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Review

Bacteriophages Therapy: Exploring Their Promising Role in Microbiome Modulation and Combatting Antibiotic Resistance

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Abstract

Bacteriophages, or phages, are viruses that infect bacteria, exhibiting specificity towards particular bacterial strains. Despite being overshadowed by traditional antibiotics in the West, interest in phage therapy has resurged due to the escalating antimicrobial resistance crisis. Understanding phage biology, selection, production, and pharmacology is crucial for their clinical application. Phages interact intricately with the human microbiome, influencing bacterial populations and potentially offering therapeutic avenues for microbiome-related diseases. Moreover, phages present a promising alternative to combat antibiotic resistance, leveraging their specificity and evolutionary adaptability. Regulatory approval and standardization challenges persist, yet ongoing research underscores the potential of phage therapy in clinical medicine.

Keywords
Bacteriophages; phage therapy; antimicrobial resistance; microbiome; microbiota

1. Introduction

Bacteriophages (or phages), literally "eaters of bacteria," are viruses that infect bacteria. Phages were initially described just over a century ago when it was noted that agents from environmental sources that could pass through small-pore filters could destroy specific subsets of bacteria [1]. Subsequently, phages were shown to be viruses capable of infecting a particular range of bacteria. The host range of infectible bacteria may be narrow for an individual phage. Still, with an estimated 10^{31} distinct phages in the planet's biosphere, every bacterium has a vast collection of phages to which it is vulnerable [2].

From the perspective of antimicrobial therapy, phages are best conceptualized as living antibiotics [3]. They were promoted as antimicrobials globally until the 1930s when interest was lost in the West as optimism about the utility of traditional antibiotics grew. Phage therapy continued unabated throughout the 20th century in Russia and the former Soviet Republics, where antibiotics were less available because of cost. Over the past five years, however, it has become apparent that the traditional antibacterial pipeline is failing to address the antimicrobial drug resistance crisis adequately, and as some well-studied (albeit anecdotal) successful uses of phages in the treatment of serious bacterial infections have been described, interest in phage therapeutics has reemerged in the United States and Western Europe [4, 5].

2. Phage Biology

Rational use and clinical development of phages require a fundamental understanding of their biological properties [6]. Phages are small 50 to 200 nm structures abundant in the biosphere. Most environmental phages comprise a head that encapsulates a segment of double-stranded DNA encoding the phage's genetic program and a tail that provides binding specificity to the phage's bacterial prey. Phages cannot reproduce independently; they replicate within a bacterial host. Bacteriophages have lytic and temperate lifestyles (Figure 1). Lytic phage infection produces a single outcome: host lysis. Temperate phage infection produces a stable incorporation of the phage genome into the host's DNA, forming a prophage, with the lysogen reproduced as the host bacterium divides, possibly to be activated to a lytic phase by environmental factors [1-3, 5].

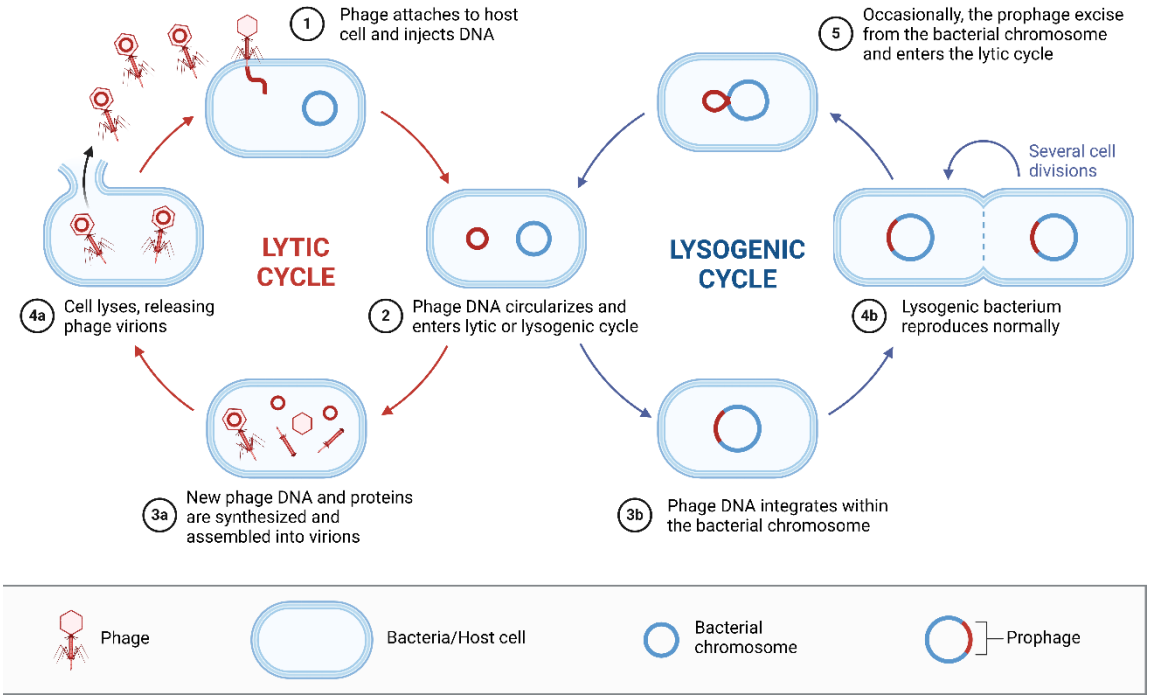

Figure 1 Lytic and isogenic life cycles of bacteriophage.

The role of bacteriophage genomes in phage-host interactions is a complex and intricate process that determines the outcome of infection [7]. Bacteriophages carry their genetic material in either DNA or RNA form, which contains all the necessary instructions for replication and assembly within their host bacteria. Through a series of molecular interactions, the phage genome hijacks the host's cellular machinery to produce more phages, ultimately leading to the lysis of the bacterial cell [8].

Understanding these genomic interactions is crucial for developing new strategies to combat bacterial infections and harnessing bacteriophages for biotechnological applications [9].

Unveiling the mechanisms of lytic enzymes and viral assembly machinery sheds light on the intricate processes involved in bacteriophage biology. Lytic enzymes break down bacterial cell walls, allowing the phage to enter and replicate within the host cell [10]. Understanding how these enzymes work can provide insights into developing new antibacterial therapies [11]. Similarly, studying the viral assembly machinery reveals the intricate steps in constructing new phage particles.

Unraveling these mechanisms enhances our knowledge of bacteriophages and opens up exciting possibilities for biotechnological applications.

Exploring the intricate relationship between bacteriophages and host cells reveals a fascinating interplay of molecular interactions that drive infection [9]. Bacteriophages have evolved unique strategies to hijack the cellular machinery of their hosts, allowing them to replicate and propagate. By studying this dynamic relationship, scientists gain insights into the molecular mechanisms that govern viral infections and host immune responses. Understanding how bacteriophages interact with host cells sheds light on fundamental biological processes and opens new avenues for developing novel therapeutic strategies against bacterial infections [6].

Lytic phage cycle: Lytic phages infect bacteria via attachment, penetration, and injection of their DNA into the bacterial host, leading to inactivation of the host's genome and hijacking the host

replicative machinery for replicating the phage genome and synthesizing and assembling new phage particles. The result is the lysis of the host cell and the release of a new crop of bacteriophage progeny. Temperate phage lysogenic cycle: Temperate phages attach and inject their DNA as described for lytic phages. However, rather than phage replication leading to lysis of the host cell, the temperate phage cycle involves the incorporation of the phage DNA into the bacterial DNA and subsequent cell division with daughter cells containing the expanded complement of DNA. Environmental factors can induce these prophage cells to enter a lytic cycle. This figure is created using BioRender.com.

2.1 Lytic Phages

Lytic phages are a bacteriophage that infects and ultimately destroys their bacterial host cells, disrupting the host's DNA [12]. These phages inject their genetic material into the host cell, taking over the cellular machinery to produce more phage particles [8]. This leads to the lysis, or bursting, of the host cell, releasing new phages to infect other bacteria. Since the timeline for this process is maybe 20 minutes, the expansion of a phage population and the collapse of the population's susceptible prey can occur on an explosive timeline. As this process proceeds, phage-resistant bacteria emerge that are, in turn, chased by evolving phage populations capable of growth in these emerging phage-resistant populations. Lytic phages are crucial in controlling bacterial populations in various environments and have been studied for their potential use in combating bacterial infections [13, 14].

2.2 Temperate Phages

Temperate phages, a type of bacteriophage, can follow either a lytic or lysogenic cycle, known as the "temperate" lifestyle, during replication within a bacterial host [15, 16].

In the temperate lifestyle, instead of immediately using the host's machinery for replication, temperate phages integrate their genetic material into the host's genome using integrases. They also produce repressor proteins that inhibit other phages from attacking the host [15]. This integrated DNA is replicated alongside the host DNA and passed to subsequent bacterial generations. Periodically, induced by stimuli, these integrated phages switch to a lytic cycle, synthesizing phage proteins and lysing the host cell [16].

However, temperate phages are not ideal for therapy because they do not immediately lyse their hosts and can carry host DNA, including genes for antibiotic resistance or increased pathogenicity [15]. Yet, recent molecular biology advances allow us to engineer temperate phages, removing their lysogenic properties or designing them synthetically for specific hosts and lytic abilities.

Temperate phages integrate their genome into the host's, remaining dormant until they enter the lytic cycle triggered by specific conditions [17]. Recombination events can alter phage proteins or groups of interdependent proteins. The host cell continues to function normally, replicating the prophage DNA alongside its own [17].

Under stress or environmental changes, the prophage can switch to the lytic cycle, destroying the host cell [16].

Temperate phages are intriguing because they can switch between two life cycles: lytic and lysogenic. Unlike virulent phages, which immediately replicate and lyse the host, temperate phages

can integrate into the host's genome, remaining as a prophage until conditions prompt them to become lytic. This dual cycle makes them significant in phage biology research [18, 19].

3. Phage Selection and Production

Bacteriophages present a promising avenue for tackling the increasing global problem of antibiotic resistance [20]. This part delves into the complex process of selecting and producing bacteriophages, focusing on fine-tuned methods for targeted antibacterial interventions. The efficiency and specificity of phages in combating bacterial pathogens hinge critically on the selection processes employed [21].

3.1 Selecting the Right Bacteriophages for Targeted Applications

The selection of appropriate bacteriophages is critical for the success of targeted antibacterial therapies. This process begins with identifying the specific bacterial host that needs to be targeted. The host range of a phage, which determines the spectrum of bacteria it can infect, is primarily defined by its receptor-binding proteins [22]. Researchers typically employ techniques such as host range determination to match phages with specific bacterial pathogens [20].

Following identifying potential phages, their genetic stability and the absence of temperate phages capable of integrating their genomes into the host bacteria, thus potentially transferring undesirable genes, are assessed [21]. This is essential to minimize the risk of transducing antibiotic resistance genes among bacterial populations.

Further, the efficacy of bacteriophages must be evaluated under conditions that closely resemble the intended application environment. For therapeutic purposes, this often involves testing in relevant biological fluids and assessing potential interactions with human cells [23]. Phages designed for food safety applications are tested in food matrices to determine their efficacy and stability under various storage and processing conditions [24].

Ultimately, the selection process is a rigorous and iterative cycle of testing and modification to optimize the phage’s specificity and effectiveness against the targeted bacterial pathogens while ensuring safety and compliance with regulatory standards.

3.2 Techniques for Optimizing Bacteriophage Production

Optimizing the production of bacteriophages involves enhancing both the yield and purity of the phages. Large-scale production is often achieved through liquid fermentation or solid-state cultivation, depending on the phage type and intended use [20]. Temperature, pH, and nutrient concentration are meticulously controlled in liquid fermentation to maximize phage replication. This method is advantageous for quickly producing large volumes of phages [21, 25].

On the other hand, solid-state cultivation is used for phages that show higher activity and stability in such conditions. It involves growing the host bacteria and phages on solid substrates such as agar, which can sometimes promote higher phage titers [23]. However, solid media extraction and purification processes are more challenging and require more steps than liquid fermentation.

Purification techniques such as ultracentrifugation, filtration, and chromatography are critical to removing bacterial debris, endotoxins, and other impurities, ensuring the phage preparation is safe for its intended application [20]. Each purification method has its trade-offs, and the choice of

technique usually depends on the balance between efficiency, cost, and the specific quality requirements of the phage preparation.

Researchers also focus on formulation techniques to enhance bacteriophage stability and shelf life, primarily when intended for therapeutic uses. These may include lyophilization, a process where the phages are freeze-dried into a powder form, and encapsulation, which protects phages from environmental factors that could degrade their effectiveness [21].

The optimal environments for the growth of phages and bacteria can be different; however, phages can control their life cycle in response to the host's physiology [26]. Thus, environments can impact phages in terms of both titers produced (phage replication) and titers retained (phage decay) [25]. By utilizing alternative environments, scientists can optimize the genetic modification process, opening up new possibilities for enhancing the capabilities and applications of bacteriophage engineering [27].

3.3 Harnessing Phage Biology for Effective Antibacterial Therapies

Bacteriophages offer a unique mechanism to combat bacterial infections through their ability to specifically target and lyse bacterial cells without harming human cells, thereby reducing the risk of dysbiosis often associated with broad-spectrum antibiotics [20]. This unique property makes phages an attractive option for developing personalized medicine strategies, particularly in cases where patients are infected with antibiotic-resistant bacteria [21].

One critical aspect of leveraging phage biology in antibacterial therapies is using phage cocktails. These cocktails combine multiple phages to broaden the spectrum of bacterial targets and prevent the development of phage-resistant bacterial strains. The formulation of these cocktails requires careful consideration of the interaction between different phages and their collective dynamics within the infection site [23].

Furthermore, advancements in genetic engineering have paved the way for the customization of bacteriophages. Techniques such as CRISPR-Cas9 have been employed to enhance phages' specificity or engineer them to carry therapeutic payloads, such as antibiotics or enzymes that degrade biofilms, thus amplifying their antibacterial effects [28].

The regulatory landscape for phage therapy, however, presents challenges that must be navigated carefully. Ensuring the safety and efficacy of phage-based treatments through clinical trials is essential for gaining regulatory approval [20]. Continuous monitoring and adaptation of regulatory guidelines are necessary to keep pace with the innovations in phage therapy.

When a patient is deemed a candidate for phage therapy, it is first necessary to identify and characterize phages to which the patient’s pathogen is susceptible [29]. This determination must be made empirically by pairing a patient’s specific bacterium with a library of phages previously identified as being active against bacteria of the same species and identifying those that specifically lyse the patient’s isolate. Suppose a laboratory has a collection of previously characterized phages that are active against the species of the bacterium with which the patient is infected. In that case, such a screening process can be conducted in 24 to 48 hours. Ideally, these phages have already been shown to have an exclusively lytic lifestyle and to be free of genetic material that encodes bacterial resistance or factors that may enhance bacterial pathogenesis. If active phages are not identified in prescreened phage libraries, environmental searches can be undertaken (typically involving sewage, soil, or other sources in which the target bacterium exists in nature). Such a

process may take weeks and is further complicated by the need to characterize any phages that emerge from the environmental search fully. When phages (at least one, preferably more) with the needed bactericidal activity are identified, they must be propagated in feeder bacteria to produce a sufficient number for a therapeutic course. Once created, the phages must be purified to remove endotoxin and other impurities that may cause toxicity and then suspended in a buffer in which they are stable [30-33].

4. Pharmacology

Phages may be administered by intravenous, oral, topical, or inhaled routes. Historically, most phage therapy was administered by topical or oral routes because the technology required to fully separate highly purified phage populations from the remnants of the bacteria in which they were propagated had not been developed. With the development of efficient techniques (e.g., CsCl gradient centrifugation, organic extraction, affinity column separation), highly purified populations of phages can be obtained and safely administered parenterally. When phages have been appropriately prepared and sufficiently depleted of endotoxin and other impurities, clinically apparent toxicity is rare [31, 34, 35].

Optimal frequency and dosing of phages is an area that remains under development [36, 37]. When administered intravenously, phages are cleared from circulation by the reticuloendothelial system within 30 to 90 minutes. Unlike antibiotics, however, once phages arrive at the site of infection, they can replicate in their bacterial hosts, depending on the bacterial population's multiplicity of infection and size and contiguity. Thus, rather than focusing on routes of clearance and systemic half-life, pharmacologic considerations center on the persistence and activity of these self-replicating antibiotics at the site of infection. From the practical perspective, well-purified phages should be administered at 10^9 to 10^{10} plaque-forming units (PFU) per dose every several hours. Most published parenteral regimens have included one or more phages administered at approximately 10^9 PFU per dose every 8 to 12 hours. Less has been done to delineate the proper dosing of topical or orally administered phage preparations. Because of the uncertain stability and distribution associated with topical administration and the added complexity of predictable absorption from the GI tract, parenteral administration is preferred for most patients with significant systemic infections. Since phages (like antibiotics) are selected for resistant bacterial populations, it is generally preferable to administer them as “cocktails” consisting of several phages with activity against the pathogen at hand that do not have overlapping resistance pathways. Principles regarding optimal valency are under development but will likely depend on the population size and the bacterial species under treatment [21, 38].

Comparing antibiotics and bacteriophages reveals distinct advantages of bacteriophages in combating bacterial infections. Antibiotics are broad-spectrum agents that can target multiple bacterial species simultaneously. Still, they have led to the rise of antibiotic-resistant bacteria, posing a significant challenge in current medical treatments [39]. On the other hand, Bacteriophages are viruses that specifically infect and kill bacteria, offering several advantages over traditional antibiotics.

One of the primary advantages of bacteriophages is their specificity. Unlike antibiotics, which can indiscriminately affect harmful and beneficial bacteria, bacteriophages target specific bacteria, reducing the impact on the body's microbiota and decreasing the likelihood of dysbiosis [40]. This

specificity also means that bacteriophages can be used to target antibiotic-resistant bacteria without affecting other microorganisms, potentially circumventing the issue of resistance development.

Another advantage is the self-amplifying nature of bacteriophages. Once a bacteriophage infects its bacterial host, it replicates within the bacterium, leading to the host's lysis and the release of new phage particles. This process allows bacteriophages to increase in number at the site of infection, enhancing their antibacterial effect without additional doses, unlike antibiotics, which require sustained administration to maintain adequate concentrations at the infection site [20].

Furthermore, the development of bacteriophage resistance by bacteria does not preclude the use of bacteriophages. Due to the co-evolutionary arms race between bacteriophages and bacteria, new bacteriophages can be isolated or engineered to overcome bacterial resistance mechanisms, offering a renewable resource in the fight against bacterial infections [41].

In summary, bacteriophages offer several advantages over antibiotics, including specificity to target bacteria, the ability to self-amplify at infection sites, and the potential to overcome bacterial resistance by isolating or engineering new phages. These characteristics position bacteriophages as a promising alternative to antibiotics, especially in the era of increasing antibiotic resistance.

5. Bacteriophages and the Microbiome

The human microbiome is a complex ecosystem comprising trillions of microorganisms, including bacteria, viruses, fungi, and archaea, residing within various human body niches, such as the skin, mouth, gut, and reproductive organs. These microorganisms are essential in maintaining human health by contributing to digestion, metabolism, immune function, and protection against pathogens. Various factors, including genetics, diet, lifestyle, and environmental exposures, influence the microbiome’s composition. Dysbiosis, or imbalance within the microbiome, has been implicated in the pathogenesis of numerous diseases, highlighting the importance of understanding and maintaining a healthy microbiome for overall well-being [42-47]. Ongoing research into the human microbiome continues to uncover its intricate connections to human health and disease, paving the way for innovative therapeutic interventions and personalized medicine approaches to modulate the microbiome to promote health and prevent illness.

Bacteriophages, the microscopic entities, have garnered significant attention in recent years due to their potential roles in shaping the microbiome, a community of microorganisms inhabiting various niches within the human body and other environments.

Within the microbiome, bacteriophages play a crucial role in regulating bacterial populations. They can influence the abundance and diversity of bacteria by infecting specific bacterial species, thereby affecting the balance of microbial communities. This intricate interplay between bacteriophages and bacteria contributes to the dynamic stability of the microbiome.

Research suggests that bacteriophages may have therapeutic potential in modulating the microbiome to promote health or combat disease. Phage therapy, the therapeutic use of bacteriophages to target pathogenic bacteria, is being explored as an alternative to traditional antibiotics. By selectively targeting harmful bacteria while leaving beneficial ones intact, phage therapy holds promise for treating bacterial infections with reduced ecological disruption to the microbiome [48-50].

Furthermore, bacteriophages are integral components of the human gut microbiome, where they regulate bacterial populations and contribute to maintaining gut homeostasis. Dysbiosis, or imbalance within the gut microbiome, has been linked to various health conditions, including inflammatory bowel diseases, metabolic disorders, and mental health disorders. Understanding the role of bacteriophages in modulating the gut microbiome could offer insights into novel therapeutic strategies for managing these conditions [51-53].

The study of bacteriophages and their interaction with the microbiome has gained significant attention in recent years due to their potential impact on human health. Bacteriophages influence the composition and function of the microbiome. Understanding the dynamics between bacteriophages and the microbiome is crucial for developing therapeutic strategies to modulate microbial communities [51-53]. While research in this area is still in its nascent stages, recent advancements in sequencing technologies have allowed for a more comprehensive analysis of bacteriophage-microbiome interactions.

Bacteriophages are crucial in shaping microbial communities and driving microbial evolution within the human microbiome. Understanding the genetic diversity and co-evolutionary dynamics of bacteriophages in the microbiome, as exemplified by these studies, is essential for elucidating their therapeutic potential in controlling bacterial infections, including acne, and sheds light on the intricate interactions shaping microbial communities and host-microbe relationships. This knowledge provides valuable insight for developing targeted phage-based therapies tailored to specific bacterial species in the microbiome [51-53].

In summary, bacteriophages are critical players in shaping the microbiome, influencing the composition and function of bacterial communities within diverse ecosystems, including the human body. Their intricate interactions with bacteria highlight their potential therapeutic applications in maintaining microbiome health and combating bacterial infections.

5.1 Impact of Bacteriophages on the Microbiome

The impact of bacteriophages on the microbiome is a pivotal aspect of microbial ecology, with significant implications for host health and ecosystem dynamics. Studies examining the response of gut bacterial and viral communities to dietary changes have revealed distinctive alterations in their composition and diversity, notably influenced by shifts from standard chow to high-fat diets [54]. These changes, particularly the shift towards more virulent viruses post-diet transition, indicate the intricate interplay between bacteriophages and bacterial populations within the microbiome. Furthermore, investigations into maternal influences on infant gut microbiome acquisition underscore the differential transmission rates of bacterial communities compared to viromes, with the former displaying a higher vertical transmission from mothers [55]. These findings emphasize the nuanced relationships between bacteriophages and bacteria in shaping the microbiome landscape and highlight the need to further explore their interplay in microbial ecosystems.

5.2 Applications of Bacteriophages in Microbiome Research

Bacteriophages have gained significant attention for their potential applications in microbiome research. The use of phages in modulating microbial communities within the microbiome shows promise in addressing dysbiosis, particularly in conditions such as acne, where microbial imbalances contribute to pathogenesis [56]. Studies have highlighted the ability of phages to target problematic

bacteria like Propionibacterium acnes in the skin microbiome, offering a targeted and potentially effective approach to rebalancing dysbiotic states. Additionally, advancements in phage therapy, including bioengineered phages and purified phage lytic proteins, open new avenues for precision microbiome interventions [56]. The evolving landscape of phage-based therapies suggests a potential alternative or supplementary strategy to conventional antibiotic treatments, emphasizing the need for further research to explore the intricate interactions between phages, bacteria, and the host microbiome. Integrating bacteriophages in microbiome research presents a novel and potentially transformative avenue for addressing dysbiosis and advancing therapeutic approaches in microbial communities.

In conclusion, the intricate relationship between bacteriophages and the microbiome is a complex and crucial area of study in microbiology. Bacteriophages play a significant role in shaping the composition and dynamics of the microbiome by selectively targeting and influencing the abundance of specific bacterial species. Through this intricate interplay, bacteriophages maintain microbial diversity, promote a balanced gut ecosystem, and potentially impact human health. Furthermore, discovering bacteriophages as potential therapeutic agents against pathogenic bacteria brings new possibilities for developing targeted treatments for infectious diseases. Understanding the interactions between bacteriophages and the microbiome is essential for advancing our knowledge of microbial ecology and developing novel strategies for combating microbial infections. Further research is needed to fully elucidate this relationship's complexities and harness the potential benefits for human health.

6. Bacteriophages and Antibiotic Resistance

The emergence and spread of antibiotic-resistant bacteria pose a significant threat to global public health. Traditional antibiotics indiscriminately kill both pathogenic and beneficial bacteria, leading to the selection and proliferation of antibiotic-resistant strains. In this context, bacteriophages offer a promising alternative or adjunct to traditional antibiotics in the fight against antibiotic resistance.

Bacteriophages have unique characteristics that make them attractive candidates for combating antibiotic-resistant bacteria. Firstly, they exhibit specificity towards their bacterial hosts, targeting particular strains or species while sparing others. This precision allows for the targeted eradication of pathogenic bacteria without disrupting the entire microbiome, thus minimizing collateral damage [5, 37].

Moreover, bacteriophages can evolve rapidly in response to bacterial resistance mechanisms. As bacteria develop resistance to phages, the phages can also grow to overcome these mechanisms, resulting in a dynamic arms race between the two entities. This evolutionary adaptability enhances the long-term efficacy of phage therapy against antibiotic-resistant bacteria [5, 57].

Phage therapy, the therapeutic application of bacteriophages to treat bacterial infections, has demonstrated promising results in clinical settings, notably where traditional antibiotics have failed. By harnessing bacteriophage specificity and evolutionary potential, researchers explore novel approaches to combat antibiotic resistance and develop personalized treatment strategies tailored to individual patients and their specific bacterial infections [5].

However, challenges remain in the widespread implementation of phage therapy, including regulatory hurdles, standardization of phage preparations, and the need for further clinical

validation. Despite these challenges, the potential of bacteriophages to address antibiotic resistance offers hope in the ongoing battle against drug-resistant infections, emphasizing the importance of continued research and development in this field.

7. Clinical Indications

Phages are not currently FDA-approved in the U.S. and must be administered as investigational agents in clinical trials or for individual patients under investigator-initiated Investigational New Drug applications. Indications for which phages may be considered include patients with severe or life-threatening bacterial or mycobacterial infections for whom antibiotic therapy has failed or is unlikely to be successful. This may consist of patients with multidrug-resistant bacterial infections. In addition, because of the capacity of phages to disrupt biofilms, patients with persistent infections on implanted devices for which removal and replacement are difficult or impossible are also candidates for therapy. Investigational efforts are underway to explore the ability of phages to influence the microbiome [43] and/or to reduce populations of specific bacteria prophylactically.

8. Ethical Considerations in Bacteriophage Therapy

Ethical considerations in implementing bacteriophage therapy, particularly in wound biofilm management and antibiotic-resistant infections, play a pivotal role in shaping the efficacy and accessibility of this innovative treatment modality. Using bacteriophages to combat biofilm formation in wounds raises ethical dilemmas surrounding patient safety, informed consent, treatment accessibility, and equitable distribution. The potential for compassionate phage therapy for antibiotic-resistant infections as a compassionate treatment option emphasizes the need for stringent ethical guidelines and central coordination to ensure this promising therapy's responsible and equitable use. As highlighted by previous research [58, 59], phage therapy's historical use and solid fundamental support accentuate its therapeutic potential, underscoring the importance of ethical frameworks to guide its integration into clinical practice. With the antibiotic resistance crisis exacerbating treatment failures, ethical considerations must be at the forefront when navigating the ethical landscape of bacteriophage therapy to safeguard patient well-being and promote effective healthcare practices within the broader microbiome ecosystem.

9. Future Prospects

The comprehensive scope of potential clinical uses for phage therapy is currently being actively explored. Determining their precise role in medical practice will necessitate thorough clinical and translational research, adhering to the established principles of evaluating traditional antibiotics. Despite the field's long history spanning over a century, the contemporary phase of phage therapy only commenced within the past decade. Consequently, our understanding of their role in clinical medicine, whether utilized independently or alongside conventional antibiotics, is still developing.

10. Current Limitations in Bacteriophage Research

As we continue exploring the world of bacteriophages, several limitations hinder its full potential. One of the most prominent limitations is our limited understanding of the complex interactions between bacteriophages and their host bacteria. In addition, there is still a lack of knowledge

regarding the mechanisms behind phage resistance development in bacteria. This makes it challenging to design effective bacteriophage treatments [60-62].

Another limitation is the inability to produce large quantities of specific bacteriophages for targeted therapies due to the difficulties in isolating and purifying them from environmental samples. The need for standardized protocols and techniques to optimize phage production remains a significant hurdle. Furthermore, the limited availability of comprehensive and efficient tools for bacteriophage research is a considerable obstacle. There is a dire need for improved genomics and proteomics technologies to enable in-depth analysis of phage-host interactions, phage replication, and other critical processes [60-62].

Recognizing these limitations, the scientific community is making significant strides to overcome them. Collective efforts are underway to develop innovative phage isolation, purification, and cultivation methods. For example, researchers are exploring next-generation sequencing technologies to identify and isolate new bacteriophage strains from diverse environments, such as soil, water, and even extreme environments like hot springs or the deep ocean. Additionally, they are working on developing novel methods for phage amplification and propagation [63].

such as efficient induction of prophages from bacterial populations and the use of lysogenic conversion. These advancements will ensure a more continuous and reliable supply of phages, ultimately improving their potential as therapeutics [63].

Another approach to address bacteriophage resistance is to explore phage cocktails, which consist of a mixture of different phages targeting multiple bacterial receptors. This strategy aims to reduce the likelihood of resistance developing, as bacteria must simultaneously evolve resistance to numerous phage strains. Researchers are also studying the possibility of combining phage therapy with other antimicrobial strategies, such as antibiotics or immune-boosting agents, to increase the overall efficacy and reduce the risk of resistance development. In these cases, phages can weaken the bacterial population and make it more susceptible to other treatments while minimizing the chances of the bacteria developing resistance [64].

In addition to these approaches, continuous research in bacteriophage biology and evolution is essential for understanding and overcoming potential challenges associated with phage resistance.

11. Conclusion

The resurgence of interest in bacteriophages heralds a new era in antimicrobial therapy and microbiome modulation. Phage therapy offers a targeted approach to combatting antibiotic-resistant bacteria while minimizing collateral damage to beneficial microbiota. As research progresses, elucidating the clinical indications and refining therapeutic strategies will be imperative. Although regulatory challenges remain, the potential of phages to address the antimicrobial resistance crisis and impact microbiome-related diseases underscores the importance of continued exploration and innovation in this field. Ultimately, integrating phage therapeutics into clinical practice holds promise for revolutionizing infectious disease management and microbiome modulation in the years to come.

Author Contributions

Ahmad R. Alsayed: Methodology, Validation, Investigation, Oririginal Writing-review and editing, Visualization. Andi Dian Permana: Writing-review and editing.

Competing Interests

The authors declare that no competing interests exist.

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