Ricos Biology Journal, June, 2025, Vol. 3 (6) 21-40.
www.ricosbiology.net/Vol.3(6)/June-2025/64

Bacteriophage Therapy: A ResurgentAlternative in the Era of Antibiotic Resistance

***Abouelhag H. A.**

[*]Department of Microbiology and Immunology, National Research Centre, Dokki, Giza, Egypt, 12622.
***Corresponding author:** Prof. Abouelhag H. A. (Email: drabouelhag5@gmail.com)

Received: 25-05-2025, **Accepted:** 20-06-2025, **Published online:** 23-06-2025
DOI: https://doi.org/10.33687/ricosbiol.03.06.64

Abstract

Phage therapy, the use of bacteriophages to combat bacterial infections, is experiencing a significant resurgence driven by the escalating crisis of antibiotic resistance. This review provides a comprehensive overview of the evolution of phage therapy, from its early 20th-century origins and subsequent decline to its current status as a promising alternative or adjunct to conventional antibiotics. We examine the fundamental mechanisms of phage action, highlighting their specificity for bacterial targets and their lytic capabilities against even multidrug-resistant strains, while often sparing the host microbiota. Current applications are explored across various domains, including the treatment of chronic and resistant infections in humans, personalized medicine approaches, veterinary uses, and food safety applications. Key innovations, fueled by advances in genomics and synthetic biology, such as phage engineering, cocktail formulations, phage-derived enzymes (e.g., endolysins), and novel delivery systems, arediscussed as crucial enhancers of therapeutic potential. Despite its promise, phage therapy faces significant challenges, including complex regulatory pathways, manufacturing and standardization hurdles, the potential for bacterial resistance to phages, and host immune responses. Addressing these limitations through rigorous clinical trials, standardized protocols, and continued research is essential. This review underscores the critical need to integrate phage therapy into modern medical paradigms as a vital tool in the global fight against antibiotic-resistant infections, outlining future directions for research and clinical implementation.

Keywords: antibiotic resistance, bacteriophages, phage therapy, clinical applications, genetic engineering, innovations, multidrug-resistant bacteria.

Introduction

The escalating crisis of antibiotic resistance (AMR) represents one of the most significant global health threats of the 21st century. Decades of widespread, and often inappropriate, use of antibiotics in human medicine, veterinary practice, and agriculture have driven the selection and proliferation of bacteria resistant to multiple drugs, rendering previously effective treatments obsolete (World Health Organization, n.d.). Common infections are becoming increasingly difficult, and sometimes impossible, to treat, leading to prolonged illness, increased mortality rates, and substantial economic burdens on healthcare systems worldwide. The World Health Organization (WHO) has repeatedly warned that without urgent, coordinated action, the world is heading towards a post-antibiotic era where common infections and minor injuries could once again prove fatal. This alarming trajectory underscores the critical need for innovative strategies and alternative therapeutic agents to combat bacterial infections, particularly those caused by multidrug-resistant (MDR) pathogens.

Amidst this challenge, there is a renewed and rapidly growing interest in a therapeutic approach that predates the antibiotic era: bacteriophage therapy. Bacteriophages, often simply called phages, are viruses that naturally infect and kill bacteria. Discovered independently by Frederick Twort in 1915 and Félix d'Hérelle in 1917 **(Summers, 1999)**, these bacterial predators are the most abundant biological entities on Earth, playing crucial roles in shaping microbial ecosystems. Phages possess remarkable specificity, typically targeting only particular strains or species of bacteria. This high degree of specificity is a key advantage of phage therapy; unlike broad-spectrum antibiotics which can disrupt the host's beneficial microbiota (leading to dysbiosis and secondary infections like *Clostridioides difficile*), phages can selectively eliminate pathogenic bacteria while leaving the commensal flora largely undisturbed **(Górski *et al.*, 2016; Sulakvelidze *et al.*, 2001)**. Furthermore, phages can replicate exponentially at the site of infection as long as susceptible host bacteria are present, essentially acting as self- amplifying drugs, and they possess diverse mechanisms to overcome bacterial defenses.

The concept of using phages therapeutically was pioneered by d'Hérelle shortly after their discovery. He demonstrated their potential by successfully treating bacterial dysentery and later applied them against other infections like cholera and typhoid fever during the 1920s **(Summers, 1999)**. Phage therapy gained considerable traction, particularly in Eastern Europe and the former Soviet Union, where institutions like the Eliava Institute in Tbilisi, Georgia, became centers for phage research and application, continuing this practice even through the antibiotic age **(Górski *et al.*, 2016)**. However, inthe Western world, the advent of penicillin and subsequent broad-spectrum antibiotics in the 1940s overshadowed phage therapy **(Principi *et al.*, 2019)**. The perceived reliability, ease of use, and broad applicability of antibiotics, coupled with methodological shortcomings in some early phage therapy studies (lack of rigorous controls, poor characterization of phage preparations, limited accessibility of research published in non-English journals), led to its decline in most parts of the world **(Principi *et al.*, 2019; Summers, 1999)**.

Today, facing the stark reality of dwindling antibiotic efficacy, the scientific and medical communities are revisiting phage therapy with renewed vigor. Driven by the urgent need for alternatives to combat AMR, modern research is leveraging advances in genomics, molecular biology, and synthetic biology to overcome the historical limitations of phage therapy and unlock its full potential **(Hatfull *et al.*, 2022)**.

Contemporary studies are exploring the use of naturally occurring phages, precisely characterized phage cocktails,and genetically engineered phages to treat a wide range of challenging infections causedby MDR bacteria.

This review aims to provide a comprehensive overview of the evolution, current status, and future prospects of bacteriophage therapy as a viable alternative and adjunct to conventional antibiotic treatments. We will delve into the fundamental mechanisms of phage action, explore their diverse applications in clinical settings, veterinary medicine,and food safety, and discuss the cutting-edge innovations shaping the field.

Furthermore, we will critically examine the challenges and limitations that must be addressed – including regulatory hurdles, manufacturing complexities, potential for phage resistance, and host immune responses – to facilitate the successful integration of phage therapy into mainstream medical practice. By synthesizing the historical context, recent advancements, and ongoing research, this review seeks to highlight the significant potential of phage therapy to contribute to the global fight against antibiotic resistance and transform the management of bacterial infections.

Mechanisms of Action

The therapeutic efficacy of bacteriophage therapy hinges on the intricate biological mechanisms governing phage-bacteria interactions and the subsequent response within the host environment. Understanding these mechanisms is fundamental to optimizing phage selection, administration strategies, and predicting treatment outcomes **(Sulakvelidze *et al.*, 2001)**. Phages employ sophisticated strategies to infect, replicate within, and ultimately destroy their specific bacterial targets.

Central to phage activity are their distinct life cycles, primarily the lytic and lysogenic cycles. The lytic cycle represents the aggressive, bacteria-killing phase most relevant for direct therapeutic action. In this cycle, a lytic phage first adsorbs to a susceptible bacterium by recognizing and binding to specific receptors on the bacterial cell surface (e.g., lipopolysaccharides, outer membrane proteins, pili, flagella). This binding event triggers the injection of the phage's genetic material (DNA or RNA) into the bacterial cytoplasm. Once inside, the phage genome hijacks the host cell's machinery, redirecting it towards the replication of phage DNA/RNA and the synthesis of phage structural components (capsid proteins, tail fibers, etc.). Crucially, lytic phages often produce enzymes like endolysins and holins late in the cycle. Holins create pores in the bacterial cytoplasmic membrane, allowing endolysins access to the peptidoglycan layer of the cell wall, which they degrade. This enzymatic breakdown weakens the cell wall, leading to osmotic lysis – the rupture of the bacterial cell – releasing hundreds of newly assembled progeny phages **(Cahill and Young, 2019; Young, 1992)**. These newly released virions can then infect surrounding susceptible bacteria, amplifying the antibacterial effect locally. This rapid replication and bacterial killing make lytic phages the preferred candidates for treating acute infections (see Figure 1).

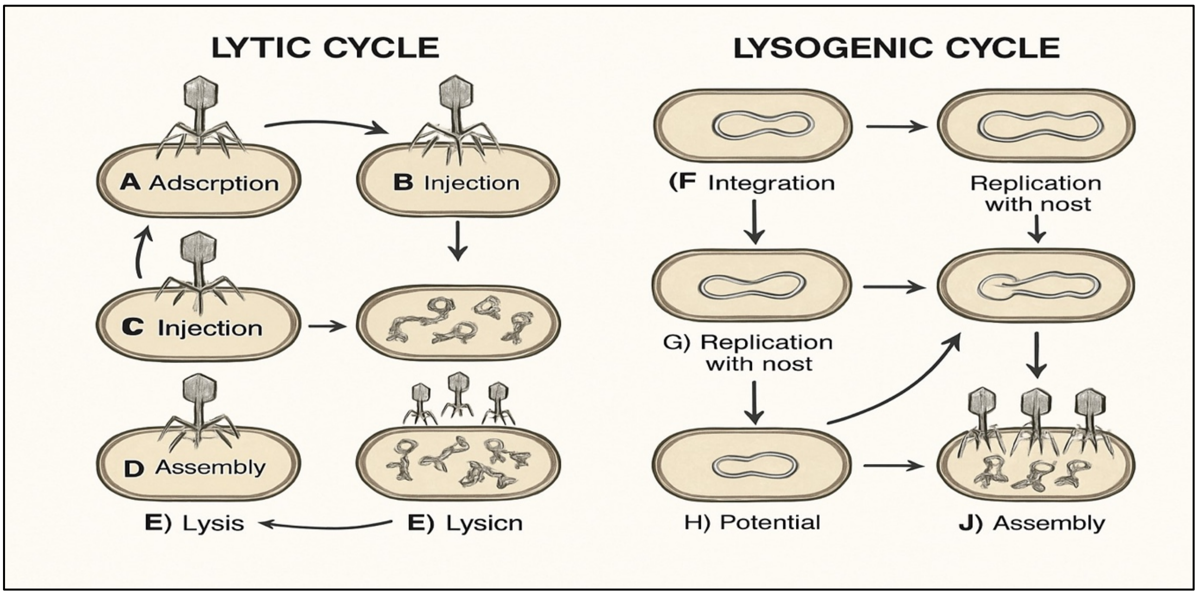

Figure 1: Comparison of lytic and lysogenic bacteriophage life cycles.

In contrast, the **lysogenic cycle** involves the integration of the phage genome (now termed a prophage) into the host bacterium's chromosome, or its maintenance as an extrachromosomal plasmid. The prophage replicates passively along with the bacterial DNA during cell division, without immediately harming the host. Bacteria carrying a prophage are termed lysogens. This state can persist for many generations, potentially conferring new properties to the bacterium, such as resistance to infection by similar phages (superinfection immunity) or even the production of virulence factors (lysogenic conversion, e.g., diphtheria toxin, cholera toxin). Under certain environmental triggers (e.g., UV radiation, chemical stress, nutrient depletion), the prophage can excise itself from the bacterial chromosome and enter the lytic cycle, leading to phage replication and lysis **(Howard-Varona *et al.*, 2017)**. While lysogenic phages are generally avoided for direct therapy due to the potential for lysogenic conversion and the lack of immediate bacterial killing, their study provides insights into phage-bacteria co-evolution, and engineered temperate phages might hold future therapeutic potential.

The remarkable host specificity of phages is a defining characteristic and a major therapeutic advantage. This specificity is primarily determined by the initial adsorption step, requiring a precise molecular match between phage attachment structures (e.g., tail fibers) and specific bacterial surface receptors **(Labrie *et al.*, 2010)**. This lock-and-key mechanism ensures that a given phage typically infects only a narrow range of bacterial strains or species, leaving non-target bacteria, including beneficial members of the host microbiota, unharmed. This contrasts sharply with the collateral damage often caused by broad-spectrum antibiotics (see Figure 2).

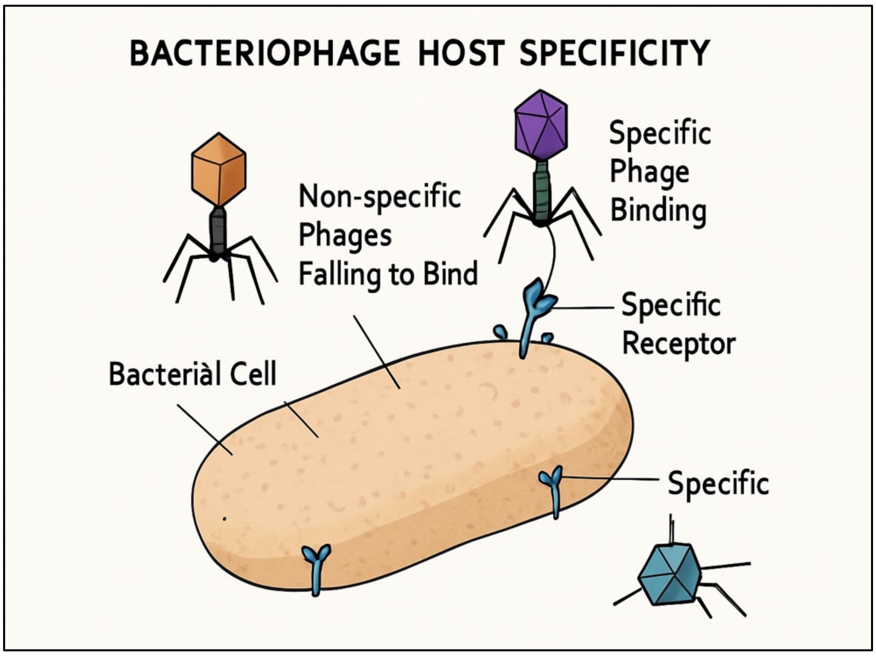

Figure 2: Bacteriophage host specificity. Only phages with matching receptors can bind and infect the bacterial cell.

However, the interaction is not solely dictated by the phage. Bacteria have evolved numerous resistance mechanisms to evade phage predation. These include modifying or masking surface receptors to prevent phage adsorption, producing extracellular matrices that block phage access, deploying restriction-modification systems that degrade foreign DNA upon injection, and utilizing CRISPR-Cas adaptive immune systemsto recognize and cleave phage genetic material based on previous encounters (Labrie et al., 2010). The dynamic co-evolutionary arms race between phages and bacteria means that bacterial resistance to specific phages can emerge, necessitating strategies like using phage cocktails (mixtures of different phages targeting the same bacterium via different receptors or mechanisms) to mitigate this risk **(Chan *et al*., 2013)** (see Figure 6).

Finally, the interaction between phages and the host immune system adds another layer of complexity. When introduced therapeutically, phages can be recognized as foreign entities, potentially triggering innate and adaptive immune responses. The production of neutralizing antibodies against phages can lead to their rapid clearance from circulation, potentially limiting the efficacy of systemic phage therapy, especially upon repeated administration **(Hodyra-Stefaniak *et al*., 2015)**. The extent of this immuneresponse depends on factors like the phage type, dosage, route of administration, and the host's immune status. While often viewed as a hurdle, the immune response is not always detrimental; in some cases, phage-induced bacterial lysis can release bacterial antigens and pathogen-associated molecular patterns (PAMPs) that stimulate a beneficial host immune response against the infection. Furthermore, some phages have evolved mechanisms to persist despite host immune responses, such as encapsulation or adaptation within the host environment, enhancing their ability to evade immune detection and prolong their activity (Hodyra-Stefaniak et al., 2015). Understanding and potentially modulating these phage-immune interactions is crucial for developing effective and safe phage therapy protocols.

Current Clinical Applications

Phage therapy is increasingly being recognized and explored as a viable clinical strategy,particularly for tackling challenging bacterial infections that are refractory to

conventional antibiotic treatments. Its applications span various medical fields, demonstrating significant potential, although widespread adoption is still hindered by regulatory and logistical challenges. The primary driver for its clinical resurgence is the urgent need to address infections caused by multidrug-resistant (MDR) bacteria, where treatment options are severely limited. One of the most notable uses of modern phage therapy is in managing chronic and persistent infections, especially those involving biofilms, which are notoriously difficultfor antibiotics to penetrate and eradicate. Phage therapy has shown promise in treating chronic wounds, osteomyelitis (bone infections), prosthetic joint infections, and chronic respiratory infections in patients with conditions like cystic fibrosis **(Fabijan *et al.*, 2020)** (see Figure 3).

For instance, successful case reports For instance, successful case reports detail the use of phage therapy, often in combination with antibiotics, to resolve long-standing infections caused by MDR pathogens like *Pseudomonas aeruginosa* and *Staphylococcus aureus*. A case involving a Siamese cat with a surgical wound infected by multidrug-resistant *P. aeruginosa* demonstrated complete healing after 14 weeks following treatment with a combination of a specific phage and antibiotics, underscoring the potential in both human and veterinary medicine **(Fabijan *et al.*, 2020)**.

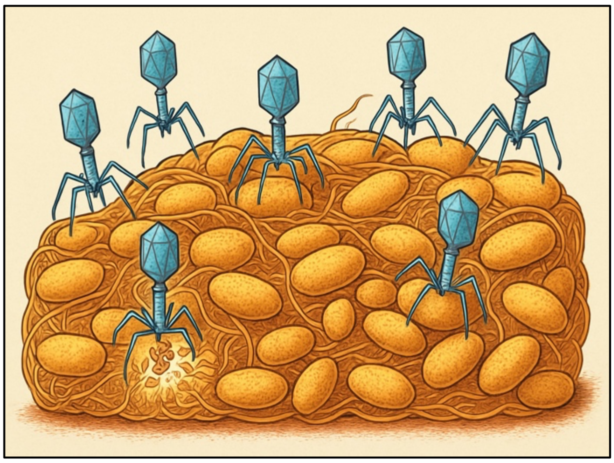

Figure 3: Bacteriophages penetrating a bacterial biofilm and lysing embedded bacteria.

Phage therapy is particularly suited for personalized medicine approaches. Given the high specificity of phages, treatment often involves identifying the specific bacterial strain causing the infection and then selecting or isolating phages that are effective against that particular strain. This tailored approach enhances therapeutic effectiveness and minimizes disruption to the patient's beneficial microbiota **(Pirnay *et al.*, 2011)**.

Several centers, particularly in countries with a longer history of phage use like Georgia and Poland, as well as emerging programs in the US, Belgium, and Australia, offer compassionate use or experimental phage therapy. These programs often involve creating customized phage preparations (sometimes cocktails of multiple phages) for patients with life-threatening or debilitating infections unresponsive to standard care. While often conducted outside large-scale randomized controlled trials (RCTs), these compassionate use cases provide valuable real-world evidence and case reports documenting both successes and challenges.

Clinical trials investigating phage therapy are gradually increasing in number and rigor,although they still lag behind those for conventional drugs. Early trials and ongoing studies are evaluating the safety and efficacy of phage preparations for various conditions, including urinary tract infections, diabetic foot ulcers, burn wound infections, and respiratory infections. For example, standardized phage cocktails targeting *E. coli*, *P. aeruginosa*, and *S. aureus* have been tested. While some trials have shown promising results regarding safety and bacterial load reduction, demonstrating definitive clinical superiority over standard care in large RCTs remains a key objective and challenge **(Jault *et al.*, 2019)**. Regulatory pathways, such as the FDA's compassionate use programs, allow access for some patients, but broader approval requires more extensive clinical validation.

Different routes of administration are employed depending on the site and type of infection. Topical application is common for wound infections and skin conditions. Oral administration is used for gastrointestinal infections or potentially for systemic effects, although phage stability in the gut environment can be a concern. Intravenous administration allows for systemic delivery to treat bloodstream infections or deep-seated infections like osteomyelitis. Aerosolized phage delivery systems are being explored for treating respiratory infections, allowing for localized treatment while minimizing systemic exposure **(Malik *et al.*, 2017)**. The optimal route and dosing regimenoften need to be determined on a case-by-case basis.

The specific bacterial targets most commonly addressed in recent clinical applications reflect the major AMR threats, including ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). Tailored phage formulations and cocktails are being developed and tested against these critical pathogens **(Jault *et al.*, 2019; Rhoads *et al.*, 2009)**. While the clinical application of phage therapy is still evolving, the accumulating evidence from case studies, compassionate use programs, and initial clinical trialsprovides a strong rationale for its continued development as a crucial tool againstantibiotic-resistant infections.

Applications Beyond Human Medicine

The potential of bacteriophage therapy extends significantly beyond human clinical applications, offering promising solutions in veterinary medicine, food safety, and agriculture, primarily driven by the need to reduce antibiotic use and combat resistance in these sectors as well.

In veterinary medicine, phage therapy is increasingly considered a viable alternative or adjunct to antibiotics for treating infections in both livestock and companion animals **(Jończyk-Matysiak *et al.*, 2021)**. Antibiotic resistance is a growing concern in animal health, impacting treatment efficacy and potentially contributing to the pool of resistant bacteria that can affect humans. Phage therapy has shown effectiveness in managing conditions such as mastitis in cattle, salmonellosis in poultry, and respiratory diseases inpigs. Studies indicate that phage treatments can significantly reduce bacterial loads and prevent disease, enhancing animal health while decreasing reliance on antibiotics **(Jończyk-Matysiak *et al.*, 2021)**. Personalized approaches, similar to those in human medicine, are also applicable. For instance, specific phages have been applied topically to successfully treat antibiotic-resistant skin infections in dogs, demonstrating how customization can improve outcomes and reduce collateral damage to beneficial bacteria **(Pirnay *et al.*, 2011)**. The use of phages in veterinary settings aligns with the 'One Health' approach, recognizing the interconnectedness of human, animal, and environmental health in tackling AMR.

Food safety represents another major area where phages hold considerable promise. Phages can be used to specifically target and eliminate pathogenic bacteria that contaminate food products, thereby improving safety and potentially extending shelf life(Endersen et al., 2014). Phage preparations have been approved by regulatory agencies like the FDA and USDA (and in the EU) for use as food processing aids, particularly against pathogens like *Listeria monocytogenes* on ready-to-eat meat and poultry products (e.g., Bacteriophage P100) **(Goodridge and Abedon, 2003)**. Research has demonstrated that phage treatments can effectively reduce the presence of pathogens such as *Salmonella* and *E. coli* in various food items, including fresh produce, meats, and dairy products. By incorporating phage treatments into food production and processing protocols (e.g., spraying onto carcasses or adding to packaging), producers can mitigate the risks associated with bacterial contamination, offering a natural and targeted biocontrol method **(Endersen *et al.*, 2014)** (see Figure 4).

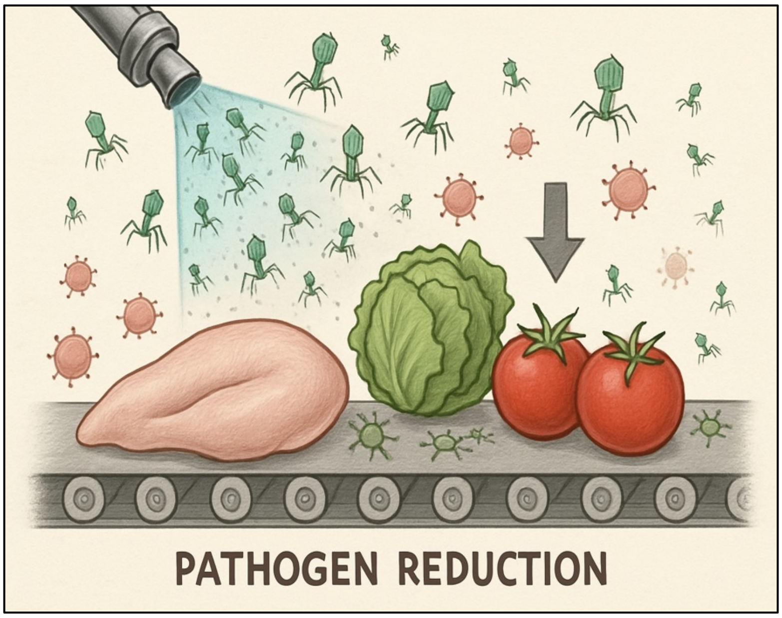

Figure 4: Application of bacteriophages in food safety to reduce pathogens on food products.

Challenges in this area Challenges in this area include ensuring phage survival and activity in complex food matrices and varying environmental conditions **(Goodridge andAbedon, 2003)**.

In agriculture, phages are being explored as biocontrol agents to combat bacterial diseases in plants. Phytopathogenic bacteria cause significant crop losses worldwide, and resistance to traditional bactericides is emerging. Phages that specifically target plant pathogens, such as *Xanthomonas* species (causing blights and spots) or *Pseudomonas syringae*, offer an environmentally friendly alternative to chemical treatments **(Jones *et al.*, 2007)**. Field trials are investigating the efficacy of phage applications in controlling diseases in various crops. Key challenges include ensuring phage stability and persistence in the agricultural environment (phyllosphere, rhizosphere) under fluctuating conditions like UV radiation and desiccation **(Jones *et al.*,2007)**.

These applications highlight the versatility of bacteriophages as targeted antibacterial agents across diverse sectors, contributing to a broader strategy for reducing antibioticdependency and managing bacterial threats in interconnected ecosystems.

Innovations and Advances in Phage Therapy

Innovations and advancements in phage therapy are significantly enhancing its efficacyand expanding its potential applications, largely driven by progress in genomics, molecular biology, and synthetic biology. Researchers are moving beyond simply isolating naturally occurring phages to actively engineering and optimizing them for improved therapeutic performance.

One of the most promising areas is phage engineering. Scientists are manipulating phage genomes to enhance desirable traits, such as improving their stability, broadening their host range (to target more bacterial strains), increasing their potency, or boosting their resistance to bacterial defense mechanisms **(Pires *et al.*, 2016)**. Techniques like CRISPR-Cas9 gene editing are being utilized to modify phages, for example, to create phages that can effectively combat antibiotic-resistant bacteria by directly targeting and disrupting bacterial DNA or essential genes **(Yosef *et al.*, 2015)** (see Figure 5).

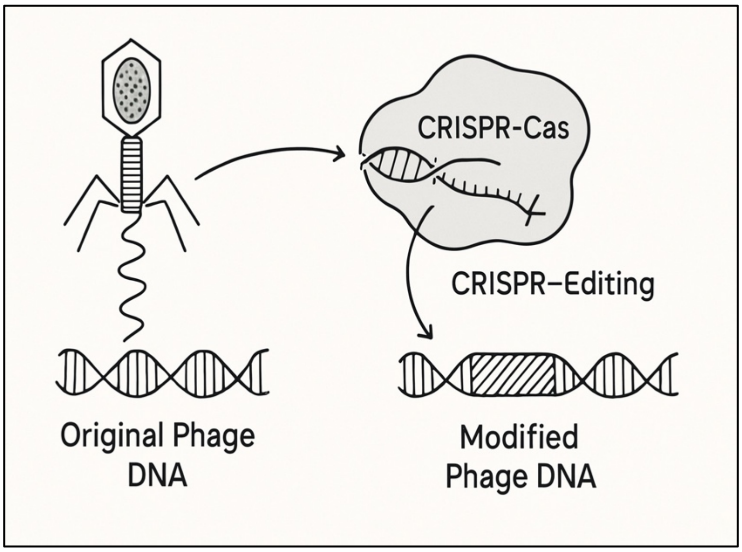

Figure 5: Conceptual diagram of phage engineering using CRISPR-Cas technology to modify phage DNA.

These engineered phages These engineered phages can be designed to overcome specific bacterial resistance mechanisms or to express antibacterial proteins themselves.

Phage display technology has revolutionized the development of extensive libraries ofgenetically engineered phages that can be screened for their ability to bind to specific bacterial targets **(Smith, 1985)**. This capability allows researchers to develop broad-spectrum phage therapies by identifying and selecting phages targeting multiple bacterial strains or species. Libraries with vast diversity (e.g., up to 10^10 different variants) enable the rapid identification of effective phages for therapeutic use.

Techniques like biopanning, involving repeated cycles of selection and amplification, are crucial for enriching phage clones with high binding affinity to targeted pathogens, thus enhancing the therapeutic arsenal against bacterial infections **(Pande *et al.*, 2010)**.

Beyond modifying whole phages, researchers are harnessing phage-derived products, particularly lytic enzymes like endolysins and depolymerases. Endolysins are enzymes produced by phages late in the lytic cycle to degrade the bacterial peptidoglycan cell wall from within, causing lysis. When applied externally (as purified recombinant proteins), endolysins can rapidly kill Gram-positive bacteria and, with modifications, Gram-negative bacteria as well. They offer advantages like high specificity, low likelihood of resistance development (as they target essential conserved structures), and the ability to kill antibiotic-resistant strains **(Fischetti, 2005)**. Depolymerases are enzymes found on some phages that degrade the capsular polysaccharides or exopolysaccharides forming bacterial biofilms, helping phages penetrate these protective layers or disrupting the biofilm structure directly.

Phage cocktails, mixtures containing multiple distinct phages targeting the same bacterial species (often via different receptors or lytic mechanisms), are a key strategy to combat the emergence of phage-resistant bacterial mutants and broaden the effective host range of a therapeutic preparation **(Chan *et al.*, 2013)**. By presenting bacteria with multiple simultaneous threats, cocktails make it significantly harder for resistance to develop against all components concurrently.

Significant innovations are also occurring in phage delivery systems to overcome challenges related to stability, bioavailability, and targeted delivery. Phages can be sensitive to environmental conditions (e.g., pH in the stomach) and host immune clearance. Encapsulation techniques using polymers, liposomes, or hydrogels can protect phages from degradation, control their release kinetics, and facilitate delivery tospecific infection sites **(Malik *et al.*, 2017; Puapermpoonsiri *et al.*, 2009)** (see Figure 7).

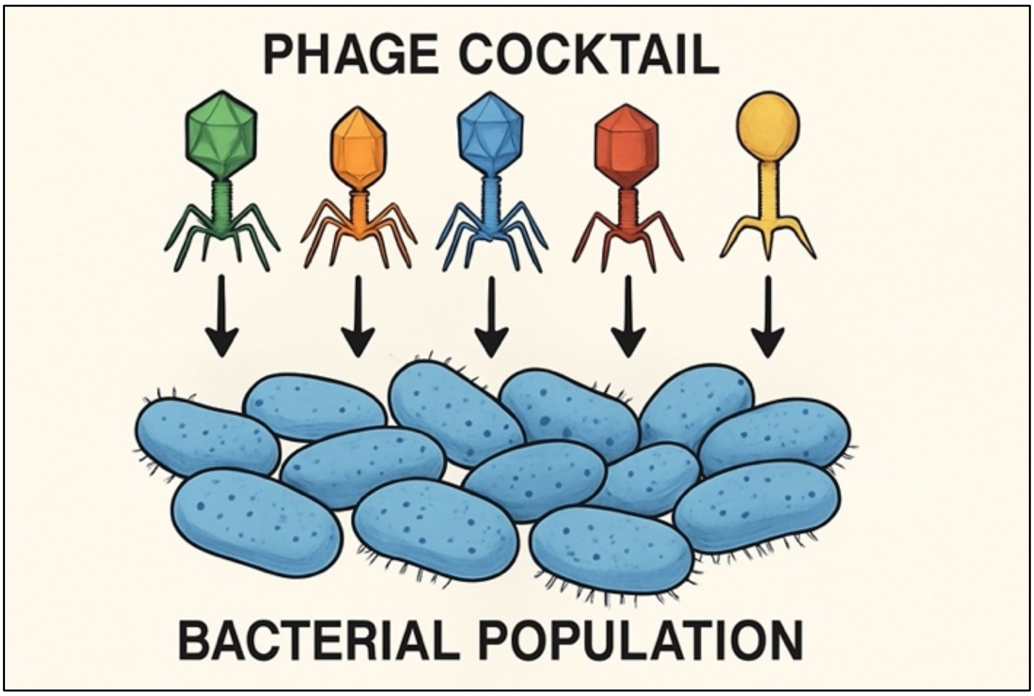

Figure 6: A phage cocktail, consisting of diverse phages, used to target a bacterial population.

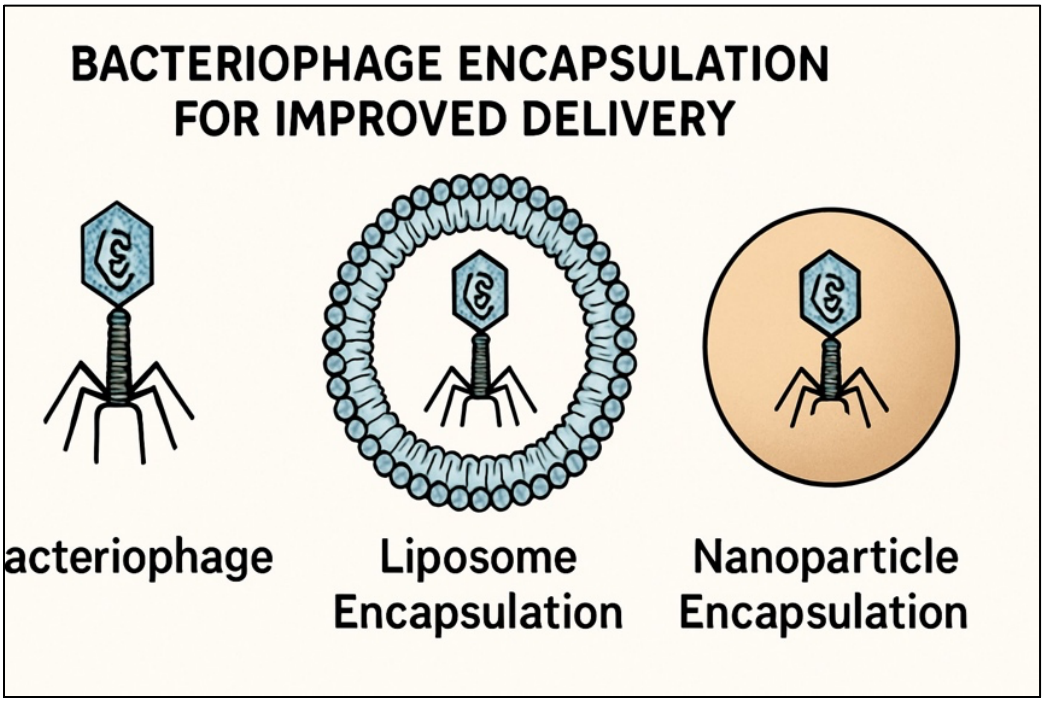

Figure 7: Encapsulation methods like liposomes and nanoparticles protect phages for improved delivery.

Nanoparticles are also being explored Nanoparticles are also being explored as carriers for targeted phage delivery and even for combined diagnostic/therapeutic (theranostic) purposes (Peng and Chen, 2021). Advancements in aerosolized phage delivery systems are being investigated for treating respiratory infections, allowing for localized treatment while minimizing systemic exposure **(Malik *et al.*, 2017)**.

Synergistic approaches, particularly combining phages with conventional antibiotics (phage-antibiotic synergy, PAS), are gaining considerable attention. Studies have shown that sub-lethal concentrations of certain antibiotics can enhance phage propagation or that phages can re-sensitize antibiotic-resistant bacteria to the drug. This combination can lead to more effective bacterial clearance, reduce the required doses of both agents, and potentially slow the development of resistance to both phages and antibiotics **(Comeau *et al.*, 2007; Tagliaferri *et al.*, 2021)**. This synergistic effect is being explored in various clinical settings, potentially leading to improved patient outcomes, especially for difficult-to-treat infections **(Chaudhry *et al.*, 2017)**.

Other emerging innovations include the development of phage-based vaccines, using phages as platforms for antigen display or delivery (Clark and March, 2004), and the exploration of oncolytic phages, engineered to specifically target cancer cells or tumor- associated bacteria **(Yacoby *et al.*, 2007)**. While still in early stages, these areas highlight the expanding versatility of phage-based technologies.

These innovations collectively aim to overcome the limitations of natural phages and traditional antibiotics, paving the way for more effective, targeted, and sustainable antibacterial strategies.

7. Challenges and Limitations

Despite the considerable promise and renewed interest, the widespread clinical implementation of phage therapy faces a complex array of challenges spanning regulatory, developmental, biological, and logistical domains. Addressing these obstacles is crucial for successfully integrating phage therapy into mainstream medical practice **(Verbeken *et al.*, 2014)**.

One of the most significant hurdles is regulatory. Unlike chemically synthesized small- molecule antibiotics, phages are biological entities capable of replication and evolution. This unique nature does not fit neatly into existing regulatory frameworks designed for conventional pharmaceuticals. Regulatory agencies like the FDA and EMA require rigorous approval processes, typically involving standardized manufacturing, preclinical safety data, and large-scale randomized controlled trials (RCTs) to demonstrate safety and efficacy **(Verbeken *et al.*, 2014)**. Proving efficacy for phages can be challenging given their high specificity (requiring precise matching to the infecting bacteria) and the potential need for personalized or adaptable phage cocktails. Early clinical studies often lacked adequate controls and employed crude preparations, complicating the interpretation of historical data and necessitating modern, high-quality trials **(Merabishvili *et al.*, 2009)**. Establishing standardized protocols for phage isolation, characterization, manufacturing (ensuring purity, potency, and freedom from contaminants like bacterial toxins or antibiotic resistance genes), and storage remains a critical need **(Pirnay *et al.*, 2011)**. The absence of universally accepted quality controls and manufacturing standards (like Good Manufacturing Practice - GMP for phages) creates significant barriers to large-scale production and clinical use.

Manufacturing and quality control present specific technical challenges. Producing well-characterized, high-titer phage preparations free from bacterial debris, endotoxins, and potentially harmful phage-encoded genes (e.g., toxins, antibiotic resistance genes) requires sophisticated purification and quality assessment methods. Ensuring the stability and maintaining the viability of phage preparations during storage and transport is also essential but can be difficult, as phages can be sensitive to physical and chemical conditions **(Pirnay *et al.*, 2011)**. Scaling up production to meet potential clinicaldemand while maintaining strict quality standards is another major logistical and economic challenge.

Biological challenges primarily revolve around bacterial resistance to phages and host immunogenicity. Just as bacteria evolve resistance to antibiotics, they can also develop resistance to phages through various mechanisms (e.g., receptor modification, CRISPR-Cas systems) **(Labrie *et al.*, 2010)**. While the use of phage cocktails can mitigate this, the potential for resistance necessitates ongoing surveillance and the continuous discovery or engineering of new phages. The immunogenicity of phages is another concern. The host immune system can recognize phages as foreign and mount an immune response,primarily through antibody production, which can lead to rapid phage clearance and reduced therapeutic efficacy, particularly upon repeated administration (Hodyra-Stefaniak et al., 2015; Łusiak-Szelachowska et al., 2014). While this response is not always detrimental and can sometimes be leveraged, minimizing adverse immune reactions through careful phage selection, purification, dosing strategies, and potentially phage engineering is important for safety and effectiveness **(Łusiak- Szelachowska *et al.*, 2014)**.

Furthermore, the narrow host range of most phages, while advantageous for specificity, can also be a limitation. It requires accurate and rapid diagnosis of the causative bacterial agent and susceptibility testing to select effective phages. This contrasts with the empirical use often possible with broad-spectrum antibiotics. Developing rapid diagnostic tools and extensive, well-characterized phage libraries is essential to overcome this practical challenge.

Finally, issues related to pharmacokinetics and pharmacodynamics (PK/PD) – how phages distribute within the body, reach the infection site at sufficient concentrations, and interact with bacteria over time – are still not fully understood and require further investigation to optimize dosing regimens and administration routes (Malik et al., 2017). Ethical considerations surrounding the use of self-replicating biological agents also need careful consideration and public discourse.

Overcoming these multifaceted challenges will require concerted efforts from researchers, clinicians, regulatory bodies, and industry stakeholders to develop standardized protocols, conduct rigorous clinical trials, and establish clear pathways for the safe and effective use of phage therapy.

8. Future Directions and Perspectives

The trajectory of phage therapy research and development points towards an increasingly important role in combating bacterial infections, particularly in the face ofescalating antibiotic resistance. However, realizing this potential requires addressing the current challenges and capitalizing on recent scientific and technological advancements. Several key future directions are emerging.

First and foremost is the critical need for large-scale, rigorously designed randomized controlled trials (RCTs). While case studies and compassionate use provide valuable anecdotal evidence, robust RCTs are essential to definitively establish the safety and efficacy of phage therapy for specific indications compared to standard-of-care treatments. These trials need to address complexities such as appropriate control groups, standardized phage preparations, defined clinical endpoints, and strategies for handling phage specificity and potential resistance development. Generating high- quality clinical evidence is paramount for gaining regulatory approval and acceptance by the broader medical community.

Developing standardized protocols and regulatory pathways is another crucial area. Collaboration between researchers, industry, and regulatory agencies (like FDA, EMA) is needed to establish clear guidelines for phage manufacturing (GMP standards), quality control, characterization, preclinical testing, and clinical trial design specifically tailored to the unique nature of phages. Harmonizing regulations internationally would also facilitate broader development and access.

Continued advancements in phage engineering and synthetic biology hold immense promise. Future research will likely focus on creating phages with enhanced properties: broader host ranges, reduced immunogenicity, improved stability and delivery characteristics, enhanced biofilm penetration capabilities, and mechanisms to actively combat bacterial resistance. Engineering phages to deliver specific payloads (e.g., enzymes, toxins targeting bacteria) or to work synergistically with the host immune system are also exciting avenues. Synthetic biology approaches may enable the de novo design and construction of phages with precisely defined characteristics.

Further exploration of phage-microbiome interactions is warranted. Understanding how therapeutic phages interact with the complex microbial communities in the human body (e.g., gut, respiratory tract) is important for predicting efficacy and potential off-target effects. Leveraging phages to selectively modulate the microbiome for therapeutic benefit is an emerging field.

Optimizing phage discovery and selection processes is also key. Developing high-throughput methods for isolating and characterizing phages against clinically relevant pathogens, including MDR strains, is essential. Building extensive, well-curated phage libraries, potentially linked to rapid diagnostic tools that identify the causative agent andits phage susceptibility profile, will be vital for implementing personalized or readily available phage therapy.

Improving delivery systems to ensure phages reach the site of infection at adequate concentrations and remain active remains a priority. Research into advanced formulations, encapsulation methods, and targeted delivery strategies will continue.

Finally, educating clinicians, policymakers, and the public about the potential and limitations of phage therapy is necessary to foster acceptance and facilitate its integration into clinical practice. Phage therapy is unlikely to completely replace antibiotics but rather will serve as a valuable alternative or adjunct, particularly for difficult-to-treat infections. Its successful integration will likely involve its use in combination therapies (e.g., phage-antibiotic synergy) and within specific clinical nicheswhere antibiotics fail.

The path forward requires sustained investment in research, interdisciplinary collaboration, and a flexible yet rigorous approach to regulation to translate the promise of phage therapy into tangible clinical benefits in the fight against AMR.

Conclusions

The era of antibiotics, while revolutionary, is facing an unprecedented challenge due to the global rise of antimicrobial resistance. As conventional therapies lose their effectiveness against increasingly resilient pathogens, bacteriophage therapy is re-emerging from its historical roots as a highly promising and scientifically validated alternative and adjunct strategy. Its inherent specificity allows for the targeted elimination of pathogenic bacteria while preserving the host's beneficial microbiota, a distinct advantage over broad-spectrum antibiotics. Furthermore, the ability of phages to self-replicate at the site of infection and their potential to overcome existing resistance mechanisms offer unique therapeutic benefits.

Significant progress has been made in understanding phage biology, developing methods for phage characterization and production, and exploring diverse applications ranging from treating MDR infections in humans and animals to ensuring food safety.

Innovations in phage engineering, cocktail formulation, delivery systems, and synergistic combinations with antibiotics are continually enhancing the potential and applicability of this therapeutic modality. Modern research, leveraging genomics and synthetic biology, is actively addressing the historical limitations and paving the way for more potent, reliable, and safer phage-based treatments.

Despite this progress, substantial challenges remain. Regulatory frameworks require adaptation, manufacturing processes need standardization and scaling, and issues like bacterial resistance to phages and host immunogenicity must be effectively managed through ongoing research and strategic development. Rigorous, large-scale clinical trials are essential to provide definitive evidence of efficacy and safety, facilitating regulatory approval and broader clinical acceptance.

In conclusion, bacteriophage therapy represents a critical component in the multifaceted approach required to combat the AMR crisis. While not a panacea, its unique mechanisms and adaptability offer a powerful tool, particularly for infections where conventional antibiotics have failed. Continued investment in research, interdisciplinary collaboration, robust clinical validation, and the development of supportive regulatory pathways are imperative to fully realize the potential of phagesand successfully integrate them into 21st-century medicine as a vital weapon againstbacterial infections.

References

Cahill, J., and Young, R. (2019). Phage lysis: Multiple genes for multiple barriers. *Advancesin Virus Research*, *103*, 33–70. https://doi.org/10.1016/bs.aivir.2018.09.003

Chan, B. K., Abedon, S. T., and Loc-Carrillo, C. (2013). Phage cocktails and the future of phage therapy. *Expert Review of Anti-infective Therapy*, *11*(8), 755–760. https://doi.org/ 10.1586/14787210.2013.819725

Chaudhry, W. N., Concepción-Acevedo, J., Park, T., Andleeb, S., Bull, J. J., and Levin, B. R. (2017). Synergy and order effects of antibiotics and phages in killing Pseudomonas aeruginosa biofilms. *PLoS One*, *12*(1), e0168615. https://doi.org/10.1371/journal.pone. 0168615

Clark, J. R., and March, J. B. (2004). Bacteriophage-mediated nucleic acid immunisation. *FEMS Immunology and Medical Microbiology*, *40*(1), 21–26. https://doi.org/10.1016/ S0928-8244(03)00301-6

Comeau, A. M., Tétart, F., Trojet, S. N., Prère, M. F., and Krisch, H. M. (2007). Phage-antibiotic synergy (PAS): β-lactam and quinolone antibiotics stimulate virulent phage growth. *PLoS One*, *2*(8), e799. https://doi.org/10.1371/journal.pone.0000799

Endersen, L., O'Mahony, J., Hill, C., Ross, R. P., McAuliffe, O., and Coffey, A. (2014). Phage therapy in the food industry. *Annual Review of Food Science and Technology*, *5*, 327–349. https://doi.org/10.1146/annurev-food-030713-092415

Fabijan, A. P., Lin, R. C. Y., Ho, J., Maddocks, S., Khatami, A., Lam, M., ... and Iredell, J. R. (2020). Safety of bacteriophage therapy in severe Staphylococcus aureus infection. *Nature Microbiology*, *5*(3), 465–472. https://doi.org/10.1038/s41564-019-0634-z

Fischetti, V. A. (2005). Bacteriophage lytic enzymes: Novel anti-infectives. *Trends in Microbiology*, *13*(10), 491–496. https://doi.org/10.1016/j.tim.2005.08.007

Fruciano, D. E., and Bourne, S. (2020). Phage therapy for animal health: Focus on aquaculture. *Viruses*, *13*(1), 1. https://doi.org/10.3390/v13010001

Goodridge, L. D., and Abedon, S. T. (2003). Bacteriophage therapy for food safety: Challenges and opportunities. *Current Opinion in Biotechnology*, *14*(3), 294–301. https:// doi.org/10.1016/s0958-1669(03)00062-9

Górski, A., Międzybrodzki, R., Węgrzyn, G., Jończyk-Matysiak, E., Borysowski, J., and Weber-Dąbrowska, B. (2016). Phage therapy: Combating infections with potential for evolving from merely a last resort to systematic application. *Cellular and Molecular LifeSciences*, *73*(23), 4473–4491. https://doi.org/10.1007/s00018-016-2286-7

Hatfull, G. F., Dedrick, R. M., and Schooley, R. T. (2022). Phage therapy for antibiotic-resistant bacterial infections. *Annual Review of Medicine*, *73*, 197–211. https://doi.org/ 10.1146/annurev-med-012221-083637

Hodyra-Stefaniak, K., Miernikiewicz, P., Drapała, J., Drab, M., Jonczyk-Matysiak, E., Lecion, D., ... and Górski, A. (2015). Mammalian host-versus-phage immune response determines phage fate in vivo. *Scientific Reports*, *5*, 11174. https://doi.org/10.1038/ srep11174

Howard-Varona, C., Hargreaves, K. R., Abedon, S. T., and Sullivan, M. B. (2017). Lysogeny innature: Mechanisms, impact and ecology of temperate phages. *The ISME Journal*, *11*(7),1511–1520. https://doi.org/10.1038/ismej.2017.16

Jault, P., Leclerc, T., Jennes, S., Pirnay, J. P., Que, Y. A., Resch, G., ... and PhagoBurn study group. (2019). Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas aeruginosa (PhagoBurn): A randomised, controlled,double-blind phase 1/2 trial. *The Lancet Infectious Diseases*, *19*(1), 35–45. https:// doi.org/10.1016/S1473-3099(18)30482-1

Jones, J. B., Jackson, L. E., Balogh, B., Obradovic, A., Iriarte, F. B., and Momol, M. T. (2007).Bacteriophages for plant disease control. *Annual Review of Phytopathology*, *45*, 245– 262. https://doi.org/10.1146/annurev.phyto.45.062806.094444

Jończyk-Matysiak, E., Weber-Dąbrowska, B., Owczarek, B., Międzybrodzki, R., Łusiak-Szelachowska, M., and Górski, A. (2021). Phage therapy in veterinary medicine: A historical and modern perspective. *Antibiotics (Basel)*, *10*(6), 680. https://doi.org/10.3390/ antibiotics10060680

Labrie, S. J., Samson, J. E., and Moineau, S. (2010). Bacteriophage resistance mechanisms.

Nature Reviews Microbiology, *8*(5), 317–327. https://doi.org/10.1038/nrmicro2315

Lin, D. M., Koskella, B., and Lin, H. C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World Journal of Gastrointestinal Pharmacology and Therapeutics*, *8*(3), 162–173. https://doi.org/10.4292/wjgpt.v8.i3.162

Loc-Carrillo, C., and Abedon, S. T. (2011). Pros and cons of phage therapy. *Bacteriophage*, *1*(2), 111–114. https://doi.org/10.4161/bact.1.2.14590

Malik, D. J., Sokolov, I. J., Vinner, G. K., Mancuso, F., Cinquerrui, S., Clokie, M. R. J., ... and Garton, N. J. (2017). Formulation, stabilisation and encapsulation of bacteriophage for phage therapy. *Advances in Colloid and Interface Science*, *249*, 100–133. https://doi.org/ 10.1016/j.cis.2017.05.014

Merabishvili, M., Pirnay, J. P., Verbeken, G., Chanishvili, N., Tediashvili, M., Lashkhi, N., ... and De Vos, D. (2009). Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. *PLoS One*, *4*(3), e4944. https:// doi.org/10.1371/journal.pone.0004944

Miedzybrodzki, R., Borysowski, J., Weber-Dabrowska, B., Fortuna, W., Letkiewicz, S., Szufnarowski, K., Pawełczyk, Z., Rogóż, P., Kłak, M., Wojtasik, E., and Górski, A. (2012). Clinical aspects of phage therapy. *Advances in Virus Research*, *83*, 73–121. https:// doi.org/10.1016/B978-0-12-394438-2.00003-7

Nilsson, A. S. (2014). Phage therapy--constraints and possibilities. *Upsala Journal of Medical Sciences*, *119*(2), 192–198. https://doi.org/10.3109/03009734.2014.902878

Pande, J., Szewczyk, M. M., and Grover, A. K. (2010). Phage display: Concept, innovations, applications and future. *Biotechnology Advances*, *28*(6), 849–858. https://doi.org/10.1016/j.biotechadv.2010.07.004

Pelfrene, E., Willebrand, E., Cavaleiro Sanches, A., Sebris, Z., and Cavaleri, M. (2016). Bacteriophage therapy: a regulatory perspective. *Journal of Antimicrobial Chemotherapy*, *71*(8), 2071–2074. https://doi.org/10.1093/jac/dkw157

Peng, H., and Chen, I. A. (2021). Phage-based diagnostic and therapeutic applications. *International Journal of Molecular Sciences*, *22*(10), 5145. https://doi.org/10.3390/ijms22105145

Pires, D. P., Cleto, S., Sillankorva, S., Azeredo, J., and Lu, T. K. (2016). Genetically engineered phages: A review of advances over the last decade. *Microbiology and Molecular BiologyReviews*, *80*(3), 523–543. https://doi.org/10.1128/MMBR.00069-15

Pirnay, J. P., De Vos, D., Verbeken, G., Merabishvili, M., Chanishvili, N., Vaneechoutte, M., ... and Lavigne, R. (2011). The phage therapy paradigm: Prêt-à-porter or sur-mesure? *Pharmaceutical Research*, *28*(4), 934–937. https://doi.org/10.1007/s11095-010-0313-5

Principi, N., Silvestri, E., and Esposito, S. (2019). Phage therapy: An additional weapon in the armamentarium against multidrug-resistant bacterial infections? *Frontiers in Pharmacology*, *10*, 194. https://doi.org/10.3389/fphar.2019.00194

Puapermpoonsiri, M., Spencer, J., and van der Walle, C. F. (2009). A freeze-dried formulation of bacteriophage K: An alternative approach to treating Staphylococcus aureus infections. *European Journal of Pharmaceutics and Biopharmaceutics*, *71*(2),222–229. https://doi.org/10.1016/j.ejpb.2008.09.011

Rhoads, D. D., Wolcott, R. D., Kuskowski, M. A., Wolcott, B. M., Ward, L. S., and Sulakvelidze, A.(2009). Bacteriophage therapy of venous leg ulcers in humans: Results of a phase I safety trial. *Journal of Wound Care*, *18*(6), 237–243. https://doi.org/10.12968/jowc. 2009.18.6.42801

Schooley, R. T., Biswas, B., Gill, J. J., Hernandez-Morales, A., Lancaster, J., Lessor, L., Barr, J. J., Reed, S. L., Rohwer, F., Benler, S., Segall, A. M., Taplitz, R., Smith, D. M., Kerr, K.,Kumaraswamy, M., Nizet, V., Lin, L., McCauley, M. D., Strathdee, S. A., Aslam, S., and Hamilton, T. (2017). Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection. *Antimicrobial Agents and Chemotherapy*, *61*(10), e00954-17. https://doi.org/10.1128/AAC.00954-17

Segall, A. M., Roach, D. R., and Strathdee, S. A. (2019). New frontiers: phage therapy againstbacterial pathogens of the opioid epidemic. *Drug and Alcohol Dependence*, *198*, 1–5. https://doi.org/10.1016/j.drugalcdep.2019.02.016

Smith, G. P. (1985). Filamentous fusion phage: Novel expression vectors that display cloned antigens on the virion surface. *Science*, *228*(4705), 1315–1317. https://doi.org/ 10.1126/science.4001944

Sulakvelidze, A., Alavidze, Z., and Morris, J. G., Jr. (2001). Bacteriophage therapy. *Antimicrobial Agents and Chemotherapy*, *45*(3), 649–659. https://doi.org/10.1128/AAC.45.3.649-659.2001

Summers, W. C. (1999). *Félix d'Herelle and the origins of molecular biology*. Yale University Press.

Tagliaferri, T. L., Compagnone, M., Sahi, J. S., Riello, G., Von Arx, C., and Ruga, S. (2021). Phage-antibiotic combination therapy: State of the art and future perspectives. *Critical Reviews in Microbiology*, *47*(6), 719–736. https://doi.org/10.1080/1040841X.2021.1969869

Verbeken, G., Pirnay, J. P., De Vos, D., Jennes, S., Zizi, M., Lavigne, R., ... and Huys, I. (2014). Optimizing bacteriophage therapy: In vivo studies matter. *Expert Review of Anti-infectiveTherapy*, *12*(7), 753–757. https://doi.org/10.1586/14787210.2014.915687

World Health Organization. (n.d.). *Antibiotic resistance*. Retrieved June 2, 2025, from https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

Yacoby, I., Bar, H., and Benhar, I. (2007). Targeted cancer therapy by employing engineered bacteriophages. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(45), 17950–17955. https://doi.org/10.1073/pnas.0704112104

Yosef, I., Manor, M., Kiro, R., and Qimron, U. (2015). Temperate and lytic bacteriophages programmed to sensitize bacteria to antibiotics. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(14), 4266–4271. https://doi.org/10.1073/ pnas.1500107112

Young, R. (1992). Bacteriophage lysis: Mechanism and regulation. *Microbiological Reviews*, *56*(3), 430–481. https://doi.org/10.1128/mr.56.3.430-481.1992

Łusiak-Szelachowska, M., Żaczek, M., Weber-Dąbrowska, B., Międzybrodzki, R., Kłak, M., Fortuna, W., ... and Górski, A. (2014). Phage neutralization by sera of patients receiving phage therapy. *Viral Immunology*, *27*(6), 295–304. https://doi.org/10.1089/vim.2013.0128