# "Bacteriophage Therapy: A Promising Solution to Combat Antibiotic Resistance"

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**ABSTRACT**:

Antibiotic resistance poses a major threat to global health, complicating the treatment of bacterial infections due to their prevalence, high mortality rates, and associated healthcare costs. Bacteriophages, which are viruses that target and destroy bacteria, offer a promising alternative for treating antibiotic-resistant infections. Although bacteriophage therapy was first utilized nearly a century ago, its progress was halted with the advent of antibiotics. Today, with the growing issue of antibiotic resistance, interest in bacteriophage therapy has resurfaced. Given its safety and potent antibacterial effects, certain bacteriophages, such as phiX174 and the Pyo bacteriophage complex liquid, have advanced to phase III clinical trials. In this review, we discuss the critical aspects of the antibiotic resistance crisis and the factors contributing to the renewed interest in bacteriophage applications. We summarize recent cutting-edge research and clinical data on bacteriophage therapy, covering (i) the pharmacological mechanisms and benefits of antibacterial bacteriophages, (ii) clinically relevant bacteriophage preparations and anti-bacterial treatment strategies derived from them, and (iii) bacteriophage therapeutics designed for various infection types and cancer treatments related to infections. Lastly, we address the challenges and key considerations for the future clinical development of bacteriophage therapy.

Key Words: Bacteriophages, Antibiotic resistance, Clinical trials, Pharmacological mechanisms.

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INTRODUCTION:

While bacteriophage therapy is not a novel treatment approach, it continues to inspire renewed optimism in the battle against antimicrobial resistance (AMR). The initial report on the effectiveness of bacteriophage therapy garnered significant attention nearly a century ago. However, following World War II, interest in these therapies waned as antibiotics became widely adopted.[1] Recent advancements in personalized bacteriophage therapy have generated significant hope for patients suffering from infections caused by antimicrobial-resistant bacteria. This review aims to highlight the crucial role of bacteriophage therapy in addressing the AMR crisis. We begin by discussing the pharmacological mechanisms and benefits of bacteriophage antibacterial agents. Following that, we summarize various formulation strategies for bacteriophage therapy, including cocktail therapy, liposomes, polymeric biological particles, microneedles, and electrospun fibers, as well as bacteriophage-derived antibacterial methods. Antimicrobial resistance (AMR) poses an increasing threat to global public health. According to the World Health Organization (WHO), approximately 700,000 deaths annually are linked to AMR, and this figure is expected to escalate significantly in the coming years. This alarming statistic underscores the urgent need for new and effective antimicrobial therapies. Unfortunately, over the past two decades, the U.S. Food and Drug Administration (FDA) and the European Medicines Agency have only approved two new classes of antibiotics targeting Gram-positive pathogens, which offer limited effectiveness against Gram-negative bacteria.[2]

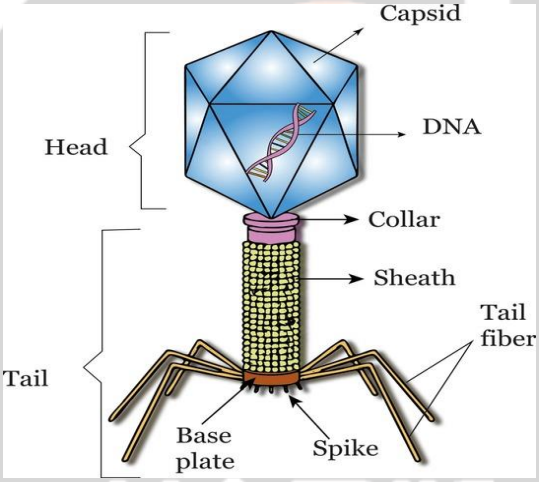
What is Bacteriophage?

Bacteriophages, or phages, are a group of viruses that specifically infect bacteria. They were discovered independently by Frederick W. Twort in the UK in 1915 and Félix d'Hérelle in France in 1917. D'Hérelle coined the term "bacteriophage," meaning "bacteria eater," to highlight their ability to kill bacteria. Phages can also infect archaea, which are single-celled prokaryotic organisms. Thousands of phage varieties exist, each typically infecting a specific type or a limited range of bacteria or archaea. Phages are classified into several virus families, including Inoviridae, Microviridae, Rudiviridae, and Tectiviridae. Like all viruses, they are simple organisms comprising a core of genetic material (either DNA or RNA) encased in a protein capsid. Their nucleic acid can be double-stranded or single-stranded, and phages generally have three basic structural forms: an icosahedral head with a tail, an icosahedral head without a tail, and a filamentous shape.

Phages are the most abundant biological entities on Earth and have demonstrated effectiveness in combating multi-drug resistant bacteria. When antibiotics fail, phages can still target and eliminate these bacteria, potentially saving lives in critical situations. The rise of "superbugs"—bacteria resistant to most, if not all, antibiotics—poses a significant threat to human health and longevity. Compounding this issue is the lack of regulatory pathways to approve phage therapy for anything less than dire emergencies, which raises serious concerns. At IPATH, we aim to make phage therapy more accessible to save lives when other treatments are no longer viable.[3]

Antibiotic Resistance:

Numerous mechanisms of drug resistance have emerged at nearly every stage of antibiotic action: (a) bacterial cell




membranes can alter their structure to block drug entry, or efflux pumps can be overexpressed to expel the drugs; (b) antibiotics can be modified or degraded by enzymes, such as β-lactamases, and the production of essential enzymes for antibiotic activation can be inhibited; (c) the drug targets can be modified, hidden, or altered to reduce drug effectiveness.[4][5] At the molecular level, the primary mechanism of antimicrobial resistance involves genetic alterations. In addition to mutations induced by drug pressure, bacteria can also acquire exogenous drug-resistant genes through processes such as transformation, conjugation, or transduction, thereby converting sensitive strains into resistant ones.[6] dditionally, bacterial cells can develop transient, non-gene-coded resistance through mechanisms such as biofilm formation, colony adaptation, metabolic dormancy, and persistence.[7]

1] By Enzymetic Destruction of Antibiotic Compounds:

The primary mechanism of β-lactam resistance involves the degradation of these compounds by β-lactamases. These enzymes break the amide bond of the β-lactam ring, rendering the antimicrobial ineffective. Although β-lactamases were first identified in the early 1940s, just a year before penicillin became available, evidence suggests that they have existed for millions of years.[8][9] Infections caused by penicillin-resistant *S. aureus* became a significant clinical issue after penicillin was widely used. The resistance mechanism was identified as a plasmid-encoded penicillinase, which could easily spread between *S. aureus* strains, leading to rapid dissemination of this resistance trait. To address this challenge, new β-lactam compounds with broader activity and reduced susceptibility to penicillinases, such as ampicillin, were developed. However, in the 1960s, a novel plasmid-encoded β-lactamase capable of hydrolyzing ampicillin was discovered in gram-negative bacteria, named TEM-1 after the patient (Temoneira) in whom it was first

identified.[10] The evolution of β-lactamases has progressed from narrow-spectrum enzymes to those capable of degrading nearly all available β-lactams, including carbapenems. Additionally, enzymes that were originally categorized based on a specific biochemical profile can evolve into new enzymes with different substrate specificities, typically through mutations in the active site. A notable example of this is TEM-3, which developed from the original TEM-1 penicillinase by acquiring the ability to hydrolyze third-generation cephalosporins and aztreonam. This expanded functional profile classifies it as an "Extended Spectrum β-Lactamase" (ESBL), resulting from two amino acid substitutions that altered its enzymatic activity.[11]

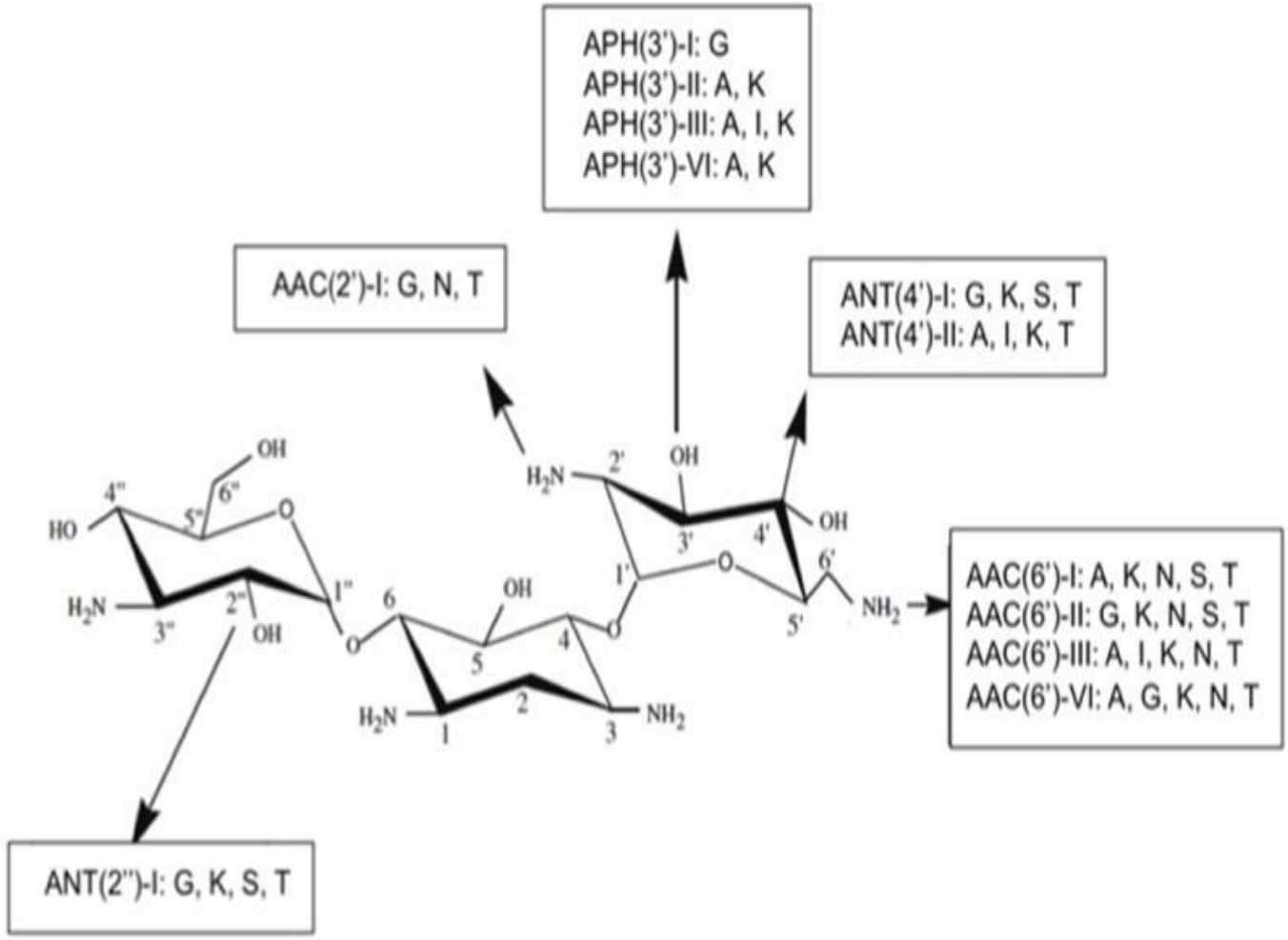



Fig.2: Distruction Of Compound By Enzyme

[2] By Mutation of Targated site:

A notable example of antibiotic resistance due to mutational changes is the resistance to oxazolidinones, specifically linezolid and tedizolid. These synthetic bacteriostatic antibiotics have broad activity against gram-positive bacteria and work by interacting with the A site of bacterial ribosomes, thereby inhibiting protein synthesis by disrupting the

positioning of aminoacyl-tRNA. Linezolid is the more widely used of the two, as tedizolid was only recently approved for clinical use. While resistance to linezolid is still relatively rare, it has been documented in many clinically significant gram-positive bacteria. The primary mechanisms of linezolid resistance include mutations in the genes encoding domain V of the 23S rRNA and/or the ribosomal proteins L3 and L4 (rplC and rplD), as well as methylation of A2503 (according to E. coli numbering) in the 23S rRNA, mediated by the Cfr enzyme.[12]

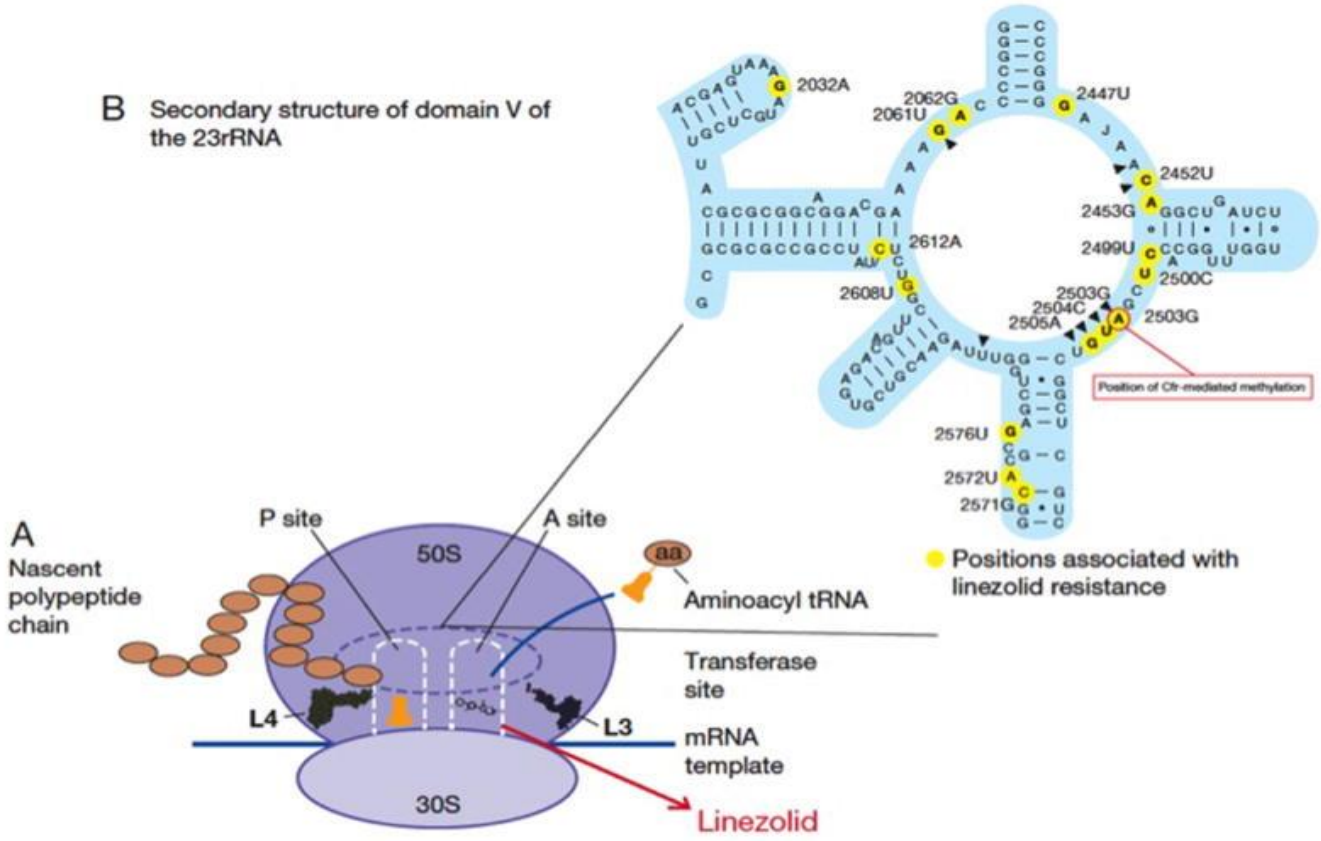



Fig.3: Mutation Of Targated Site

Mechanism Of Action Of Bacteriophage:

While antibiotics are chemical substances, phages are strict parasites of bacteria, functioning as biological entities that engage in complex co-evolutionary relationships with their hosts. Phages can be classified as either virulent or temperate, depending on their life cycles: lytic (which results in the destruction of the bacterial cell) or lysogenic (where the phage's genetic material integrates into the bacterial DNA, granting the bacterium immunity to reinfection by the same phage). For therapeutic applications, only virulent phages—those that can perform lytic cycles—are utilized. The lytic cycle of phages begins with the adsorption phase, during which the phage attaches to the bacterial membrane. This step is highly specific; a given phage typically binds only to specific bacterial species, and sometimes to particular strains (genetic variants) within that species. After attachment, the phage injects its genetic material into the bacterium. The bacterial enzymes then replicate the phage DNA and synthesize the proteins and lipids needed to form new capsids. Once the components are assembled into new virions, the bacterium is lysed, releasing between 50 and 200 new phages that can infect other bacteria, thereby restarting the cycle.

In therapeutic contexts, it is essential to isolate active phages that target the bacteria responsible for the patient's infection, amplify these phages, and administer them in a manner that ensures contact with the pathogen. For example, in cases of osteoarticular infections, a phage solution may be used to wash the wound before closing it at the end of surgery. As phages multiply in the presence of host bacteria, they will continue to spread as long as there are susceptible cells to infect. Once the pathogen is eliminated, the phages, which cannot survive without a host, will degrade.

The specific mode of action of lytic phages allows for tailored therapeutic approaches based on the history of the infection. Phages offer the potential to treat an increasing number of critical cases, especially as antimicrobial resistance (AMR) becomes more prevalent. They may serve as an alternative to antibiotics or be used in combination with them. Some studies indicate that phages can enhance antibiotic effectiveness and reduce the likelihood of resistance development in bacteria.[13] They could be used to decolonize patients before surgery or to treat carriers of *Staphylococcus aureus*, particularly in immunocompromised individuals.[15]

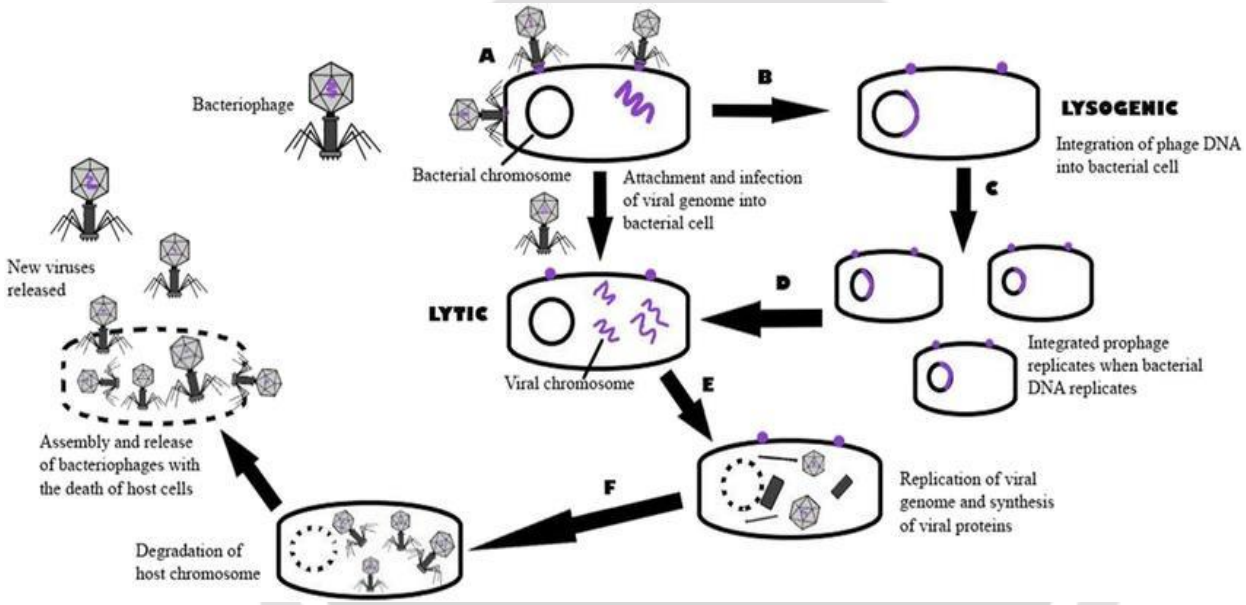



Fig.4: Mechanism Of Action Of Bacteriophage

Historical Clinical Trials Supports the Effectiveness of Phage Therapy:

Building on the promising results achieved by d'Hérelle, phages began to be used more widely by other physicians. Between 1925 and 1935, Lieutenant Colonel John Morison of the British Colonial Army worked with d'Hérelle to administer phage preparations as a preventive measure to populations in the Naogaon region of India (now Bangladesh), which was heavily impacted by cholera outbreaks.[16] During this time, Naogaon did not experience an epidemic, while neighboring Habiganj saw over 1,500 deaths from cholera in 1933 alone. In Brazil, José da Costa Cruz from the Oswaldo Cruz Institute used isolated phages to combat dysentery, reporting recoveries within a few days—an unprecedented outcome in the Brazilian medical community.[17] In 1930, U.S. physicians Crutchfield and Stout treated 57 patients with staphylococcal skin infections, achieving an over 90% success rate.[18] Additionally, American bacteriologist Thurman Rice applied phages to 300 patients suffering from suppurative illnesses, successfully curing nearly all of them.[19] In 1932, American physicians Ward McNeal and Frances Frisbee used phage preparations to treat 15 patients with staphylococcal septicemia, successfully curing seven of them, which accounts for a 47% success rate.[20] Pseudomonas aeruginosa has been the focus of another clinical study conducted by University College London in partnership with Biocontrol Limited in the United Kingdom.[21] Twelve patients

with chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa* infections were treated with a single dose of a cocktail containing six phages (BC-BP, 1 to 6) at a combined concentration of 10^5 PFU, and their outcomes were compared to a placebo group (n = 12). All patients in the treatment group demonstrated significant improvements in clinical scores and reductions in *P. aeruginosa* load, similar to the patients in the placebo group. Additionally, a case report noted the absence of adverse side effects when using phage OMKO1 in treating prosthetic vascular graft infections caused by *P. aeruginosa.*[22]

Potential Advantage of Bacteriophage (BP) Use to Treat Bacterial Infections:

Bacteriophages have a very narrow spectrum of activity, which addresses a key issue associated with antibiotic use: the impact on the entire microbiome. Unlike antibiotics that can eliminate beneficial bacteria, potentially leading to secondary pathogen overgrowth and the emergence of resistant strains, phages specifically target harmful bacteria without disrupting the overall microbial community [23] Use of BPs without modification of the microbiota has been reported by several studies in both animals and humans. In mice, oral administration of four T4-like BPs effective against diarrhea-associated E. coli did not lead to any collateral damage of non-pathogenic bacteria of the same species.[24] In humans, the specificity of bacteriophage (BP) action was demonstrated in a study conducted by Sarker et al. In this study, an oral cocktail of nine T4-like E. coli BPs was administered to 15 healthy adults over a period of two days. After a 5-day washout period, while the BPs could be detected in the feces of nearly all treated subjects, no significant changes in gut microbiota composition were observed.[25] Compared to antibiotics, bacteriophages (BPs) are believed to offer several advantages. They are considered significantly safer and better tolerated since they replicate exclusively within their target bacteria and do not infect mammalian cells. This conclusion is supported by historical experiences from Eastern Europe as well as recent studies conducted in experimental animals and humans, which have not reported significant adverse events following BP administration.[26] Additionally, the administration of bacteriophages (BPs) is more straightforward, as they do not require repeated doses over several days, unlike antibiotics. BPs can persist in the human body for relatively long periods—up to several days (Bogovazova et al., 1991, 1992). Generally, only a few doses are needed due to the increase in BP concentration at the infection site following initial administration. Unlike antibiotics, the effect of BPs is localized to the site of infection, even when bacteria are located in areas that are difficult for antimicrobials to penetrate. For instance, the lytic phage EC200(PP), which targets the fatal neonatal meningitis *E. coli* strain S242, was evaluated in meningitis models with a 100% fatality rate. Despite low titers of the BP being detected in the central nervous system, treatment administered 1 and 7 hours post-infection successfully rescued all treated pups.[27]

Conclusion:

In conclusion, the growing issue of antibiotic resistance poses a serious challenge to global health, making it essential to explore new treatment options. Bacteriophage therapy has emerged as an exciting alternative, offering a precise way to tackle infections caused by antibiotic-resistant bacteria. This review underscores the unique ways that bacteriophages work, their ability to specifically target harmful pathogens, and the wealth of historical and modern evidence supporting their effectiveness and safety. Recent developments in phage therapy, such as personalized treatments and phage cocktails, have shown promising results in clinical settings, especially against tough-to-treat strains. One of the standout benefits of bacteriophages is their capacity to leave beneficial bacteria in the microbiome intact while focusing on the pathogens, addressing a significant drawback of traditional antibiotics. Though there are still hurdles to overcome in terms of regulatory approval and standardization, ongoing research and clinical trials are moving us closer to making phage therapy a routine option in healthcare. As the urgency surrounding antibiotic resistance continues to grow, bacteriophage therapy offers a hopeful path forward in the fight against infectious diseases, deserving our attention and investment.

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