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# BACTERIOPHAGE THERAPY: A PROMISING ALTERNATIVE TO ANTIBIOTICS IN THE ERA OF MULTIDRUG RESISTANCE

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## ABSTRACT

Bacteriophages, viruses that selectively infect and lyse bacteria, have gained renewed attention as a potential alternative to antibiotics in the fight against multidrug-resistant infections. This article provides an overview of the biology of bacteriophages, including their morphology, classification, host range specificity, and interaction mechanisms with bacteria. The therapeutic potential of phages is discussed through preclinical animal studies, biofilm, and their synergistic or antagonistic interactions with antibiotics. Although phage therapy offers significant advantages such as high specificity, ability to target antibiotic-resistant strains, and minimal disruption to normal flora, it also faces limitations including immune response, bacterial resistance, and lack of standardized regulatory frameworks. To overcome these barriers, strategies such as phage cocktails, genetic engineering, encapsulation, and phage banks are being explored. Beyond human medicine, phage therapy also demonstrates applications in veterinary practice, food safety, and environmental protection. Overall, bacteriophage therapy represents a promising and innovative approach that could complement or even replace antibiotics in the near future, provided that regulatory, pharmacological and clinical challenges are systematically addressed.

**KEYWORDS:** Bacteriophages, antibiotics, Host specificity, Biofilms, Multidrug-resistant infections.

## INTRODUCTION

Bacteriophages, or phages, were independently identified by Felix d'Herelle and Frederick Twort over a century ago as viruses that specifically infect and destroy bacteria. Their discovery introduced the concept of "phage therapy," a novel approach to treating bacterial infections at a time when diseases such as cholera, diphtheria, tuberculosis, and typhoid fever were leading causes of mortality. In the early 1900s, life expectancy in the United States was only 46–48 years, largely due to these infections.[1]

The arrival of antibiotics in the mid-20th century revolutionized infection control. With broad-spectrum activity, ease of use, and rapid effect, antibiotics quickly became indispensable. Their impact, alongside advances in sanitation and public health, contributed to a dramatic rise in life expectancy—surpassing 70 years in the U.S. by 1970. Phage therapy, meanwhile, persisted mainly in Eastern Europe and the former Soviet Union, notably at the Eliava Institute in Georgia.

Today, antimicrobial resistance (AMR) has reignited interest in phages. Overuse and misuse of antibiotics in medicine, agriculture, and industry have accelerated the spread of resistance genes, including those targeting β-lactams, aminoglycosides, and tetracyclines. The ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.) exemplify multidrug-resistant (MDR) threats. In the U.S., resistant infections cause over 2 million illnesses and at least 23,000 deaths annually, costing approximately $55 billion. Globally, AMR accounts for around 700,000 deaths each year, with projections of up to 10 million annually by 2050.[2]

The situation is worsened by the slowdown in antibiotic development: between 1983 and 1987, 16 new antibiotics were approved by the U.S. FDA, but only six between 2010 and 2016. The emergence of carbapenem-resistant bacteria, often with mortality rates of 40–50%, underscores the urgency of alternative treatments.

Phages offer several advantages over antibiotics, including high host specificity, self-replication at infection sites, biofilm degradation, and minimal toxicity to human cells. Advances in genomics, sequencing technologies, and molecular tools have renewed momentum in phage research, paving the way for targeted and effective therapies. With ongoing clinical trials and experimental evidence, phage therapy is once again positioned as a promising strategy against multidrug-resistant bacterial infections.[2]

**Historical Background of Bacteriophage**

In 1896, British bacteriologist Ernest Hanbury Hankin, working in India, found that water from the Ganga and Yamuna rivers contained a substance that could kill cholera-causing bacteria. This agent could pass through filters that usually stop bacteria. Hankin reported his findings in the Annals of the Pasteur Institute.

In 1915, British microbiologist Frederick Twort discovered that some bacterial cultures contained a filterable, transparent material that could break bacteria into small granules. This “agent” could infect new bacterial cultures but could not grow without them. Twort suggested it might be a type of “ferment” made by bacteria for unknown reasons.

Two years later, in 1917, Félix d’Herelle independently found a similar agent while studying patients recovering from dysentery. He filtered stool samples and found something that could kill Shiga bacilli, describing it as a “bacteriophage” — a virus that infects bacteria. He also tested phages in animals, suggesting their possible medical use.

The first clinical application was reported in Belgium by Bruynoghe and Maisin, who treated skin boils with staphylococcal phages. Patients improved within 48 hours, showing less pain, swelling, and fever.

At the time, scientists debated what phages really were. The roles of DNA and RNA were not yet known, and some believed phages were just proteins produced by bacteria. Gradually, studies confirmed that phages were viruses. The discovery of lysogeny — where phages stay inside bacteria without killing them immediately — showed that phage

genetic material could integrate into bacterial DNA. Researchers like Frank Macfarlane Burnet and Schlesinger helped confirm that phages were made of nucleoproteins and had diverse forms.

The invention of the electron microscope in the 1930s allowed Helmut Ruska to see phages clearly. Luria and Anderson later described their structure: a round head with a thin tail, resembling sperm, and observed the stages of bacterial infection and lysis.

Phage research continued in places like the Eliava Institute in Georgia and the Hirsfeld Institute in Poland, but in many Western countries, interest declined after antibiotics became common. It returned in the 1980s with animal studies and in the 2000s with human trials, including a phase I study in the US in 2009.

In 2004, the Phage Summit in Florida brought together over 350 scientists, marking a major revival of interest. Today, bacteriophages are among the most widely studied microbes, with tens of thousands of publications worldwide.[3]

**Bacteriophage biology**

Bacteriophages, or simply phages, are viruses that exclusively infect bacterial cells and have been known to science for more than a century. Owing to their simple yet fascinating structure, they have often been used as model systems to explore fundamental questions about the nature of life, becoming central to the early development of molecular biology. While they were initially studied for their basic biological significance, interest in bacteriophages has recently surged due to their potential as alternatives or complements to traditional antibiotics—particularly in combating multidrug-resistant bacteria—thanks to their remarkable specificity and unique infection mechanisms.

Phages lack their own metabolic machinery, which makes them obligate intracellular parasites; they depend entirely on bacterial hosts for replication, hijacking the host's biochemical systems via their genetic material. They are also significant drivers of bacterial evolution, influencing genome variation and, in some cases, contributing to the emergence of bacterial pathogenicity.[4]

**Morphology of a Typical Bacteriophage**

- **Capsid (Head)**
  - Icosahedral (20-sided) protein shell.
  - Protects the genetic material (DNA or RNA).

- **Genetic material**
  - Usually double-stranded DNA in most phages.
  - Stored inside the capsid.

- **Tail Sheath**
  - Spiral, contractile tube surrounding a central core.
  - Contracts to push DNA into the host cell.

- **Central Core Tube**
  - Hollow pipe inside the tail sheath.
  - Acts as a channel for DNA injection into the bacterium.

- **Baseplate**

➢ Hexagon-shaped structure at the tail end.

➢ Controls attachment and tail fiber movement.

- **Long Tail Fibers**

➢ Detect bacterial host by sensing specific surface receptors.

- **Short Tail Fibers**

➢ Emerge from beneath the baseplate.

➢ Anchor the phage firmly to the bacterial surface.

- **Function in infection**

➢ Long fibers find the host → short fibers lock it → sheath contracts → DNA injected into bacterium.[4]

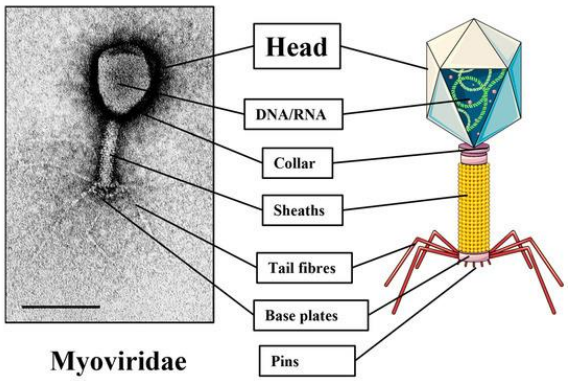


**Figure 1.**[5]

**Classification of Bacteriophages**

| Family | Morphology | Nucleic acid | Characteristic |
|---|---|---|---|
| *Myoviridae* | | Linear dsDNA | contractile tail, Non-enveloped |
| *Siphoviridae* | | Linear dsDNA | Long non-contractile tail, Non-enveloped |
| *Podoviridae* | | Linear dsDNA | Short non-contractile, Non-enveloped tail |
| *Tectiviridae* | | Linear dsDNA | Isometric , Non-enveloped |
| *Corticoviridae* | | Circular dsDNA | Isometric, Non-enveloped, |
| *Lipothrixviridae* | | Linear dsDNA | rod-shaped, Enveloped |
| *Plasmaviridae* | | Circular dsDNA | Pleomorphic, Enveloped |
| *Rudiviridae* | | Linear dsDNA | Rod-shaped , Enveloped |
| *Fuselloviridae* | | Circular dsDNA | lemon shaped , Non-enveloped |
| *Inoviridae* | | Circular ssDNA | Filamentous, Non-enveloped |
| *Microviridae* | | Circular ssDNA | Isometric, Non-enveloped |
| *Leviviridae* | | Linear ssDNA | Isometric, Non-enveloped |
| *Cystoviridae* | | Segmented dsDNA | Spherical , Enveloped, |

**Host Range specificity**

Phages can generally be divided into two main categories based on their host range: monovalent and polyvalent. Monovalent phages are those that have a limited host range, typically infecting only a single bacterial genus. In contrast, polyvalent phages have a wider host range and are capable of infecting multiple bacterial genera. Most phages reported tend to be specific to particular species or even strains.

However, terms like “broad host range” and “narrow host range” are often used inconsistently within the scientific community, which reduces their clarity. Some researchers define broad host range phages as those that can infect multiple bacterial species, while others use the term to describe phages that infect several strains within the same species. Similarly, the term “polyvalent” was originally used to describe phages active against different bacterial genera, but it is now sometimes used interchangeably with broad-host range phages.

Moreover, the terms “narrow” and “broad” host range are frequently confused or used interchangeably with “monovalent” and “polyvalent.” There is currently no standardized guideline regarding how many strains or species a phage must infect to be classified as broad-host range. For example, some studies have called a phage polyvalent if it infects around 60% of tested strains, while others have labelled a phage broad host range even when it infected only a few strains across different species. Because of these differences and the variations in bacterial strain collections used by different researchers, descriptions of phage host range can be quite inconsistent.[7]

**Phage-Bacteria interaction mechanism**

**Phages can follow three main life cycles—lytic, lysogenic, and chronic.**

**1) Lytic cycle**

- Virulent phage attaches to host cell and injects its genetic material.
- Host machinery is used to make many new phages.
- Cell bursts (lysis) → releases newly formed phages.

**2) Lysogenic cycle**

- Temperate phage DNA may integrate into the host chromosome (prophage).
- Prophage stays inactive but replicates with host DNA.
- Can transfer beneficial genes to the host (lysogenic conversion).
- Stress triggers (e.g., UV light, chemicals) can activate the prophage → switch to lytic cycle.

**3) Chronic cycle**

- Found in some archaeal viruses and filamentous phages.
- New viruses are released continuously without killing the host.
- Host cell survives but grows more slowly.[8]

**Attachment and penetration**

Bacterial cells have a cell wall made of polysaccharides, which play a key role in protecting them from host immune responses and the action of antibiotics. These polysaccharides also act as important virulence factors.[9]

Bacteriophages attach to their host by recognizing and binding to specific receptors on the bacterial surface, such as lipopolysaccharides, teichoic acids, proteins, or flagella. This receptor specificity limits each phage to infecting only

bacteria that possess compatible binding sites, defining its host range. Some phages carry polysaccharide-degrading enzymes that break down the host's protective capsule during the first step of infection. Additionally, the growth conditions of the host can affect how efficiently a phage attaches and enters the cell.[10] Myoviruses deliver their genetic material using a mechanism similar to a hypodermic syringe. After recognizing the correct receptor, their tail fibers bend to bring the base plate closer to the bacterial surface—this stage is called reversible binding. Once firmly attached (irreversible binding), the tail contracts, likely aided by ATP stored in the tail, to inject the genetic material through the bacterial membrane. This injection involves a bending motion of the tail shaft, moving sideways, contracting toward the cell, and then pushing back up. In contrast, podoviruses lack the long contractile tail of myoviruses. Instead, they use short, tooth-like tail fibers equipped with enzymes to break down part of the bacterial cell wall before transferring their genetic material.[11]

**Synthesis of proteins and nucleic acid**

A few minutes after a phage infects a bacterium, the bacterial ribosomes start making viral proteins from the phage's mRNA. In RNA phages, RNA replicase is made early to help copy the viral RNA. Some viral proteins change the bacterial RNA polymerase so it only makes viral mRNA. This stops the bacterium from making its own proteins and forces it to make viral parts instead. These parts include proteins for new viruses, helper proteins for assembly, and enzymes for breaking the cell open. In 1972, Walter Fiers (University of Ghent, Belgium) sequenced the first complete gene, and in 1976, he sequenced the full genome of bacteriophage MS2.[12]

**Virion assembly**

In T4 phages, the formation of new virus particles is supported by helper proteins, which work in a catalytic manner to aid in the process of phage assembly.[13] In T4 phages, the base plates are made first, followed by the tails. The head capsids are built separately and later attach to the tails on their own. The process follows a specific sequence where phage-encoded proteins interact in order. The right balance of these proteins is important for proper virus assembly.[14] The viral DNA is tightly packed inside the head, and the entire process is completed in roughly 15 minutes.[16]

**Release of virions**

Phages can exit a host cell in different ways—through lysis, extrusion, or sometimes budding. In lysis, tailed phages use the enzyme endolysin to break the bacterial cell wall. Filamentous phages release new viruses continuously without killing the host, while budding occurs in some Mycoplasma phages. In the lysogenic cycle, phages stay inside the host as prophages without causing cell death.[15]

**Phage therapy: Preclinial trials**

For nearly a hundred years, various institutions in Eastern Europe have conducted human trials on phage therapy, with two of the most notable being the Eliava Institute of Bacteriophage and the Institute of Immunology and Experimental Therapy in Wroclaw, Poland. The Eliava Institute, in particular, has played a major role in both preclinical and clinical applications of phages, targeting a wide range of bacterial pathogens, including Staphylococcus aureus, Escherichia coli, Streptococcus species, Pseudomonas aeruginosa, Proteus species, Shigella dysenteriae, Salmonella species, and Enterococcus species.[17]

In a 1938 trial, 219 bacterial dysentery patients (138 children, 81 adults) received only a phage cocktail targeting multiple pathogens, including Shigella, E. coli, Proteus, P. aeruginosa, Salmonella, Staphylococcus, Streptococcus, and

Enterococcus. The phages were given orally and rectally. Blood in stools disappeared in 28% of cases within 24 h, with another 27% improving in 2–3 days, and overall 74% showing recovery or symptom relief.[18]

**In vivo animal trials of phage therapy**

For phage therapy studies, smaller models like the nematode (C. elegans), fruit fly (D. melanogaster), wax moth (G. mellonella), and zebrafish are often used, while larger animals such as chickens, rabbits, hamsters, and mice serve as higher vertebrate models.[19] Using invertebrates and small vertebrates like zebrafish in research saves time and costs, but higher vertebrate models remain important. In birds, oral phage therapy has been applied both for prevention and treatment of diseases like salmonellosis, colibacillosis, and campylobacteriosis, which are major poultry health concerns.[20,21] Zebrafish, especially in the larval stage, are becoming a preferred model for exploring host–bacteria interactions.[22] Their developed innate immunity, ease of genetic manipulation, and transparent body make them valuable for studying infection processes not easily examined in traditional models. Recently, zebrafish models have been developed to investigate phage therapy against bacterial infections like E. faecalis and P. aeruginosa.[23,24] In a zebrafish embryo study, bacteria were injected to cause systemic infection, followed by phage delivery through the same route. Treatment led to higher survival, recovery from infection-related damage, and reduced bacterial load within five days, showing phage effectiveness in a quick, low-cost aquatic model.[25] Rabbits, which can naturally carry S. aureus like humans, are useful for studying wound infections. Infections were induced under the skin, followed by phage application at the same site. After 4–6 days, bacterial levels in abscesses were measured to assess treatment success.[25] Animal models have been key to studying how phages work and proving their effectiveness in vivo.

Invertebrate and vertebrate models offer faster, cheaper, and more ethical alternatives to human trials, while vertebrates give deeper insight into immune and inflammatory responses. Research has explored ways to boost phage action—such as improving delivery, using phage cocktails, combining with antibiotics, and applying them preventively—all of which can be tested in animals before moving to human use.[25] Most of these are uncontrolled clinical trials, making it hard to compare safety and effectiveness with placebo-controlled, blinded studies. Since a key aim of modern phage therapy is to quickly find phages for compassionate use, animal testing can help check the safety of specific phages before treating patients. While personalized phage use is not yet regulated, pre-screening in animals can help reduce some of the related challenges.[26]

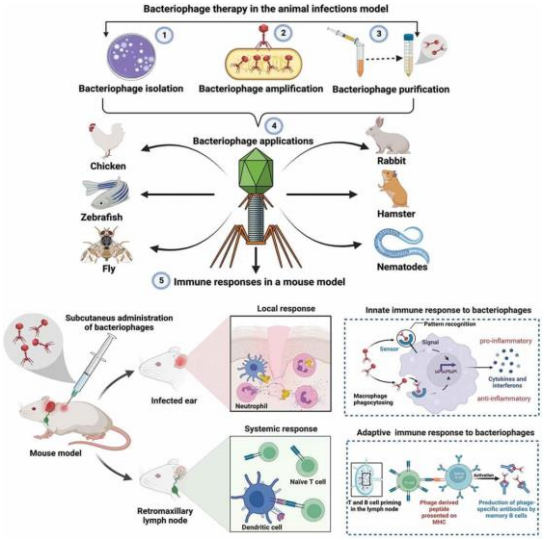


**Figure: 2**[27]

Figure shows the bacteriophage isolation process, animal infection models, and the immune responses to phages in a mouse model.

**Biofilms**

Human pathogenic bacteria frequently exist in the form of biofilms—complex, structured communities of bacterial cells encased in a self-produced polymeric matrix. Within these biofilms, the bacteria are largely immobile and differ both phenotypically and physiologically from their free-floating (planktonic) counterparts. Such biofilm-embedded cells show markedly reduced susceptibility to antimicrobial agents, partly because drug penetration is limited. In addition, they often display heightened activity of resistance mechanisms, such as efflux pumps, which actively transport antibiotics out of the cell, further reducing treatment effectiveness.[28,29] These resistance mechanisms make conventional antibiotic therapy challenging; therefore, phages capable of penetrating biofilms present a promising approach for treating bacterial infections. In numerous well-designed biofilm studies, both in vitro and in vivo, phages have proven their ability to penetrate mature biofilms and eliminate bacteria. Phages that produce enzymes such as depolymerases, which chemically break down the extracellular polymeric substances in biofilms, have shown particular effectiveness in this role. Notably, phages have demonstrated strong preventive effects against biofilm formation by pathogenic bacteria involved in nosocomial, oral, urinary tract, and wound infections in pre-treatment studies.

Furthermore, phages have been found to work excellently as an adjunct to antibiotics in eradicating Staphylococcus aureus within biofilms, reinforcing the benefit of combined phage-antibiotic therapy.[30]

**Phage-Antibiotic Combinations**

Phage therapy began to emerge as a treatment for infectious diseases in the early 20th century. However, after the discovery of penicillin in 1928, antibiotics quickly became the preferred treatment in clinical practice due to their more straightforward and well-understood mechanisms of action. From the 1940s onward, various studies explored the use of combined phage and antibiotic therapies to enhance treatment effectiveness.[31]

**Phage–Antibiotic Synergy (PAS)**

For multidrug-resistant (MDR) pathogens, bacteriophages used together with antibiotics can be particularly beneficial. In a disk diffusion test on a urine sample, Comeau et al. reported that phage plaques on uropathogenic Escherichia coli were noticeably larger in zones with low (sub-inhibitory) concentrations of aztreonam and cefixime, suggesting a synergistic interaction between these β-lactam antibiotics and the phages. This phenomenon, where certain antibiotics at low levels enhance phage activity, was termed phage–antibiotic synergy (PAS). Since then, PAS has become an important focus of phage research, contributing to several clinical successes.

In general, synergy describes a combined antimicrobial effect greater than the sum of individual effects, while antagonism is when the combination performs worse than either alone . Initially, PAS was explained as antibiotics directly boosting phage growth, but more recent work considers evolutionary and ecological factors in phage–host interactions.[32]

**Combination of Phages with Sulphonamides and Penicillin**

During the early 20th century, phages were sometimes administered alongside sulphonamides for the treatment of infectious diseases. The synergistic potential of this approach was first reported in 1941, when Zaytzeff-Jern and

colleagues demonstrated that combining phages with sulphanilamide and sulphapyridine could inhibit the in vivo growth of Staphylococcus aureus and Escherichia coli.[33] In the same year, Neter et al. observed that combining phages with sulphanilamide, sulphapyridine, and sulphathiazole restored their antibacterial activity against phage-resistant Staphylococcus strains in broth cultures.[34] In 1942, MacNeal and colleagues evaluated phage therapy against a combined phage–sulphathiazole regimen in 56 rabbits with staphylococcal meningitis. The combined approach showed clear superiority: all 23 untreated rabbits died; all 5 given a delayed phage–sulphathiazole combination also died; only 1 of 6 survived with early sulphathiazole alone; whereas 13 of 22 rabbits survived when given an early phage–sulphathiazole combination. In a subsequent 1943 study, the same group reported that administering phages together with sulphonamides in humans with acute malignant staphylococcal infections resulted in 24 recoveries out of 55 treated patients.[35,36] After penicillin was discovered in 1928, several researchers investigated its combined use with phages to treat bacterial infections. In 1945, Himmelweit et al. cultured Staphylococcus at a density of 130 million cells/mL and tested three treatments: (a) penicillin alone, (b) phages alone, and (c) a phage–penicillin combination. Complete lysis occurred after 6 h 3 min with penicillin, 1 h 55 min with phages, and just 1 h 25 min with the combined treatment.[37]

**Antagonistic Phage–Antibiotic Combinations**

As many phages depend on bacterial machinery for essential central dogma processes, phage–antibiotic antagonism can arise from reduced phage replication caused by antibiotics that target components such as bacterial RNA polymerase or ribosomes. This phenomenon has been observed for several specific phage–host pairs, and examples have previously been reviewed elsewhere.[32] In 1951, studies on several bacterial species tested quinones, clavacin, subtilin, penicillic acid, polymyxin B, and streptomycin in combination with phages, showing no synergistic or additive effects, but rather antagonism.[38] In 1954, experiments with Staphylococcus aureus using aureomycin, oxytetracycline, and chloramphenicol also demonstrated antagonistic interactions with phages . In 1977, studies on E. coli treated with rifampicin revealed antagonistic effects on phage activity. In 2021, experiments with E. coli and Bacillus cereus using aminoglycosides demonstrated antagonistic interactions with phages.[38]

**Timing of Application**

The success of different treatment sequences—whether antimicrobials are given at the same time, phages are applied before antibiotics, or antibiotics are given before phages—can be influenced by the mechanisms behind phage–antibiotic synergy (PAS) or antagonism. For example, when P. aeruginosa PA14 biofilms were treated simultaneously with phages NP1 and NP3 alongside gentamicin or tobramycin, the outcome was no better than using the antibiotic alone—likely because the antibiotic interfered with phage infection . In comparison, applying the antibiotic 24 hours after the phage treatment led to a much greater reduction in bacterial density than either agent used on its own.[39] In a separate study, simultaneous treatment of S. aureus biofilms with phage SATA-8505 showed antagonistic effects when combined with the cell wall synthesis inhibitor cefazolin, and a concentration-dependent antagonism with vancomycin. However, when the phage was applied first and followed 24 hours later by vancomycin, cefazolin, linezolid, or tetracycline, the combination produced either synergistic or additive antimicrobial effects.[40] While these findings support the idea of administering phages before antibiotics, some studies indicate that simultaneous application can, in certain cases, be more beneficial. For instance, despite gentamicin's potential to hinder phage replication, Akturk et al. reported that multiple rounds of simultaneous treatment with phages EPA1 and SAFA plus gentamicin were more effective at eliminating mixed P. aeruginosa and S. aureus biofilms compared to phage-first sequential strategies.

Applying both agents together may better restrict the development of resistance to either phages or antibiotics than sequential approaches.[41]

**Advantages of Phage Therapy**

The benefits of phage therapy compared to chemical antibiotics come from the unique properties of phages.

**Bactericidal agents**

Bacteria targeted by strictly lytic phages are completely destroyed and cannot recover, whereas some antibiotics, like tetracycline, only inhibit growth without killing, which can allow bacteria to evolve resistance more easily.[42,43]

**Auto "dosing"**

During infection, phages can multiply at the sites where their bacterial hosts are present, although this requires a sufficiently high bacterial population. This self-amplification is often called 'auto-dosing,' since the phages help determine their own effective dose.

**Low inherent toxicity**

Phages are mainly made of proteins and nucleic acids, so they are generally non-toxic. However, they can interact with the immune system, which in theory could cause harmful responses, though such effects are rarely observed in practice. For some therapies, using highly purified phage preparations is important to avoid allergic reactions to bacterial components like endotoxins that may be present in crude lysates. Similarly, when phages kill bacteria, they can release bacterial substances, a process comparable to what happens with antibiotics that disrupt cell walls.

**Minimal disruption of normal flora**

Because phages are highly specific to their bacterial hosts—sometimes infecting only a few strains of a species and occasionally affecting more than one related genus—they have little effect on beneficial normal bacteria. In contrast, many antibiotics have broad activity and can disrupt the microbiome, leading to superinfections such as C. difficile colitis or Candida yeast infections. However, there is now growing interest in developing narrower-spectrum antibiotics.

**Lack of cross-resistance with antibiotics**

Phages kill bacteria using methods different from antibiotics, so bacterial resistance to antibiotics does not make them resistant to phages. This makes phages especially useful for treating infections caused by antibiotic-resistant bacteria, including multi-drug-resistant Staphylococcus aureus.

**Rapid discovery**

Phages targeting many harmful bacteria can be easily found, often in sewage or other waste rich in bacteria. However, isolating phages can be more challenging if the host bacteria are hard to grow, and different bacteria vary in how many phage types can infect them. Unlike some antibiotics, phages generally show little or no toxicity and can be obtained for most bacterial targets.

**Biofilm clearance**

Biofilms are often much more resistant to antibiotics than free-floating bacteria. Phages, however, can sometimes remove biofilms, either by breaking down one bacterial layer at a time or by producing enzymes called depolymerases that degrade the biofilm's protective matrix.

**Relatively low cost**

Phages are mainly produced by growing their host bacteria followed by purification. The cost of growing the host depends on the bacterial species, but purification is becoming cheaper with advancing technology. Overall, the per-unit cost of producing phages is comparable to other pharmaceuticals, and discovering and characterizing new phages can be relatively inexpensive.[44]

**Limitations of Phage therapy**

**Pharmacological limitations**

Phages are much larger than antibiotics—about a million times heavier—which leads to slower absorption and transport in the body. Their protein-rich shells can also be recognized by the immune system, causing them to be cleared rapidly. As a result, phages show complex behavior in the body, referred to as pharmacokinetics and pharmacodynamics. Oral administration, the most common route for drugs, has also been tested for phages.[45] the oral bioavailability of phages is very low. most phages are sensitive to an acidic environment: they get inactivated at pH values below 3.[46]

Delivering phages to the site of infection presents several challenges. Phage particles can trigger immune responses and be rapidly removed by the body. They are also sensitive to stomach acid and digestive enzymes, which can inactivate them.[47]

**Immune response**

Phages are composed of DNA or RNA enclosed within a protein shell. Their natural immunogenicity allows them to interact with both the innate and adaptive immune systems. As early as 1964, Aronov and colleagues observed that phagocytic cells could engulf phages during phagocytosis. Specifically, macrophages and neutrophils were seen ingesting the E. coli T2 phage, and the phage particles inside the cells broke down after about 15 minutes of incubation.[48] When phages used in therapy are highly purified and free of allergens, they can still trigger immune responses. Once phages cross the epithelial barrier, they reach lymphoid organs, where antigen-presenting cells process them and present them to T-cells. Repeated phage injections can lead to the production of antibodies, including non-neutralizing IgM and IgG. Studies have confirmed that phage therapy can induce anti-phage antibodies. For example, in Wroclaw, researchers measured anti-phage antibodies in the blood of 19 patients treated with Staphylococcal phages and 20 healthy volunteers using ELISA.[49] After the third dose of the ENB6 phage, IgM and IgG levels increased by approximately 5-fold and 3800-fold, respectively.[50] T4-like phage-specific antibodies are present in about 81% of human sera tested. These immune responses are mainly directed against the major head protein (gp23) and the highly antigenic outer capsid protein (Hoc). The rise in anti-Hoc and anti-gp23 antibodies can reduce T4 phage activity both in vitro and, to some extent, in vivo.[51]

**Phage resistance**

Even though phages have different pharmacokinetics and pharmacodynamics compared to antibiotics, bacteria can still evolve resistance over time. The ongoing co-evolution of phages and bacteria often leads to the development of phage-resistant bacterial strains. Bacteria can resist phage infection through various mechanisms. For example, in a study examining phenotypic changes, Pseudomonas fluorescens SBW25 was co-cultured with the lytic phage phi2 in both soil and nutrient broth. Researchers observed that the emergence of mucoid bacterial phenotypes was linked to the presence of phages, suggesting a phenotypic shift as a resistance strategy.[52] Another way bacteria can resist phages is through point mutations in surface structures that act as phage receptors. In one study, researchers sequenced four candidate genes from eight phages with distinct phenotypes and examined eight evolved phages. They found that each evolved phage had a unique combination of mutations. Most importantly, many of these mutations occurred in the phage tail fiber gene, which is critical for the phage to attach to the bacterial cell surface.[53]

**Lack of standard regulatory framework**

Phage therapy is not yet approved for human use by the Food and Drug Administration (FDA) or the European Medicines Agency (EMA), mainly due to strict regulatory and legal requirements. At present, there is no comprehensive legal framework for phage therapy in most countries. However, it has been authorized for medical use in a few nations, including Poland, Russia, and Georgia.[54] Phage therapy is considered a type of personalized medicine and differs from standard drugs in several ways. Traditional drugs have a fixed, predetermined composition, whereas phage cocktails can vary widely in their make-up. Moreover, phages are capable of evolving over time, which presents a unique challenge for regulators. As a result, conventional drugs fit within existing regulatory frameworks, but phage therapy does not.[55]

**Strategies to Overcome Limitations**

**Phage cocktails**

Owing to the vast diversity of phages present in natural environments, formulating an effective phage cocktail presents a far greater challenge than designing conventional combination antibiotic regimens. The selection and composition of phages within a cocktail play a pivotal role in determining the therapeutic success of phage-based treatments.[57]

Phage therapy may involve the use of either a single phage preparation or a combination of multiple phages in the form of a cocktail. Compared to monophage treatments, phage cocktails offer distinct advantages, including a reduced likelihood of resistance development and a broader range of antibacterial activity. Evidence from studies indicates that phage cocktails can markedly inhibit biofilm formation. Moreover, when combined with agents such as antibiotics, disinfectants, or honey, these cocktails have demonstrated enhanced efficacy in biofilm removal.[58]

**Engineering Strategies for Phage Genome**

Phages offer promising applications in areas such as therapeutic interventions, precision medicine, and bacterial infection control. Yet, the practical use of naturally occurring lytic phages remains limited due to factors including regulatory hurdles, restricted host range, bacterial resistance, manufacturing difficulties, and potential side effects linked to bacterial lysis and delivery. Many of these challenges can potentially be addressed through genetic modification of phages. Homologous recombination (HR) in vivo is one of the earliest and widely used strategies for phage genome modification, enabling targeted gene insertion, replacement, or deletion. Using this approach, linear dsDNA phages—such as Mycobacterium smegmatis phage L5 and Pseudomonas aeruginosa phage PaP1—can be

engineered. HR is generally effective only when the host strain is competent for donor DNA uptake and lacks restriction activity. However, the process often suffers from low efficiency, sometimes below 0.1%, making the identification of recombinant phages labor-intensive. Incorporating selection markers into donor DNA, such as the trxA gene (essential for phage replication but nonessential for host growth), can improve screening efficiency. The CRISPR–Cas9 system has become a widely adopted tool for targeted gene knockout, insertion, and site-specific mutagenesis of phage genomes. Studies from the Martel lab demonstrated its effectiveness in introducing mutations and large-fragment deletions in the Streptococcus thermophilus phage 2972 genome, with all examined plaques carrying the intended modifications. In 2017, the Aslan lab employed the native CRISPR–Cas10 system of Staphylococcus epidermidis to modify the staphylococcal lytic phage Andhra, achieving silent mutation rates of up to 100%. Similarly, use of the CRISPR–Cas12a system enabled deletion of large segments (11–29 kb) from the phage T4 genome, producing exclusively mutant plaques. Type I-E CRISPR–Cas systems have also proven effective, as shown by editing of Vibrio cholerae phage ICP1_2011_A, where 33 bp and 2670 bp deletions were achieved with efficiencies of 100% and 58%, respectively. Editing efficiency in CRISPR-based approaches can vary depending on factors such as donor fragment homologous-arm length and target sequence size.[59]

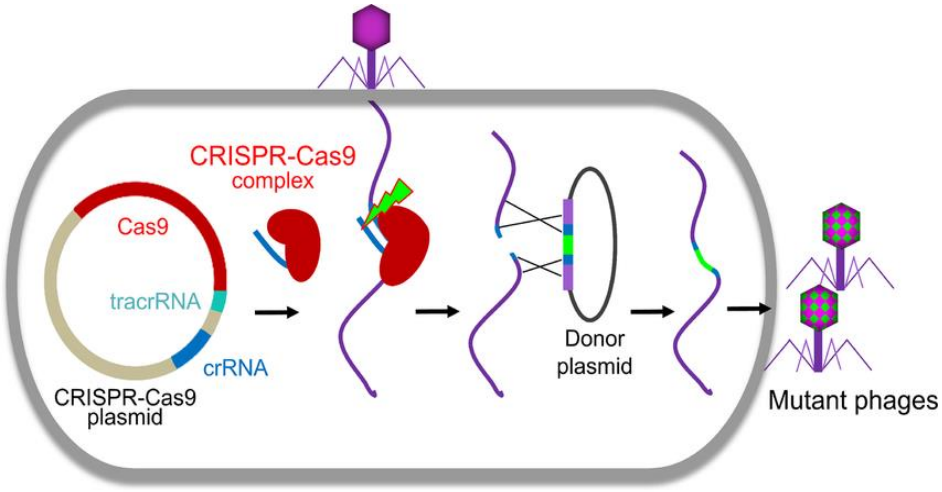


**Figure 3.**[60]

**Encapsulation**

Encapsulation involves enclosing active agents within a carrier matrix, resulting in uniform particles that remain stable during production and application. The resulting microcapsules typically consist of a core containing the therapeutic agent, enclosed within a protective shell that shields it from environmental factors. This method has been widely explored for enabling oral administration of bacteriophages. Polymeric encapsulation can improve the effectiveness of orally delivered phages by safeguarding them against the acidic and enzymatic conditions of the stomach.[61]

**Phage banks**

An essential aspect of clinical phage microbiology involves establishing extensive local repositories, or phage banks, which maintain phage preparations in a ready-to-use form for rapid clinical application. Bacterial isolates from patients considered for phage therapy are first tested for antibiotic susceptibility and then screened against bacteriophages from existing phage banks, using techniques such as plaque assays and growth kinetics analysis. In situations requiring urgent intervention, rapid matching methods can be applied to identify effective phages and commence treatment without delay. If no suitable phage is identified, developing phage cocktails or expanding the phage–antibiotic

compatibility screening may be necessary, with particular attention to cases involving biofilms or mixed bacterial infections. Phages stored in banks should undergo complete genome sequencing to confirm the absence of undesirable genes and to determine whether they are strictly lytic or possess lysogenic characteristics. Phage products intended for clinical application must be produced under Good Manufacturing Practice standards, accompanied by rigorous quality control and purification processes to verify identity, potency, and purity. Because phage titres can decline over time, maintaining functional phage banks demands continuous preparation of fresh working stocks. The Phage Bank ATM initiative proposes maintaining extensive stocks of ready-to-use phage vials across clinical facilities worldwide.[62]

## Applications beyond Human Medicines

### Phage Therapy in Veterinary Medicine

In recent years, while research on the therapeutic potential of phages for human bacterial infections has grown rapidly, there has also been increasing attention on their use in veterinary medicine, especially in livestock such as cattle and poultry.[63]

**Companion Animals**

| Animal | Condition / Target Pathogen | Key Findings | Outcome / Notes |
|---|---|---|---|
| Dogs | *Pseudomonas aeruginosa* (otitis externa) | Antibiotic-resistant infections are difficult to treat; phage therapy used in a 2006 case | Successful recovery; no side effects; no bacteria detected for 9 months |
| Cats | Uropathogenic *E. coli* | No clinical trials; only in vitro studies available | Promising potential for treating antibiotic-resistant urinary infections |
| Horses | *Staphylococcus aureus* (superficial pyoderma) | Topical phage cocktail tested in 20 horses | No clinical improvement; suggests need for broader phage cocktails and better management factors |

**Livestock**

| Animal Group | Condition / Target Pathogen | Key Findings | Outcome / Notes |
|---|---|---|---|
| Ruminants (Cattle) | *Staphylococcus aureus* (mastitis, including MRSA) | Early phage (phage K) trial limited by inactivation; newer phages (e.g., MSA6) show strong in vitro results | Promising but requires more in vivo studies; combination with probiotics shows potential |
| Pigs | *E. coli* infections | Microencapsulated phage A221 tested in piglets | Improved health, reduced bacterial load; effectiveness similar to antibiotics without harming gut flora.[64] |
| Poultry (Chickens) | *Salmonella enterica* (Pullorum) | Early phage trials (1926) ineffective due to inactivation in digestive system | Later research revisited concept; highlights importance of delivery method.[65] |

### Phage research to improve food safety

Various strategies have been explored to improve bacterial removal from fresh produce, including radiation, edible coatings, nitrogen oxides, ultraviolet light, controlled storage conditions, potassium permanganate, water washes, and in some cases, viral proteins. Among these, microbial agents, such as bacteriophages, are considered cost-effective and environmentally friendly alternatives because they do not compromise the taste or quality of fresh produce, unlike some chemical treatments. Studies have examined the potential of viral formulations to control foodborne pathogens responsible for outbreaks associated with fruits and vegetables. However, inconsistent results have limited their broader application in local food systems. These inconsistencies are largely attributed to insufficient viral dosing during production and a limited understanding of bacteriophage ecology.[66]

**Environmental Applications**

Researchers have explored the use of bacteriophages in combination with other control strategies and found promising results. For example, mixing phages with fungicides such as copper hydroxide and mancozeb showed synergistic effects in controlling plant pathogens by enhancing cell penetration. Similarly, the biocontrol agent Pantoea agglomerans became more effective against fire blight when used together with phages. In tobacco bacterial wilt, combining phages with bacteriocin-producing Ralstonia solanacearum also led to remarkable improvements. Another compound, Acibenzolar-S-methyl (ASM), commonly used as a fungicide, demonstrated stronger control of tomato bacterial diseases when applied with phages. Since ASM also acts as a systemic acquired resistance (SAR) inducer, its combination with phages further enhanced plant defense against several pathogens.[67]

**Future Prospects**

In the future, advances in biotechnology may help overcome challenges like patenting and large-scale production of phage-based drugs. Genome editing tools such as CRISPR/Cas, sequencing, and DNA recombination can be used to design phages that specifically target antibiotic-resistant bacteria while sparing the normal microflora. These engineered phages or phage cocktails will also be easier to commercialize and patent. Establishing global phage libraries and biobanks will be important to ensure quick access to suitable phages for both personalized treatments and larger-scale therapies. At the same time, strict quality control—covering sterility, stability, and safety—will be necessary to produce effective phage preparations.

Although phages hold great promise, most experts agree that they cannot completely replace antibiotics. Instead, phage therapy may work best when combined with antibiotics or used as a last option for infections that do not respond to conventional treatments. With the rising number of multidrug-resistant infections worldwide, revisiting phage therapy and developing it as a practical alternative to antibiotics has become more important than ever.[68]

## CONCLUSION

The review article on bacteriophage therapy emphasizes its growing importance as a potential alternative to antibiotics in managing bacterial infections, particularly those caused by multidrug-resistant strains. The biological characteristics of bacteriophages, including their specificity, ability to disrupt biofilms, and interactions with antibiotics, highlight their therapeutic potential. At the same time, challenges such as immune response, bacterial resistance, and lack of a standardized regulatory framework limit their widespread clinical use. The article also discusses strategies to overcome these limitations, including the development of phage cocktails, genome engineering, encapsulation methods, and the establishment of phage banks. Furthermore, applications beyond human medicine, such as veterinary practice, food safety improvement, and environmental management, expand the scope of phage research. Despite existing challenges, advancements in biomedical research and innovative therapeutic approaches indicate that phage therapy holds great promise. The review concludes that with continued scientific progress and regulatory support, bacteriophages could emerge as a sustainable and effective future alternative to antibiotics.

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