Journal of Immunology and Infectious Diseases
Volume 13 | Issue 1

Review Article Open Access

Bacteriophage Intervention for Multi-Drug Resistance in Pathogenic Bacteria: A Literature Review

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Citation: Khalid Parwez, Suman Veerappa Budihal, Asif Zawed (2026) Bacteriophage Intervention for Multi-Drug Resistance in Pathogenic Bacteria: A Literature Review. J Immunol Infect Dis 13(1): 101

Received Date: December 16, 2025 **Accepted Date:** December 31, 2025 **Published Date:** January 03, 2026

Abstract

The discovery and commercialization of antibiotics marked a new era in medicine, revolutionizing healthcare, improving quality of life, and increasing life expectancy for humankind. However, this revolution has led to the emergence of antibiotic resistance genes among bacterial populations, resulting in resistance to common antibiotics, including β-lactams, aminoglycosides, chloramphenicol, and tetracycline. This situation weakens the effectiveness of standard antibiotics in treating common infections.

Concerns about the possibility of returning to a pre-antibiotic era have prompted scientists and clinicians to search for new antibiotics or alternative treatments for antimicrobial resistance in bacteria. Phage therapy is being reintroduced as an alternative to antibiotic treatment for pathogenic microorganisms. Addressing this critical issue in modern medicine requires innovative approaches.

Researchers have proposed two primary strategies for phage therapy over the past few years. The first involves formulating engineered bacteriophages as sequence-specific antimicrobials that can effectively kill pathogens, including phage-resistant mutants. We refer to this method as Smart-Antimicrobials (SAM) in this report. The second strategy involves using species-specific bacteriophages in combination with antibiotics, known as Antibiotic-Phage Combination (PAC) therapy. This approach applies dual selection pressure to combat antimicrobial resistance (AMR) in pathogenic bacteria.

The article reviews both historical and contemporary uses of phage therapy, highlighting that phage cocktails can be utilized

in clinical and commercial contexts, potentially offering solutions for infections that antibiotics can no longer effectively treat. Despite the promise of phage therapy, challenges remain, particularly concerning regulatory frameworks in Western medicine. We propose a regulatory approach similar to that for vaccine development, allowing for periodic adjustments to formulations based on emerging infections. This strategy could facilitate the integration of phage therapy into modern medical practice and address the urgent need for new antibacterial treatments.

Keywords: CRISPR-Cas system, Smart Antimicrobials (SAM), Antimicrobial Resistance (AMR), Phage Therapy, Priority Pathogen, Phage-Antibiotic Combination (PAC) Therapy, Phage Cocktail.

1. Introduction

Since the late 1930s, the global consumption of antibiotics has reached approximately 200,000 tons annually [1,2]. This extensive production and application of antibiotics across various domains, including healthcare, agriculture, and horticulture, have contributed to a notable decline in bacterial susceptibility to these agents. This decline is primarily due to the emergence of antibiotic resistance genes within bacterial populations, resulting in resistance to commonly utilized antibiotics such as β-lactams, aminoglycosides, chloramphenicol, and tetracycline [3].

The increasing prevalence of Antibiotic Resistant Pathogenic Bacteria (ARPB) and Multi-Drug Resistant (MDR) organisms poses a significant threat to public health [4], raising concerns about a potential return to the "pre-antibiotic era," characterized by inadequate treatment options for bacterial infections. Both the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have recognized antimicrobial resistance (AMR) as a critical global health issue [5,6]. This concern was further emphasized during the United Nation General Assembly meeting on September 21, 2016, where AMR was described as "the greatest and most urgent global risk" [7].

In response to this pressing challenge, the Indian Priority Pathogen List (IPPL) was created through a collaborative effort involving the Department of Biotechnology and WHO India, officially released on March 9, 2021[8]. Additionally, the Government of India has classified AMR as an inter-ministerial priority, acknowledging the need for a coordinated national response to this pervasive health threat.

The rise of antibiotic resistance genes among prevalent bacterial strains, including Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species—collectively referred to as ESKAPE [9] pathogens—has significantly compromised the efficacy of essential antibiotics.

Furthermore, the emergence of escape mutants resistant to bacteriophages presents an additional challenge. Genetic analyses indicate that genomes of various pathogenic microorganisms frequently contain integrated prophage sequences [10] and the genomic structures of many bacteriophages exhibit extensive mosaicism [11].

To combat AMR in pathogenic bacteria effectively, the reverse-engineering of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated proteins (CRISPR-Cas) represents a promising approach. This methodology can facilitate the targeted modification of antibiotic resistance genes and their associated plasmids, thereby re-sensitizing bacteria to antibiotics and enhancing understanding of the modular evolution of bacterial populations and related bacteriophages [12].

This strategic initiative aims to address AMR in pathogenic microorganisms through selective targeting and sensitization in the following manners:

I. The utilization of programmed CRISPR-Cas-based nucleases to target antibiotic resistance genes located on plasmids will enable the selective elimination of pathogenic microorganisms while preserving the beneficial microbiota.

II. This approach is also intended to alleviate both hospital-acquired and community-acquired nosocomial infections, thereby contributing to the restoration of effective treatments for bacterial infections.

2. Therapeutic Bacteriophage

The bacteriophage, a virus that infects bacteria, was formally rediscovered and named by the French scientist Felix d'Herelle in 1917 [13] during his tenure at the Pasteur Institute in Paris. Presently, phage therapy is being reexamined as a viable alternative to antibiotics for the treatment of bacterial infections. This therapeutic approach involves employing bacteriophages to target and eliminate pathogenic bacteria directly at the infection site. It is noteworthy that this method of treatment predates the discovery of antibiotics in the late 1930s [14].

However, after World War II, the scientific community's interest in phage therapy diminished, as antibiotics emerged as the preferred treatment modality for bacterial infections. While antibiotics have remained in use to the present day, the rise of antibiotic-resistant pathogens and multidrug-resistant (MDR) bacteria, including Methicillin-resistant Staphylococcus aureus (MRSA), has raised significant concerns within both the scientific community and society at large.

Thus, there exists an urgent imperative to develop alternative strategies for the prevention and management of antibiotic-resistant pathogens, with the pursuit of phage therapy posited as a potentially vital component of our medical arsenal.

To facilitate a comprehensive understanding of this subject, Table 1 compares the prophylactic and therapeutic applications of phages versus antibiotics. Table 2 summarizes published findings on the effectiveness of phage therapeutics in human and animal models. Finally, Table 3 enumerates current market products related to phages and phage therapy, reflecting the renewed interest in this innovative area of medical research.

Table 1: Comparative analysis of antibiotics and bacteriophage as therapeutic agents

Antibiotics	Bacteriophage	References
Antibiotics have a non-specific action, which means they target both harmful pathogens and the normal flora in the patient's body. This can create an imbalance and potentially lead to secondary infections, such as yeast infections. Additionally, side effects like allergies and intestinal disorders are also associated with the use of antibiotics.	Bacteriophages specifically target pathogenic bacterial species, minimizing secondary infection risks by preserving normal flora. No side effects or secondary infections have been reported in phage therapy.	[15]
The antibiotics are quickly metabolized and eliminated by the host, resulting in a bioavailability of less than 10% at the infection site.	The bacteriophage replicates itself inside the bacteria at the site of infection, making it readily available for use.	[16]
The bacterium may develop resistance to antibiotics by acquiring resistant genes after prolonged usage.	Bacteria that are resistant to one type of phage may remain susceptible to another phage that targets a similar feature.	[15]
Developing new antibiotics to combat antibiotic-resistant bacteria will be costly and time-consuming.	Selecting phages against phage-resistant and multidrug-resistant bacteria is less time-consuming and cheaper. This can be accomplished within a week or less.	[17,18]

Table 2: Existing findings of Phage therapy

Disease conditions	Causative organism	Study model	Mode of delivery	Dose/ Administration of phage	Clinical findings	References
Dysentery	*Shigella dysenteriae*	Human	oral	$1x10^{7}$ cfu/ml	All patients recovered	[14]
Tuberculosis	*Mycobacterium tuberculosis*	Mouse	inhalation	$5x10^{7}$ cfu/ml	Significant decrease in lungs bacterial load	[56]
Sepsis	*Pseudomonas aeruginosa*	Murine	IP/IM (intraperitoneal /intra muscular injection)	$2x10^{6}$ cfu/ml	Significant reduction in bacterial loads in lungs, liver and spleen	[19,57]
Bacteremia	Vancomycin-resistant *Enterococcus faecium*	Mouse	Intraperitoneal injection	$2x10^{9}$ cfu/ml	Reduced mortality by 100%	[20]
Bacteremia	β -lactamase producing *Escherichia coli*	Murine	Intraperitoneal injection	$5x10^{9}$ cfu/ml	Reduced mortality by 100%	[21]
Bacteremia	Imipenem-resistant *P. aeruginosa*	Murine	Intraperitoneal injection	$2x10^{6}$ cfu/ml	Reduced mortality by 100%	[22]
Meningitis and Sepsis	*Escherichia coli*	Murine	Intraperitoneal injection	$5x10^{9}$ cfu/ml	Reduced mortality by 100% for meningitis50% for sepsis	[23]
Wound infection	*S. aureus*	Rabbit	Subcutaneous injection	$6x10^{8}$ cfu/ml	Infection prevented	[24]
Diabetic foot ulcer	MDR *S. aureus*	Human	Topical	$6x10^{8}$ cfu/ml	100% recovery of the patient	[25]
Gastroenteritis	*Vibrio parahaemolyticus*	Mouse	Intraperitoneal injection	$2x10^{7}$ cfu/ml	56% recovery	[58]
Typhoid	*Salmonella typhi*	Human	oral	$1.5x10^{8}$ cfu/ml	5 times decrease of typhoid cases compare to placebo (18577 children cohort)	[26]

The world of therapeutic bacteriophages is utterly captivating, offering a unique glimpse into the interplay between viruses and bacteria. Most therapeutic phages are classified under the order Caudoviridae, which is further divided into three notable families: Siphoviridae, Myoviridae, and Podoviridae [40].

Phages from the Siphoviridae family are characterized by their long, slender, non-contractile tails. These phages typically exhib-

it temperate behavior, which means they often enter a lysogenic cycle after infecting their bacterial hosts. This trait, while interesting, makes them less suitable for therapeutic applications, as they do not immediately kill the bacteria.

In contrast, members of the Myoviridae and Podoviridae families possess distinct structural characteristics that enhance their therapeutic potential. Myoviridae phages feature contractile tails that allow them to effectively penetrate bacterial cells, leading to a rapid lytic cycle—wherein they actively replicate and subsequently destroy their bacterial hosts. On the other hand, Podoviridae phages possess shorter non-contractile tails and also engage in a lytic cycle upon infection, further establishing their effectiveness in phage therapy.

Moreover, it is important to recognize that therapeutic bacteriophages can be categorized into seven distinct types. This classification is based not only on the methods of application and types of formulations but also on the regulatory frameworks they navigate in modern medicine. Each type plays a crucial role in the ongoing exploration and application of phage therapy as an innovative solution to combat bacterial infections.

i) Monophage

The monophage approach employs a single type of bacteriophage specifically tailored to target a distinct strain or species of bacteria. This method is particularly advantageous when a particular pathogen has been accurately identified, allowing for a precise match between the bacteriophage and the bacterial invader. The administration process is typically straightforward, minimizing the risk of adverse interactions that can arise from using multiple phages simultaneously. However, the narrow spectrum of monophage therapy may pose challenges, especially in situations where bacteria mutate or when a variety of strains contribute to an infection [41].

ii) Polyphage or Phage Cocktail

In contrast, the polyphage approach consists of a carefully selected combination of multiple phages, aimed at addressing a wider array of bacterial strains or species. This strategy is especially useful for treating infections characterized by diverse bacterial populations or when the exact strain remains uncertain. By incorporating a broader spectrum of phages, the likelihood of effectively combating the infection is significantly enhanced, while simultaneously decreasing the chances of bacterial resistance emerging. Nevertheless, the development of polyphage cocktails is inherently more complex, potentially resulting in higher costs of production and navigating stringent regulatory hurdles [42].

iii) Customized or Tailored Phage

The customized or tailored phage therapy focuses on individualizing treatment plans for patients by selecting specific phages from a phage bank, based on the unique bacterial strains isolated from their infections. This method is particularly advantageous in the realm of personalized medicine, especially for patients suffering from infections that resist conventional treatments. While this tailored approach can significantly boost treatment efficacy against the specific strain responsible for the infection, it demands advanced laboratory capabilities and extended timeframes to identify effective phages, as well as a well--maintained and diverse phage bank [43].

iv) Preformulated or Off-the-Shelf Phage Cocktails

Preformulated or off-the-shelf phage cocktails are standard formulations designed to address common types of infections rather than targeting specific bacterial strains. These cocktails are particularly suitable for routine infections where the variability of bacterial strains is already understood, creating a readily accessible treatment option. While they offer consistency and ease of availability, their effectiveness may be compromised against unique or resistant bacterial strains that are not included in

the formulation [44].

v) Modifiable Phage Cocktails

This category encompasses standardized phage cocktails that are periodically updated with new phage strains to ensure ongoing effectiveness against evolving bacterial populations. An exemplary practice is seen in countries like Georgia, where products such as "Pyophage" and "Intestiphage" are routinely modified to match the most prevalent or virulent bacterial strains. This approach provides a solid balance between the immediate accessibility of off-the-shelf formulations and the adaptability needed to respond to shifting patterns of bacterial resistance. However, the necessity for continuous monitoring and updates introduces complexities in maintaining regulatory compliance and production consistency [45].

vi) Genetically Modified or Engineered Phages

Genetically modified or engineered phages represent a cutting-edge strategy, involving the alteration of bacteriophages at a genetic level to enhance their efficacy. Such modifications can expand the host range of the phages, reduce the likelihood of bacterial resistance, or improve the stability of the phages in various environments. Although currently largely experimental, this innovative approach harbors the potential to target particularly resistant bacterial strains more effectively. Nevertheless, it faces substantial regulatory scrutiny and ethical dilemmas associated with the implications of genetic modification [46].

vii) Oral, Topical, and Systemic Phage

Phages can also be classified based on their route of administration: oral (for gastrointestinal infections), topical (for treating wounds or skin infections), or systemic (for addressing bloodstream infections). Each of these routes is specifically suited to different types of infections, and the chosen delivery method can significantly influence the stability and overall efficacy of the phages. Targeted delivery mechanisms are designed to aim phages directly at infection sites, thereby enhancing patient outcomes. It's important to note that phage stability can vary considerably depending on the administration route; for instance, oral phages need to endure the acidic environment of the stomach, whereas systemic applications must utilize highly purified phages to mitigate potential immune reactions [47].

In the evolving landscape of modern medicine, these various phage therapy classifications serve as the foundation for innovative and targeted therapeutic strategies. These therapies can be integrated with existing antibiotic regimens or stand alone as crucial tools in the fight against the escalating issue of antibiotic resistance. Regulatory frameworks are adapting to these diverse methodologies, striving to find a balance between therapeutic efficacies, safety considerations, and ensuring broad accessibility for patients.

Table 3: Products related to phage and phage therapy currently available in the market

No.	Applications	Product name	Company
1.	Treatment of Topical infection (*Staphylococcus aureus*)	a) PhagoBioDerm b) Phagoderm	a) Intralytix, Republic of Georgiab) Micro World, Russia
2.	Treatment of purulent infection(*Pseudomonas auroginosa*)	a) PYO Bacteriophage b) Septaphage	a) Eliava BioPreparationb)Biochimpharm, Republic of Georgia
3.	Treatment of diarrheal infections(*Salmonella typhi*)	a) Intesti Bacteriophage b) Dysentery Bacteriophage c) Travelphage (from); d) Intesti Bacteriophage e) Intestifag	a) Eliava BioPreparationb) Microgen, Russiac) Biochimpharm , Republic of Georgiad) Microgen, Russiae) NeoProbioCare, Ukraine

4.	Food safety	a) ListShield (targeting *Listeria monocytogenes*); b) EcoShield (targeting *Escherichia coli*); c) SalmoFresh (targeting highly pathogenic *Salmonella*-serotypes); and d) ShigaShield (targeting three major *Shigella species; S. flexneri, S. sonnei and S. dysenteriae*)	

A systematic review on the topical application of phage therapy revealed that phages can be used in various forms, such as sprays, droplets, or soaked bandages and gauze, with concentrations ranging from 10^6 to 10^10 phages per milliliter for treating different types of topical or cutaneous infections [48]. For example, PhagoBioDerm utilizes a hydrogel for phage treatment.

3. CRIPR-Cas System

There are three types of CRISPR-Cas systems present in bacteria: Type I, Type II, and Type III. Each type is characterized by its unique Cas (CRISPR-associated) protein. For example, the Type I CRISPR system is associated with Cas3, the Type II system with Cas9, and the Type III system with Cas6 nuclease. The Proto Spacer Adjacent Motif (PAM) plays a crucial role in recognizing the proto-spacer of the invading genome in the Type I and Type II systems. In contrast, the Type III CRISPR-Cas system does not rely on the PAM sequence for the incorporation of proto-spacers into the CRISPR locus.[31]

The CRISPR-Cas action occurs in three stages:

i. Adaptation

ii. Expression

iii. Interference

In the Type I CRISPR system, the Cas3 nuclease is involved. The PAM sequence aids in the integration of the proto-spacer from the invading genome into the CRISPR locus of the cas operon. Once integrated, the proto-spacer is referred to as a spacer sequence. The CRISPR locus containing the spacer undergoes transcription to produce a CRISPR RNA transcript known as crRNA. The crRNA, in combination with Cas3, directly targets and cleaves the invading DNA within the spacer sequence [28].

The Cas9 protein is the double-stranded DNA nuclease associated with the Type II CRISPR immune system in bacteria. When a bacterium is invaded by a bacteriophage, the PAM helps incorporate the proto-spacer of the invading genome into the CRISPR locus, forming spacers. The CRISPR locus with the spacers is then expressed to produce crRNA. The CRISPR-associated complex that defends the host bacterium becomes activated, binding to the crRNA, which is subsequently cleaved by the housekeeping RNase III in the presence of Cas9 proteins. In this system, crRNA combined with Cas9 directly targets the invading genome [30,34], Fig.1.

The prominent distinction between the Type III system and the Type I and II systems is that the incorporation of proto-spacers into the CRISPR locus does not require PAM sequences in the Type III system. The role and mechanism of how PAM sequences help recognize proto-spacers in the Type I and Type II systems remains unclear. Once the proto-spacers are integrated into the CRISPR locus with the involvement of Cas1 and Cas3, they are termed spacers and undergo transcription to form crRNA. The crRNA associated with Cas9 can cleave both DNA and RNA of the invading genome through the subtype III-A and subtype III-B systems. The subtype III-A system specifically targets and cleaves invading DNA, while the subtype III-B system cleaves invading RNA [33].

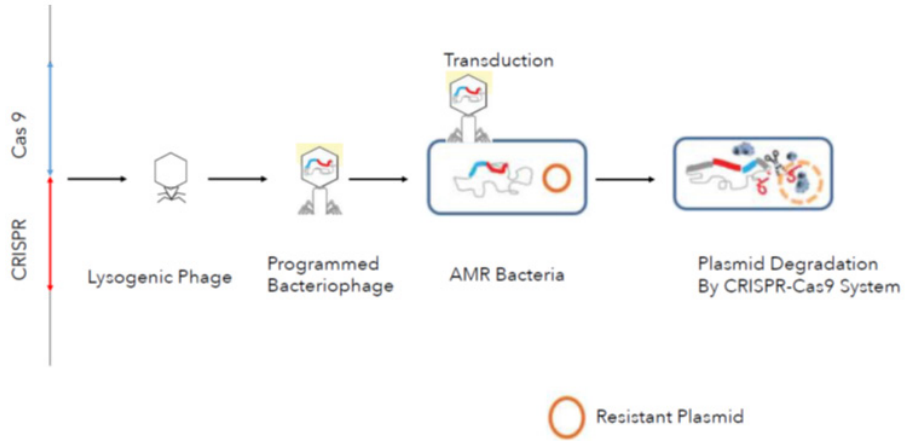

Figure 1: Scheme for Sensitization and Elimination of AMR Bacteria

4. CRISP-Cas based sequence specific antimicrobials

Recent research suggests that bacteriophages possess their own CRISPR systems, which allow them to evade the immune defenses of their hosts. The CRISPR sequence can be engineered to target bacterial or plasmid genomes and delivered to the host via bacteriophages using a phagemid vector. Phagemids are designed to be encapsulated within the phage capsid, facilitating the transfer of reprogrammed CRISPR-Cas antimicrobials into the host bacterium through a process known as transduction [27,35].

These engineered plasmids specifically target antibiotic resistance genes present in the host's genome or in plasmids carrying such resistance genes. The CRISPR-Cas antimicrobials are sequence-specific, meaning they precisely target the antimicrobial resistance (AMR) genes in the host bacteria. One scientific report indicates that a reprogrammed CRISPR-Cas9 nuclease was utilized to specifically target the staphylococcal plasmid that contains the aph-3 kanamycin resistance genes [29,32,].

These sequence-specific antimicrobials represent a groundbreaking approach to addressing AMR in pathogenic bacteria. We refer to them as smart antimicrobials (SAM) because they can both target specific virulent bacterial populations and immunize avirulent populations, thus preventing the spread of plasmid-mediated resistance within bacterial communities [37].

5. Phage-Antibiotic combination (PACs) therapy

To survive the selective pressure of antibiotics, bacterial populations acquire random mutations in their genomes through natural selection. These mutant traits are then passed down to their offspring, ultimately leading to the evolution of an entirely new generation that is resistant to antimicrobial agents (AMR). Bacteriophages possess the capability to bypass the resistance developed under the selective pressure of antibiotics [38].

Evidence shows that bacteria can develop resistance against both antibiotics and bacteriophages through various mechanisms, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and Restriction-Modification systems. These immune responses evolved in prokaryotes as survival strategies against bacteriophages.

Using CRISPR-Cas antimicrobials in conjunction with antibiotics creates two distinct selective pressures on bacterial growth. This dual approach forces the bacterial population to further adapt and mutate. Additionally, reverse-engineered phages can either kill or sensitize resistant bacteria, making them susceptible to traditional antibiotics. This combination strategy is likely to be more effective than using either method alone, as it facilitates targeted killing or sensitization of resistant strains [39].

Phage cocktail as personalized therapeutics

The specificity of a therapeutic phage formulation can be established at the time of drug approval or can remain adaptable for future reformulation. This distinction highlights the contrast between a "one-size-fits-all" approach and a personalized medical approach in phage therapy. These approaches are referred to as 'prêt-à-porter' (ready-to-wear) and 'sur-mesure' (custom--made). Additionally, there is a "modifiable" middle ground between these "off-the-shelf" and "bespoke" strategies, which will be explored further in this section.

With the rise of personalized medicine, omics-based diagnostics—technologies that analyze biological data such as genomics and proteomics—are increasingly being used in clinical settings. These tools provide insights into disease risks and support the development of tailored prevention and treatment plans. Phage therapy could leverage omics technologies to create custom cocktails aimed at specific bacterial infections in patients. However, due to our limited understanding of phage-bacteria interactions, this approach might currently only suggest potentially effective phages rather than guarantee their efficacy. In the short term, phage-based methods for identifying bacteria, along with well-established phage libraries, may offer practical alternatives, although personalized phage therapy could still take days to initiate [49,50].

Table 2 outlines several broad strategies for treating bacterial infections. Personalized phage therapy can involve using phages from a phage bank (monophage therapy) or developing customized, patient-specific phage cocktails (sur-mesure cocktails). Alternatively, preformulated single cocktails, known as 'prêt-à-porter' cocktails, represent the opposite end of the spectrum, offering a standardized product for each infection type. This standardized product aims to include a broad enough range of phages to target common bacterial strains associated with the infection. However, without the option of personalized or alternative phage formulations, prête-à-porter cocktails may fail if the bacterial strain is not susceptible. This standardized model aligns with the 'western pharmaceutical model' of phage therapy, which addresses regulatory and practice-based needs for consistency in drug formulations across different times and locations [51,52].

Between these extremes, there are intermediate approaches. One involves developing multiple preformulated cocktails, each tailored to a single bacterial species rather than to broad, multi-species infections. This way, treatments could start with cocktails targeting the most probable bacteria, shifting to cocktails for other species if the initial treatment is ineffective. This cocktail bank model strikes a balance by using fewer types of phages overall, although it requires higher costs for development, production, and distribution [53].

The second intermediate approach avoids the complexity of multiple cocktails and highly personalized options by using a single, adaptable cocktail that can be modified over time. This method, seen in the former Soviet Republic of Georgia, involves 'prêt-à-porter' phage products with relatively stable formulations that are updated periodically. For example, 'Pyophage' targets bacteria such as E. coli, Proteus, Pseudomonas, Staphylococcus, and Streptococcus for treating wound infections, while 'Intestiphage' addresses gastrointestinal infections by targeting over a dozen pathogens. These cocktails are often revised twice a year, adding phages to address the most current bacterial strains and adapting existing phages to combat phage-resistant bacterial strains, thereby extending the lifespan of effective phage isolates. These formulations can vary by region and manufacturer, allowing for some local customization [54].

7. Regulatory issues

Phage therapy is currently in its early clinical stages in both Southeast Asia and the English-speaking Western world. This innovative approach is gaining attention as it signals the dawn of a new era in alternative medicine, particularly in the battle against antimicrobial resistance (AMR) in bacterial infections. However, a significant challenge lies in the absence of a defined regulatory pathway for the approval of programmed bacteriophages as therapeutic agents in these regions. This regulatory gap poses a

formidable barrier to the advancement and wider adoption of programmed phage therapies. To address this challenge effectively, we propose the establishment of a comprehensive protocol that fosters collaboration between researchers and regulatory agencies. Such a framework would be crucial in navigating the complexities of regulatory approval and promoting the development of safe and effective phage-based treatments [55].

8. Conclusion

Phage therapy is an innovative treatment approach that leverages the natural ability of bacteriophages, or phages, to target and eliminate specific bacteria. The process begins with the application of sericin films integrated with lytic phages to the surface of a wound. When these phages come into contact with their specific bacterial hosts, they attach to the bacteria and inject their nucleic acid into the bacterial cells. The phage genome then utilizes the bacteria's cellular machinery to replicate and assemble new phage particles. As the process concludes, the bacterial host cell undergoes lysis, releasing a multitude of new phages that can then infect other bacterial cells in the wound, effectively eradicating the harmful bacteria and facilitating the healing process.

In addition to phage therapy, the bacterial population often develops resistance to antibiotics through random mutations driven by natural selection, which helps them survive the selective pressure imposed by these treatments. The use of CRISPR-Cas antimicrobials, in conjunction with antibiotics, introduces two distinct forms of selective pressure that can influence bacterial growth. Notably, reverse-engineered phages can either directly kill resistant bacteria or sensitize them, making them vulnerable to conventional antibiotics. This combination strategy is expected to be significantly more effective than using either method alone for the targeted elimination or sensitization of pathogenic bacteria in humans.

While monophage therapy (using a single type of phage) has shown promise, researchers are increasingly using phage cocktails—mixtures of different phage types—to expand the range of bacteria that can be targeted and to reduce the development of phage-resistant bacterial strains.

Authorship Statement

All the authors meet the authorship criteria and have contributed equally towards the preparation of this manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

Funding

No funding of any kind is received.

Transparency declarations

None to declare.

Clinical trial number

Not applicable

References

1. Hicks LA, Bartoces MG, Roberts RM, et al. (2015) US outpatient antibiotic prescribing variation according to geography, patient population, and provider specialty in 2011 Clin Infect Dis. 60: 1308-16.

2. Laxminarayan R, Duse A, Wattal C, et al. (2013) Antibiotic resistance-the need for global solutions. Lancet Infect Dis. 13: 1057-98.

3. Zhang XX, Zhang T, Fang HH. (2009) Antibiotic resistance genes in water environment. Appl Microbiol Biotechnol. 82: 397-414.

4. Laxminarayan R, Duse A, Wattal C, et al. (2013) Antibiotic resistance-the need for global solutions. Lancet Infect Dis. 13: 1057-98.

5. Centers for Disease Control. Antibiotic Resistance: The Global Threat. 2015; Accessed Sept 11, 2020.

6. World Health Organization. Antibiotic resistance - a threat to global health security. 2013.

7. United Nations. PRESS RELEASE: High-Level Meeting on Antimicrobial Resistance. 2016.

8. New indian priority pathogen list to guide discovery of effective and affordable antibiotics. Available at: https://www.who.int/india/news/detail. Accsesed March 09, 2021.

9. Zhang XX, Zhang T, Fang HH. (2009)Antibiotic resistance genes in water environment. Appl Microbiol Biotechnol. 82: 397-414.

10. Lindsay, J. A (2014) Staphylococcus aureus genomics and the impact of horizontal gene transfer. Int J Med Microbiol. 304, 103–9.

11. Xia G. Wolz C. (2014) Phages of Staphylococcus aureus and their impact on host evolution. Infect Genet Evol. 21, 593–601.

12. Lin DM, Koskella B, Lin HC. (2017) Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World J Gastrointest Pharmacol Ther. 8(3): 162-73

13. D'Herelle, F. (1917) Sur un microbe invisible antagoniste des bacilles dysent´eriques. C. R. Acad. Sci. 165:373–5.

14. Chanishvili N (2012) Phage therapy--history from Twort and d'Herelle through Soviet experience to current approaches. Adv Virus Res. 83: 3-40

15. Chernomordik, A. B. (1989) Bacteriophages and their therapeutic-prophylactic use. Med. Sestra. 6:44–7.

16. Smith HW, Huggins MB (1982) Successful treatment of experimental Escherichia coli infections in mice using phages: its general superiority over antibiotics. J. Gen. Microbiol. 128:307–18.

17. Chopra IJ, Hodgson B, (1997) The search for antimicrobial agents effective against bacteria resistant to multiple antibiotics. Antimicrob. Agents Chemother. 41: 497–503.

18. Silver LL, Bostian KA. (1993) Discovery and development of new antibiotics: the problem of antibiotic resistance. Antimi-

crob. Agents Chemother. 37:377–83.

19. Heo YJ, Lee YR, Jung HH, et al. (2009) Antibacterial efficacy of phages against Pseudomonas aeruginosa infections in mice and Drosophila melanogaster. Antimicrob Agents Chemother. 53: 2469–74.

20. Biswas B, Adhya S, Washart P, et al. (2002) Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant Enterococcus faecium. Infect Immun. 70: 204-10.

21. Wang J, Hu B, Xu M, et al. (2006) Therapeutic effectiveness of bacteriophages in the rescue of mice with extended spectrum beta lactamase producing Escherichia coli bacteremia. Int J Mol Med. 17: 347-55.

22. Wang J, Hu B, Xu M, et al. (2006) Use of bacteriophage in the treatment of experimental animal bacteremia from imipenem-resistant Pseudomonas aeruginosa. Int J Mol Med. 17: 309-17.

23. Pouillot F, Chomton M, Blois H, et al. (2012) Efficacy of bacteriophage therapy in experimental sepsis and meningitis caused by a clone O25b: H4- ST131 Escherichia coli strain producing CTX-M-15. Antimicrob Agents Chemother. 56: 3568-75.

24. Wills QF, Kerrigan C, Soothill JS. (2005) Experimental bacteriophage protection against Staphylococcus aureus abscesses in a rabbit model. Antimicrob Agents Chemother. 49: 1220-1.

25. Fish R, Kutter E, Wheat G, et al. (2016) Bacteriophage treatment of intransigent diabetic toe ulcers: a case series. J Wound Care. 25 Suppl 7: S27-33.

26. Kutateladze M, Adamia R (2008) Phage therapy experience at the Eliava Institute. Med Mal Infect. 38: 426-30.

27. Garneau JE. Dupuis MÈ, Villion M, et al. (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature. 468: 67–71.

28. Marraffini LA, Sontheimer EJ (2010) Self versus non-self discrimination during CRISPR RNA-directed immunity. Nature. 463: 568–71.

29. Marraffini LA, Sontheimer EJ (2008) CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science. 322:1843–5.

30. Sontheimer EJ, Marraffini LA. Slicer for DNA. Nature, 468: 45–6.

31. Makarova KS, Wolf YI, Iranzo J, et al. (2020) Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. Nat Rev Microbiol. 18: 67-83.

32. Marraffini LA, Sontheimer EJ. (2008) CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science. 322: 1843–5.

33. Hale CR, Zhao P, Olson S, et al. (2009) RNA-guided RNA cleavage by a CRISPR RNA-Cas protein complex. Cell. 139: 945–56.

34. Seed KD, Lazinski DW, Calderwood SB. et al. (2013) A bacteriophage encodes its own CRISPR/Cas adaptive response to evade host innate immunity. Nature. 494: 489–91.

35. Melnikov AA, Tchernov AP, Fodor, I et al. (1984) Lambda phagemids and their transducing properties. Gene. 28, 29–35.

36. Bikard D, Euler CW, Jiang W, et al. (2014) Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. Nat biotechnol. 32: 1146-50.

37. Samson JE, Magadán AH, Sabri M, et al. (2013) Revenge of the phages: defeating bacterial defences. Nat Rev Microbiol. 11: 675– 87.

38. Torres BC, Hochberg ME (2016) Evolutionary rationale for phages as complements of antibiotics. Trends Microbiol. 24: 249 –56.

39. Gordillo FL, Barr JJ (2019) Phage therapy in the post antibiotic era. Clin Microbiol Rev. 32: e00066-18.

40. Weinbauer MG, Rassoulzadegan F (2004) Are viruses driving microbial diversification and diversity. Environ Microbiol. 6: 1–11.

41. Delbrück M (1940) The growth of bacteriophage and lysis of the host. J Gen Physiol. 23: 643– 60.

42. Larbi D, Decaris B, Simonet JM (1992) Different bacteriophage resistance mechanisms in Streptococcus salivarius subsp. thermophilus. J Dairy Res. 59: 349 –57.

43. Filippov AA, Sergueev KV, He Y, et al. (2011) Bacteriophage-resistant mu- tants in Yersinia pestis: identification of phage receptors and attenuation for mice. PLoS One. 6: e25486.

44. Marraffini LA, Sontheimer EJ (2007) CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. Nat Rev Genet. 11: 181–90.

45. Barrangou R, Fremaux C, Deveau H, et al. (2007) CRISPR provides acquired resistance against viruses in prokaryotes. Science. 315: 1709–12.

46. Hale CR, Zhao P, Olson S, et al. (2009) RNA-guided RNA cleavage by a CRISPR RNA-Cas protein complex. Cell. 139: 945–56.

47. Walter N, Mirzaei M, Deng L, et al. (2024) The Potential of Bacteriophage Therapy as an Alternative Treatment Approach for Antibiotic Resistant Infections. Med Prin Pract. 33: 1-9.

48. Duplessis CA, Biswas B. (2020) A Review of Topical Phage Therapy for Chronically Infected Wounds and Preparations for a Randomized Adaptive Clinical Trial Evaluating Topical Phage Therapy in Chronically Infected Diabetic Foot Ulcers. Antibiotics. 9: p.377.

49. Yosef I, Manor M, Kiro R, et al. (2015) Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic resistant bacteria. Proc Natl Acad Sci. 112: 7267-72.

50. Ullah F, Ahmad SS, Khan MA, et al. (2024) Bacteriophage Therapy against Antimicrobial Resistant Crisis. J Health Sci Med Ther. 2: 1-8.

51. Hitchcock NM, Devequi GN, Shiach J, et al. (2023) Current clinical landscape and global potential of bacteriophage therapy. Viruses. 15: 1020.

52. Durr HA, Leipzig ND (2023) Advancements in bacteriophage therapies and delivery for bacterial infection. Mat Adv. 4:

1249-57.

53. Pirnay JP, Djebara S, Steurs G, et al. (2023) Retrospective, observational analysis of the first one hundred consecutive cases of personalized bacteriophage therapy of difficult to treat infections facilitated by a Belgian consortium. Med Rxiv. 2023-08.

54. Chan BK, Stanley GL, Kortright KE, et al. (2023) Personalized inhaled bacteriophage therapy decreases multidrug resistant Pseudomonas aeruginosa. MedRxiv. 24: 2023-01.

55. Young J, Lee SW, Shariyate MJ, et al. (2024) Bacteriophage Therapy and Current Delivery Strategies for Orthopedic Infections: A Scoping Review. J Inf. 17: 106125.

56. Carrigy NB, Larsen SE, Reese V, et al. (2019) Prophylaxis of Mycobacterium tuberculosis H37Rv infection in a preclinical mouse model via inhalation of nebulized bacteriophage D29.Antimicrob Agents Chemother. 16.

57. Oechslin F, Piccardi P, Mancini S, et al. (2017) Synergistic interaction between phage therapy and antibiotics clears Pseudomonas aeruginosa infection in endocarditis and reduces virulence. J Infect Dis. 215: 703–12.

58. Jun JW, Shin TH, Kim JH, et al. (2014) Bacteriophage therapy of a Vibrio parahaemolyticus infection caused by a multiple-antibiotic-resistant O3:K6 pandemic clinical strain. J Infect Dis. 210: 72–8.