defined to be easily detected and enumerated by the classical DLA technique, others give rise to small, turbid plaques that are difficult to detect and count

accurately. In these cases, the classical plaque assay can be rather unsatisfactory and sometimes highly unreliable[5],[39],[40],[41].

The plaque assay exploits the ability of bacteriophages to kill their host bacteria. It enables phages to propagate on a confluent lawn of bacterial host cells immobilized in an agar layer. After incubation, a circular transparent area of lysed cells will develop, and each plaque represents a single phage particle in the original sample. The bacteriophages are usually mixed with host bacteria in molten agar and poured onto a bottom agar layer. Bottom layers usually contained 1–1.5% agar, while top layers contained 0.45–0.75% agar. Alternatively, bacteriophages are spotted onto the agar [41].

Plaque morphology

Ever since the discovery of bacteriophages (phages), the prominent clearings they produce on bacterial lawns (lysis plaques) have fascinated countless microbiologists. In fact, the name bacteriophage, literally meaning "bacteria eater," was derived, at least in part, from the phage's ability to form clearings [13]. Besides a few exceptions, such as phage T7, for which the plaque continues to increase in size [43],[44], most phage plaques, after a period of incubation, assume a certain size and acquire a definite boundary, either with a fuzzy or a clear-cut edge. The ability to form plaques is not restricted to phages, since animal and plant viruses also form plaques and lesions on cell cultures [45], host tissues [46], or leaf surfaces[47]. It is usually assumed that a single virus particle initiates each plaque on plates, although not all virus particles in the sample can initiate infections [48].

A virus initiates plaque formation and results in a cycle of infection in which progeny viral particles infect the bacterial host [49]. It is assumed that plaque size is limited by the number of infection cycles within the plaque. The infection cycle ceases as the embedded bacteria form a lawn and become unproductive on the agar media [50].

A virulent (follows lytic cycle) bacteriophage produces clear plaques, while a temperate (follows lysogenic cycle) phage tends to produce turbid plaques. The difference in plaque morphology is due to the phage life cycle. Virulent phages tend to have a single cyclic pattern of infection and immediate lysis of the host. At the same time, temperate phages tend to integrate their genome into the host genome, thereby halting the cycle of lysis and allowing bacterial growth, thereby forming cloudiness (looks like a bullseye) within a zone of plaque[50]. The size and morphology of the plaque can vary depending on the method and conditions used during the Experiment [51]. Liquid medium tends to have increased bacterial clearance due to enhanced phage mobility, thereby facilitating bacterial-phage interaction. However, solid media restrict phage motility after a specified interval, thus confining host lysis. A few phages exhibit diffusion in semi-solid media, thereby increasing plaque size upon prolonged incubation [52],[53].

Numerous researchers have studied the simplicity of the phage plaque, and many mathematical models have been developed based on the results. The first mathematical model was devised by Koch, who modeled plaque diffusion using reversible and irreversible adsorption. At present, many of Abedon's studies have examined the theoretical concept of phage plaque . The simple and universal governing factors of the plaque formation, as stated by Abedon and coworkers, are: 1) rate of phage multiplication, 2) lysis time, and 3) the diffusion of phages [54]. However, none of the mathematical models are accepted by the scientific community, as they tend to show low agreement with laboratory findings [55]. A theoretical analysis by Abedon and others showed that the major factors governing plaque formation in phage are the rate of phage adsorption, the lysis time (latency period), and the number of progeny particles produced during infection. In contrast with the theoretical models available in the database, the laboratory models by Gallet and others. It has been shown that phages produce large plaques with a shorter latency period (the time the phage spends inside the host) [55].

Phage therapy

At present, the main treatment for bacterial infection is antibiotics. Due to the extensive use of antibiotics, numerous problems have arisen, including the emergence of antibiotic-resistant bacteria, immunosuppression, antibiotic residues in animal products, and environmental pollution. According to statistics, 75% of bacteria in the United States are resistant to at least one

antibiotic, and nearly 2 million people's health is at risk each year due to drug-resistant bacteria [56]. In addition, more than half of clinical Staphylococcus isolates in Japan are multidrug-resistant [57]. A report from the British government in 2016 estimated that deaths caused by drug-resistant bacteria could reach about 700,000 each year [58]. It is estimated that by the year 2050, upwards of 10 million people will die each year due to antimicrobial resistance. In 2017, the World Health Organization (WHO) published a list of global priority pathogens comprising 12 bacterial species, which were categorized into critical, high, and medium priority based on their level of resistance and available therapeutics. It is crucial to discover, design, and develop new and alternative antimicrobial therapies. The current rate of resistance emergence far exceeds the pace of antibiotic discovery and development, representing a global public health challenge.

The ability of bacteriophages to infect and kill bacteria led to the exploration of their therapeutic potential against bacterial pathogens in a clinical approach known as bacteriophage therapy/phage therapy, almost immediately after their innovation, with the first therapeutic use in humans described in 1919, just two years after their innovation by d'Herelle [59],[60]. Phages can support the progression of an inflammatory response against bacteria by degrading bacterial cell walls, thereby triggering the immune system [61]. Thus, bacteriophage therapies, i.e., the administration of bacteriophage cocktails to patients infected with bacteria, besides the direct removal of bacterial cells, further stimulate the immune system to fight against infection [62].
Introduced in the early 1900s [60], phage therapy is the application of bacteriophages (phages) to combat uncontrolled or unwanted bacteria, such as those associated with infectious diseases [63]. In reviews of phage therapy [64] , authors commonly list the advantages of employing phages as antibacterials. These lists can be used as talking points of why, in this age of epidemic antibiotic resistance, phage therapy should not be overlooked. As lists vary from author to author, it is useful to condense them into a coherent whole. Here, we highlight the strengths and weaknesses of individual assertions. We also consider possible limitations to phage use as antibacterials.
Phage-based treatments comprise phage therapy with single phages or phage cocktails, phage-derived enzymes, phages in combination with antibiotics, and genetically modified phages [65]. These focus on lytic phages because they lack integrases and other enzymes involved in horizontal gene transfer. Lysins and depolymerases are phage-derived enzymes useful for removing biofilms. Lysins are peptidoglycan hydrolases that exert a bactericidal effect against susceptible bacteria. They cleave peptidoglycan bonds, degrading the bacterial cell wall and biofilm structure; hence, they are useful against Gram-positive bacteria. Depolymerases are enzymes that degrade extracellular substances produced by encapsulated bacteria, thereby exposing phage receptors. They can degrade the chains of capsular polysaccharides, exopolysaccharides, and O-polysaccharides from lipopolysaccharides and peptidoglycan, which are components of the biofilm matrix. Bacteriophage recombinant lytic proteins (lysins and depolymerases) can be used as enzybiotics [66]. The most common cause of opportunistic infections is the ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.) group of organisms, most of which are MDR isolates [67]. A sub-lethal dose of antibiotics can stimulate phage virulence under certain conditions known as phage-antibiotic synergy (PAS) [68],[69].
The combination of phage with antibiotics can have a variety of outcomes, including additive, synergistic, ineffective, or antagonistic effects [70],[71]. Principi and others [72] suggested that bacteriophages reduce the minimum inhibitory concentration of AR bacteria to that of sensitive bacteria. Temperate phages with phenotypic traits useful for biofilm removal can be engineered to become virulent phages. Lytic phages are used to destroy bacteria, and temperate phages are useful for delivering programmable DNA nucleases associated with CRISPR to reverse antibiotic resistance and destroy plasmids that confer it.

Potential Advantages of Phage Therapy

Bacteriophages are natural antibacterials that can regulate bacterial populations by inducing bacterial lysis. They are active against gram-positive [73,74], as well as gram-negative bacteria [75,76,77], including MDR pathogens. Indeed, because the mechanism of action of phage lysis is

distinct from that of antibiotics, it retains activity against bacteria exhibiting multiple mechanisms of antibiotic resistance [78]. Because of its specificity, phage therapy has a narrow antibacterial spectrum, limited to a single species or, in some cases, a single strain within a species. This limits the "pressure" and the heavy collateral damage to bystander, non-targeted bacteria caused by antibiotics. The patient's entire microbiome is altered by antibiotics, not just the intended target pathogen. In contrast, Chibani-Chennoufi and others reported little impact on the gut microbiota in mice after oral administration of phage therapy targeting E. coli [79] . Preservation of much of the existing microbiome during phage therapy has been confirmed in careful microbial surveys of adult healthy volunteers who ingested a 9-phage cocktail [80],[81]. Phage therapy also avoids the potential overgrowth of secondary pathogens.

Over the last 2 or 3 decades, the worldwide emergence and spread of antibiotic-resistant bacteria have become a major therapeutic challenge [82],[83]. For instance, MRSA infections in the US were reported with an incidence of about 100,000 serious infections in 2005, contributing to 20,000 deaths [84]. The limited therapeutic options remaining to treat major multi-drug resistant (MDR) bacteria, known by the acronym as the ESKAPE pathogens (for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp.), have now become a looming healthcare crisis in many ICUs worldwide [85]. Treating patients with MDR pathogens has been shown by Morales et al. to increase the total cost of care and prolong hospital length of stay[86].
An ethical imperative exists throughout the health care professions to do all we can to preserve the efficacy of antibiotics and to recognize that this precious resource is being squandered by the often unnecessary and inappropriate use of antibiotics, thereby promoting the acquisition and dissemination of antibiotic resistance genes [87]. Antibiotic drug resistance is now recognized as a health care emergency, and appeals for the development of novel means to combat it have been voiced by many; however, antibiotics are developed based on free-market criteria rather than on direct public benefit [88]. However, despite the call for the development of new antibiotics in the European Union (EU) and in the United States (US) [89],[90], there is a dearth of new antibiotics in the developmental pipeline [88],[91],[92]. An entirely novel, non-antibiotic approach to treat bacterial pathogens is certainly needed. The redeployment of phage therapy could become a welcome alternative to antimicrobial chemotherapy amid the progressive spread of MDR bacterial pathogens and the paucity of new antibiotics to combat them.
Furthermore, the need for phage applications certainly extends beyond human infections. Indeed the use of bacteriophages has been described in various situations including (but not limited to): food safety [92], agriculture [93], veterinary applications [94], industry [93], and clinical diagnostic application such as detection and typing of bacteria [95] in human infection.

Table 1: Potential benefits of bacteriophage therapy against antibiotic therapy [96].

Characteristics features	phage therapy	Antibiotics treatment
Specificity	Highly specific	Wide range of actions
Toxicity	Almost totally nontoxic	Variable levels of toxicity ranging from mild to severe
Biofilm penetration	Effective penetrating ability	Unable to penetrate except in high doses
Possibility of resistance	Almost non-existent	High possibility of resistance
Simplicity of administration	not require repeated doses	Require repeated doses
Cost of treatment	Cheaper	More expensive

Molecular identification of bacteriophage

Alongside the isolation procedure, the characterization of bacteriophages is also essential, including functional tests, genomic sequencing, and morphological examination. First, the structural properties of isolated phages are determined using electron microscopy, which enables researchers to group them by common morphotypes. This morphological classification can provide important information about a phage's host range and its uses in the scientific and

medical fields. Researchers using Transmission electron microscopy (TEM) have discovered that the majority of bacteriophages found in the environment are viruses with double-stranded DNA (dsDNA). The best-characterized phage families in marine, soil, and sediment environments are Podoviridae, Siphoviridae, and Myoviridae [97]. The next important step in understanding the genetic variance among isolated bacteriophages is genomic characterization. Researchers may identify the genetic components of phages using techniques such as whole-genome sequencing (WGS), polymerase chain reaction (PCR), and metagenomic analysis, which help them better understand their life cycles, host specificity, and operational mechanisms. In addition, the characterization of genetic elements has made the world aware of the bacteriophage's lytic cycle and its unknown proteins and functions, which may be advantageous in many ecological and medical environments [98]. For example, concerns about antibiotic resistance genes have led to the identification of the absence of lysogenic, virulence, toxic, and antibiotic resistance genes in some phages, such as EH-B-A (A1), EP-M-S, EP-B-K (E2), etc., highlighting their significance for phage therapy [99]. Finally, functional characterization is also essential in evaluating the phage's potential for therapeutic uses.

Phage typing

It is a microbiological technique used to identify and classify specific bacterial strains by using bacteriophages specific to them. When a phage infects a susceptible bacterium, it causes the bacterium to lyse. This can be observed on the plate as a zone of bacterial growth inhibition. This is visible as a distinct zone of translucence. This phenomenon underlies phage typing [100,101]. The whole procedure involves several steps. First, a pure culture of the strain to be tested is prepared. Then, it is spread evenly on the plate. Later, various bacteriophages are applied to it, each targeting a different bacterial strain. The plate is then incubated, after which a clear zone of translucence can be observed where the phage is applied. The results are then compared with phage typing patterns, and, based on this, the corresponding strain can be identified [102,103].

Bacterial detection

Bacteriophage-based approaches for bacterial detection were developed using the bacteriophages' typical ability to bind host bacteria. Only the recognition of a suitable and viable host offers the opportunity for virion proliferation and completion of the viral life cycle. This offers an advantage over other widely used techniques, such as PCR or mass spectroscopy-based methods, which might produce false-positive results when dead bacteria are present. Bacteriophages are far less expensive and simpler to prepare than antibodies, while conventional biochemical or culturing approaches take much longer to yield results [104,105]. Because bacteriophages infect only live hosts, bacterial cells can be quickly and precisely identified using bacteriophage detection methods. Bacteriophages can be adjusted, lysed, isolated, and extracted from their hosts to permit detection in a variety of methods. The three main categories of phage-mediated detection techniques are reporter bacteriophages, bacteriophage amplification, and bacteriophage capture [106]. Genetically modified reporter phages allow the entry of reporter genes into the bacterial host, where the gene is expressed, a signal is detected, and the pathogen is recognized. To enable gene expression upon phage infection of live hosts, reporter genes encoding luciferases or fluorescent proteins have been introduced into the phage genome [107]. The formation of offspring phages or the demise of the bacterial host serves as a detection signal in bacteriophage multiplication assays [108]. Typically, plaque formation on a Petri plate is used to gauge bacteriophage growth. Plaques develop when infected hosts lyse, releasing offspring phage that allows bacterial reinfection.

Exclusion Criteria

Only peer-reviewed articles published in English and directly related to bacteriophage isolation methods and applications were included in this review. Review articles, experimental studies, and methodological research papers were considered eligible. In contrast, articles with insufficient data, non-English publications, conference abstracts, non-peer-reviewed publications, duplicate studies, and articles lacking sufficient methodological details were excluded.

Study selection

A total of 40 studies published between 2010 and 2025 were initially identified through the literature search as potentially relevant to the review topic. Following screening of titles and abstracts, full-text articles were retrieved and assessed for eligibility against the predefined inclusion and exclusion criteria. Of the 40 studies evaluated, 28 met the eligibility criteria. It was included in the final review and analysis, while 12 studies were excluded due to insufficient data, lack of relevance to the review objectives, or failure to meet the inclusion criteria.

Future Perspectives

The future of bacteriophage research is highly promising, particularly in addressing antimicrobial resistance through personalized phage therapy and engineered phage cocktails. Advances in genetic engineering and synthetic biology are expected to improve phage safety, broaden host range, and enhance therapeutic effectiveness. Phage-derived enzymes such as endolysins also represent powerful alternatives against multidrug-resistant and biofilm-forming bacteria. Beyond medicine, bacteriophages have expanding applications in oncology, agriculture, food safety, and microbiome-based therapies. Nevertheless, successful integration of phage technologies into clinical practice will depend on the development of standardized regulations, ethical frameworks, and international collaboration. Overall, bacteriophages are expected to play an increasingly important role in future medicine, biotechnology, and global health.

5. Conclusion

Bacteriophages are highly specialized bacterial viruses that possess significant applications in medicine, microbiology, biotechnology, and food safety. The reviewed literature highlights their remarkable structural diversity, host specificity, and considerable therapeutic importance. In addition to their therapeutic importance, bacteriophages play valuable roles in bacterial detection, phage typing, biofilm disruption, and molecular biology research.

Bacteriophages are a potential alternative for treating bacterial infections, including those caused by MDR pathogens. Indeed, phage therapy displays several advantages, and few adverse events are reported, but underreporting cannot be ruled out. However, further well-conducted studies are required to define the role and safety of phage therapy in daily clinical practice to treat patients with various infections. Moreover, the direct use of phage-encoded proteins such as endolysins, exopolysaccharidases, and holins has demonstrated their potential as a promising alternative to antibacterial products.

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References

[1] G. Mayer, "Bacteriophage," in *Bacteriology,* ch. 7. University of South Carolina School of Medicine, 2006.

[2] M. Breitbart and F. Rohwer, "Here a virus, there a virus, everywhere the same virus?" *Trends Microbiol.*, vol. 13, no. 6, pp. 278–284, 2005.

[3] S. Chibani-Chennoufi, A. Bruttin, M. L. Dillmann, and H. Brüssow, "Phage-host interaction: An ecological perspective," *J. Bacteriol.*, vol. 186, no. 12, pp. 3677–3686, 2004.

[4] R. W. Hendrix, "Bacteriophages: Evolution of the majority," *Theor. Popul. Biol.*, vol. 61, no. 4, pp. 471–480, 2002.

[5] P. Serwer, S. J. Hayes, S. Zaman, K. Lieman, M. Rolando, and S. C. Hardies, "Improved isolation of undersampled bacteriophages: Finding of distant terminase genes," *Virology*, vol. 329, no. 2, pp. 412–424, 2004.

[6] P. Serwer, S. J. Hayes, J. A. Thomas, and S. C. Hardies, "Propagating the missing bacteriophages: A large bacteriophage in a new class," *Virol. J.*, vol. 4, no. 1, Art. no. 21, 2007.
[7] M. R. Clokie, A. D. Millard, A. V. Letarov, and S. Heaphy, "Phages in nature," *Bacteriophage*, vol. 1, no. 1, pp. 31–45, 2011.
[8] L. M. Kasman and L. D. Porter, "Bacteriophages," in *Brenner's Encyclopedia of Genetics*, 2nd ed., pp. 280–283, 2022, doi: 10.1016/B978-0-12-374984-0.00131-5.
[9] S. Singh, G. Nath, and A. Maheshwari, "Bacteriophages," *Newborn*, vol. 2, no. 4, pp. 297–309, 2023, doi: 10.5005/jp-journals-11002-0078.
[10] N. Trun and J. Trempy, *Fundamental Bacterial Genetics*. Hoboken, NJ, USA: John Wiley & Sons, 2003.
[11] E. Hankin, "L'action bactéricide des eaux de la Jumna et du Gange sur le vibrion du choléra," *Ann. Inst. Pasteur*, vol. 10, p. 511, 1896.
[12] F. W. Twort, "An investigation on the nature of ultra-microscopic viruses," *Lancet*, vol. 2, pp. 1241–1243, 1915.
[13] F. d'Hérelle, "Sur un microbe invisible antagoniste des bacilles," 1917.
[14] F. L. Gordillo Altamirano and J. J. Barr, "Phage therapy in the postantibiotic era," *Clin. Microbiol. Rev.*, vol. 32, no. 2, 2019.
[15] E. Kutter and A. Sulakvelidze, Eds., *Bacteriophages: Biology and Applications*. Boca Raton, FL, USA: CRC Press, 2004.
[16] International Committee on Taxonomy of Viruses, "Current ICTV taxonomy release," ICTV. [Online]. Available: https://ictv.global/taxonomy. Accessed: May 4, 2023.
[17] D. Turner, A. M. Kropinski, and E. M. Adriaenssens, "A roadmap for genome-based phage taxonomy," *Viruses*, vol. 13, no. 3, Art. no. 506, 2021.
[18] D. Turner *et al*., "Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee," *Arch. Virol.*, vol. 168, no. 2, Art. no. 74, 2023.
[19] H. W. Ackermann and M. S. Dubow, "General properties of tailed phages," in *Viruses of Prokaryotes*, vol. 2, p. 28, 1987.
[20] A. Lopes, P. Tavares, M. A. Petit, R. Guérois, and S. Zinn-Justin, "Automated classification of tailed bacteriophages according to their neck organization," *BMC Genomics*, vol. 15, no. 1, Art. no. 1027, 2014.
[21] H. W. Ackermann, "5500 phages examined in the electron microscope," *Arch. Virol.*, vol. 152, no. 2, pp. 227–243, 2007.
[22] S. Kumar, *Textbook of General Virology*. USA, pp. 119–120, 2025.
[23] R. Sharp, "Bacteriophages: Biology and history," *J. Chem. Technol. Biotechnol.*, vol. 76, pp. 667–672, 2001, doi: 10.1002/jctb.434.
[24] M. B. Dion, F. Oechslin, and S. Moineau, "Phage diversity, genomics and phylogeny," *Nat. Rev. Microbiol.*, vol. 18, pp. 125–138, 2020, doi: 10.1038/s41579-019-0311-5.
[25] F. Rohwer and A. M. Segall, "A century of phage lessons," *Nature*, vol. 528, pp. 46–47, 2015, doi: 10.1038/528046a.
[26] H. G. Hampton, B. N. J. Watson, and P. C. Fineran, "The arms race between bacteria and their phage foes," *Nature*, vol. 577, pp. 327–336, 2020, doi: 10.1038/s41586-019-1894-8.
[27] M. Zhang, T. Zhang, M. Yu, Y.-L. Chen, and M. Jin, "The life cycle transitions of temperate phages: Regulating factors and potential ecological implications," *Viruses*, vol. 14, Art. no. 1904, 2022, doi: 10.3390/v14091904.
[28] M. G. Weinbauer, "Ecology of prokaryotic viruses," *FEMS Microbiol. Rev.*, vol. 28, no. 2, pp. 127–181, 2004.
[29] J. G. Cappuccino and C. Welsh, *Microbiology: A Laboratory Manual*, 12th ed. Pearson, 2020.
[30] R. White *et al*., "Holin triggering in real time," *Proc. Natl. Acad. Sci. USA*, vol. 108, no. 2, pp. 798–803, 2011.
[31] Z. Drulis-Kawa, G. Majkowska-Skrobek, and B. Maciejewska, "Bacteriophages and phage-derived proteins—Application approaches," *Curr. Med. Chem.*, vol. 22, no. 14, pp. 1757–1773, 2015.

[32] B. Maciejewska, T. Olszak, and Z. Drulis-Kawa, "Applications of bacteriophages versus phage enzymes to combat and cure bacterial infections: An ambitious and also a realistic application?" *Appl. Microbiol. Biotechnol.*, vol. 102, no. 6, pp. 2563–2581, 2018.
[33] D. R. Roach *et al*., "Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen," *Cell Host Microbe*, vol. 22, pp. 38–47.e34, 2017.
[34] M. H. Adams, *Bacteriophages*. 1959.
[35] A. M. Kropinski, A. Mazzocco, T. E. Waddell, E. Lingohr, and R. P. Johnson, "Enumeration of bacteriophages by double agar overlay plaque assay," in *Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions*. Totowa, NJ, USA: Humana Press, 2009, pp. 69–76.
[36] L. J. Alvarez, P. Thomen, T. Makushok, and D. Chatenay, "Propagation of fluorescent viruses in growing plaques," *Biotechnol. Bioeng.*, vol. 96, no. 3, pp. 615–621, 2007.
[37] D. Lillehaug, "An improved plaque assay for poor plaque-producing temperate lactococcal bacteriophages," *J. Appl. Microbiol.*, vol. 83, no. 1, pp. 85–90, 1997.
[38] M. R. McLaughlin, "Simple colorimetric microplate test of phage lysis in *Salmonella enterica*," *J. Microbiol. Methods*, vol. 69, no. 2, pp. 394–398, 2007.
[39] D. Fraser and J. Crum, "Enhancement of mycoplasma virus plaque visibility by tetrazolium," *Appl. Microbiol.*, vol. 29, no. 2, pp. 305–306, 1975.
[40] M. R. McLaughlin and M. F. Balaa, "Enhanced contrast of bacteriophage plaques in *Salmonella* with ferric ammonium citrate and sodium thiosulfate and tetrazolium red," *J. Microbiol. Methods*, vol. 65, no. 2, pp. 318–323, 2006.
[41] P. A. Pattee, "Use of tetrazolium for improved resolution of bacteriophage plaques," *J. Bacteriol.*, vol. 92, no. 3, pp. 787–788, 1966.
[42] J. Cormier and M. Janes, "A double layer plaque assay using spread plate technique for enumeration of bacteriophage MS2," *J. Virol. Methods*, vol. 196, pp. 86–92, 2014.
[43] J. Yin, "A quantifiable phenotype of viral propagation," *Biochem. Biophys. Res. Commun.*, vol. 174, no. 2, pp. 1009–1014, 1991.
[44] J. Yin, "Evolution of bacteriophage T7 in a growing plaque," *J. Bacteriol.*, vol. 175, no. 5, pp. 1272–1277, 1993.
[45] R. Dulbecco, "Production of plaques in monolayer tissue cultures by single particles of an animal virus," *Proc. Natl. Acad. Sci. USA*, vol. 38, no. 8, pp. 747–752, 1952.
[46] H. Fraenkel-Conrat, *Molecular Basis of Virology*. 1968.
[47] J. Piacitelli and V. Santilli, "Relationship of tobacco mosaic virus lesion number and concentration to the rate of lesion production on pinto bean," *Nature*, vol. 191, no. 4788, pp. 624–625, 1961.
[48] A. Kleczkowski and J. Kleczkowski, "The ability of single phage particles to form plaques and to multiply in liquid cultures," *Microbiology*, vol. 5, no. 2, pp. 346–356, 1951.
[49] S. T. Abedon and R. R. Culler, "Optimizing bacteriophage plaque fecundity," *J. Theor. Biol.*, vol. 249, no. 3, pp. 582–592, 2007.
[50] S. M. Krone and S. T. Abedon, "Modeling phage plaque growth," in *Bacteriophage Ecology*, vol. 15, pp. 415–438, 2008.
[51] J. P. Payet and C. A. Suttle, "To kill or not to kill: The balance between lytic and lysogenic viral infection is driven by trophic status," *Limnol. Oceanogr.*, vol. 58, no. 2, pp. 465–474, 2013.
[52] N. Ramesh, L. Archana, M. Madurantakam Royam, P. Manohar, and K. Eniyan, "Effect of various bacteriological media on the plaque morphology of *Staphylococcus* and *Vibrio* phages," *Access Microbiol.*, vol. 1, no. 4, Art. no. e000036, 2019.
[53] N. Mitarai, S. Brown, and K. Sneppen, "Population dynamics of phage and bacteria in spatially structured habitats using phage λ and *Escherichia coli*," *J. Bacteriol.*, vol. 198, no. 12, pp. 1783–1793, 2016.
[54] K. Krysiak-Baltyn, G. J. Martin, A. D. Stickland, P. J. Scales, and S. L. Gras, "Computational models of populations of bacteria and lytic phage," *Crit. Rev. Microbiol.*, vol. 42, no. 6, pp. 942–968, 2016.

[55] S. T. Abedon, "Detection of bacteriophages: Phage plaques," in *Bacteriophages: Biology, Technology, Therapy*. Cham, Switzerland: Springer International Publishing, 2021, pp. 507–538.
[56] R. Gallet, S. Kannoly, and I.-N. Wang, "Effects of bacteriophage traits on plaque formation," *BMC Microbiol.*, vol. 11, no. 1, Art. no. 181, 2011.
[57] J. Perry, N. Waglechner, and G. Wright, "The prehistory of antibiotic resistance," *Cold Spring Harb. Perspect. Med.*, vol. 6, no. 6, Art. no. a025197, 2016.
[58] C. R. Merril, D. Scholl, and S. L. Adhya, "The prospect for bacteriophage therapy in Western medicine," *Nat. Rev. Drug Discov.*, vol. 2, no. 6, pp. 489–497, 2003.
[59] A. Górski *et al*., "Phage therapy: Combating infections with potential for evolving from merely a treatment for complications to targeting diseases," *Front. Microbiol.*, vol. 7, Art. no. 1515, 2016.
[60] E. Kutter, "Bacteriophage therapy in humans," in *Bacteriophages: Biology and Applications*, 2005, pp. 381–436.
[61] W. C. Summers, "Bacteriophage therapy," *Annu. Rev. Microbiol.*, vol. 55, no. 1, pp. 437–451, 2001.
[62] K. L. Hess and C. M. Jewell, "Phage display as a tool for vaccine and immunotherapy development," *Bioeng. Transl. Med.*, vol. 5, no. 1, Art. no. e10142, 2020.
[63] W. C. Summers, "The strange history of phage therapy," *Bacteriophage*, vol. 2, no. 2, pp. 130–133, 2012.
[64] S. T. Abedon, "Kinetics of phage-mediated biocontrol of bacteria," *Foodborne Pathog. Dis.*, vol. 6, no. 7, pp. 807–815, 2009.
[65] S. T. Abedon and C. Thomas-Abedon, "Phage therapy pharmacology," *Curr. Pharm. Biotechnol.*, vol. 11, no. 1, pp. 28–47, 2010.
[66] D. P. Pires, L. D. Melo, D. V. Boas, S. Sillankorva, and J. Azeredo, "Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections," *Curr. Opin. Microbiol.*, vol. 39, pp. 48–56, 2017.
[67] R. Schuch, D. Nelson, and V. A. Fischetti, "A bacteriolytic agent that detects and kills *Bacillus anthracis*," *Nature*, vol. 418, no. 6900, pp. 884–889, 2002.
[68] M. S. Mulani, E. E. Kamble, S. N. Kumkar, M. S. Tawre, and K. R. Pardesi, "Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review," *Front. Microbiol.*, vol. 10, Art. no. 539, 2019.
[69] C. Torres-Barceló and M. E. Hochberg, "Evolutionary rationale for phages as complements of antibiotics," *Trends Microbiol.*, vol. 24, no. 4, pp. 249–256, 2016.
[70] A. M. Motlagh, A. S. Bhattacharjee, and R. Goel, "Biofilm control with natural and genetically modified phages," *World J. Microbiol. Biotechnol.*, vol. 32, no. 4, Art. no. 67, 2016.
[71] S. T. Abedon, "Phage-antibiotic combination treatments: Antagonistic impacts of antibiotics on the pharmacodynamics of phage therapy?" *Antibiotics*, vol. 8, no. 4, Art. no. 182, 2019.
[72] C. Gu Liu *et al*., "Phage-antibiotic synergy is driven by a unique combination of antibacterial mechanism of action and stoichiometry," *mBio*, vol. 11, no. 4, 2020.
[73] N. Principi, E. Silvestri, and S. Esposito, "Advantages and limitations of bacteriophages for the treatment of bacterial infections," *Front. Pharmacol.*, vol. 10, Art. no. 457104, 2019.
[74] S. Matsuzaki *et al*., "Experimental protection of mice against lethal *Staphylococcus aureus* infection by novel bacteriophage ϕMR11," *J. Infect. Dis.*, vol. 187, no. 4, pp. 613–624, 2003.
[75] B. Biswas *et al*., "Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*," *Infect. Immun.*, vol. 70, no. 1, pp. 204–210, 2002.
[76] C. S. Vinodkumar, Y. F. Neelagund, and S. Kalsurmath, "Bacteriophage in the treatment of experimental septicemic mice from a clinical isolate of multidrug-resistant *Klebsiella pneumoniae*," *J. Commun. Dis.*, vol. 37, no. 1, pp. 18–29, 2005.
[77] J. Wang *et al*., "Use of bacteriophage in the treatment of experimental animal bacteremia from imipenem-resistant *Pseudomonas aeruginosa*," *Int. J. Mol. Med.*, vol. 17, no. 2, pp. 309–317, 2006.

[78] J. Wang *et al*., "Therapeutic effectiveness of bacteriophages in the rescue of mice with extended-spectrum β-lactamase-producing *Escherichia coli* bacteremia," *Int. J. Mol. Med.*, vol. 17, no. 2, pp. 347–355, 2006.
[79] G. W. Hanlon, "Bacteriophages: An appraisal of their role in the treatment of bacterial infections," *Int. J. Antimicrob. Agents*, vol. 30, no. 2, pp. 118–128, 2007.
[80] S. Chibani-Chennoufi, J. Sidoti, A. Bruttin, E. Kutter, S. Sarker, and H. Brüssow, "In vitro and in vivo bacteriolytic activities of *Escherichia coli* phages: Implications for phage therapy," *Antimicrob. Agents Chemother.*, vol. 48, no. 7, pp. 2558–2569, 2004.
[81] A. Bruttin and H. Brüssow, "Human volunteers receiving *Escherichia coli* phage T4 orally: A safety test of phage therapy," *Antimicrob. Agents Chemother.*, vol. 49, no. 7, pp. 2874–2878, 2005.
[82] S. A. Sarker *et al*., "Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh," *Virology*, vol. 434, no. 2, pp. 222–232, 2012.
[83] H. Giamarellou, "Multidrug-resistant Gram-negative bacteria: How to treat and for how long," *Int. J. Antimicrob. Agents*, vol. 36, pp. S50–S54, 2010.
[84] C. A. Arias and B. E. Murray, "Antibiotic-resistant bugs in the 21st century—A clinical super-challenge," *N. Engl. J. Med.*, vol. 360, no. 5, pp. 439–443, 2009.
[85] R. M. Klevens *et al*., "Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States," *JAMA*, vol. 298, no. 15, pp. 1763–1771, 2007.
[86] J. N. Pendleton, S. P. Gorman, and B. F. Gilmore, "Clinical relevance of the ESKAPE pathogens," *Expert Rev. Anti-Infect. Ther.*, vol. 11, no. 3, pp. 297–308, 2013.
[87] E. Morales *et al*., "Hospital costs of nosocomial multidrug-resistant *Pseudomonas aeruginosa* acquisition," *BMC Health Serv. Res.*, vol. 12, no. 1, Art. no. 122, 2012.
[88] M. J. Selgelid, "Ethics and drug resistance," *Bioethics*, vol. 21, no. 4, pp. 218–229, 2007.
[89] A. E. Aiello, N. B. King, and B. Foxman, "Ethical conflicts in public health research and practice: Antimicrobial resistance and the ethics of drug development," *Am. J. Public Health*, vol. 96, no. 11, pp. 1910–1914, 2006.
[90] L. Gravitz, "Turning a new phage," *Nat. Med.*, vol. 18, no. 9, pp. 1318–1320, 2012.
[91] World Medical Association, "Statement on resistance to antimicrobial agents." [Online]. Available: www.wma.net/e/policy/a19htm.
[92] L. Freire-Moran *et al*., "Critical shortage of new antibiotics in development against multidrug-resistant bacteria—Time to react is now," *Drug Resist. Updates*, vol. 14, no. 2, pp. 118–124, 2011.
[93] D. Jabes, "The antibiotic R&D pipeline: An update," *Curr. Opin. Microbiol.*, vol. 14, no. 5, pp. 564–569, 2011.
[94] I. U. Haq, W. N. Chaudhry, M. N. Akhtar, S. Andleeb, and I. Qadri, "Bacteriophages and their implications on future biotechnology: A review," *Virol. J.*, vol. 9, no. 1, Art. no. 9, 2012.
[95] R. J. Atterbury, "Bacteriophage biocontrol in animals and meat products," *Microb. Biotechnol.*, vol. 2, no. 6, pp. 601–612, 2009.
[96] P. Jain *et al*., "ϕ2GFP10, a high-intensity fluorophage, enables detection and rapid drug susceptibility testing of *Mycobacterium tuberculosis* directly from sputum samples," *J. Clin. Microbiol.*, vol. 50, no. 4, pp. 1362–1369, 2012.
[97] O. Adesanya, T. Oduselu, O. Akin-Ajani, O. M. Adewumi, and O. G. Ademowo, "An exegesis of bacteriophage therapy: An emerging player in the fight against antimicrobial resistance," *AIMS Microbiol.*, vol. 6, no. 3, p. 204, 2020.
[98] R. A. Sandaa, "Viruses, environmental," in *Encyclopedia of Microbiology*, 3rd ed., M. Schaechter, Ed., 2009, pp. 553–567.
[99] M. Imam *et al*., "vB_PaeM_MIJ3, a novel jumbo phage infecting *Pseudomonas aeruginosa*, possesses unusual genomic features," *Front. Microbiol.*, vol. 10, Art. no. 2772, 2019.
[100] T. S. Sada, D. H. Alemayehu, K. M. Tafese, and T. S. Tessema, "Genomic analysis and characterization of lytic bacteriophages that target antimicrobial-resistant *Escherichia coli* in Addis Ababa, Ethiopia," *Heliyon*, vol. 10, no. 22, Art. no. e40342, 2024.
[101] C. Kirchhelle, "The forgotten typers: The rise and fall of Weimar bacteriophage-typing, 1921–1935," *Notes Rec.*, vol. 74, no. 4, pp. 539–565, 2020.

[102] P. Lisotto *et al*., "Long-read sequencing-based in silico phage typing of vancomycin-resistant *Enterococcus faecium*," *BMC Genomics*, vol. 22, no. 1, Art. no. 758, 2021.
[103] R. Capita, C. Alonso-Calleja, L. Mereghetti, B. Moreno, and M. D. C. García-Fernández, "Evaluation of the international phage typing set and some experimental phages for typing of *Listeria monocytogenes* from poultry in Spain," *J. Appl. Microbiol.*, vol. 92, no. 1, pp. 90–96, 2002.
[104] J. Rocourt *et al*., "A multi-centre study on the phage typing of *Listeria monocytogenes*," *Zentralbl. Bakteriol. Mikrobiol. Hyg. A*, vol. 259, no. 4, pp. 489–497, 1985.
[105] J. Y. Lee, W. J. Chung, and G. Kim, "A mechanically improved virus-based hybrid scaffold for bone tissue regeneration," *RSC Adv.*, vol. 6, no. 60, pp. 55022–55032, 2016.
[106] Z. Hosseinidoust, A. L. Olsson, and N. Tufenkji, "Going viral: Designing bioactive surfaces with bacteriophage," *Colloids Surf. B Biointerfaces*, vol. 124, pp. 2–16, 2014.
[107] H. J. Jones, C. G. Shield, and B. M. Swift, "The application of bacteriophage diagnostics for bacterial pathogens in the agricultural supply chain: From farm-to-fork," *Phage*, vol. 1, no. 4, pp. 176–188, 2020.
[108] S. Meile, S. Kilcher, M. J. Loessner, and M. Dunne, "Reporter phage-based detection of bacterial pathogens: Design guidelines and recent developments," *Viruses*, vol. 12, no. 9, Art. no. 944, 2020.
[109] C. Rees and G. Botsaris, "The use of phage for detection, antibiotic sensitivity testing and enumeration," in *Understanding Tuberculosis—Global Experiences and Innovative Approaches to the Diagnosis*. Rijeka, Croatia: InTech, 2012, pp. 293–306.