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Bacteriophage Therapy: An Innovative Approach to Combating Antibiotic Resistance in Bacteria

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Finally, we extend our thanks to all those who contributed to our education and supported us along the way.

Dedication

First of all, I thank Allah, the Almighty, for granting us the courage, health, and patience to complete this work.

To my dear parents, ***Abdelhak*** *and* ***Zahira****, and my beloved sisters,* ***Zohra, Hakima****, and* ***Khaoula*** *— thank you for your unwavering support, encouragement, and the countless sacrifices you have made.*

I would also like to express special thanks to all those who supported and helped me, especially ***Lalami Hamza****,* ***Merouche Atef****,* ***Meguenni Malik Amin****,* ***Mahsas Yacine****, and* ***Nouara Haythem****.*

RAGOUB Abdelhalim

Dedication

First of all i thank Allah, the Almighty for giving us courage, health and patience to complete this work.

To my dear parents and family GHANEM and brothers for their contribution in every work I have done and for all the sacrifices they made.

GHANEM Bilal

List of abbreviation

AMR	Antimicrobial Resistance
CBD	C-terminal cell wall binding domain
CRAB	Carbapenem-Resistant Acinetobacter baumannii
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DISARM	Defense Island System Associated with Restriction-Modification
DNA	Deoxyribonucleic Acid
E.	Escherichia
EAD	N-terminal enzymatically active domain
EPS	Extracellular Polysaccharides
ICU	Intensive Care Uni
LPS	Lipopolysaccharides
MDR	Multidrug-Resistant
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MTase	Methyltransferase
Omps	Outer Membrane Porine
PAS	Phage-Antibiotic Synergy
PG	Peptidoglycan
REase	Restriction Endonuclease
RM systems	Restriction–Modification systems
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
UTIs	Urinary Tract Infections
VAPGHs	Virion-Associated Peptidoglycan Hydrolases
WHO	World Health Organization

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Abstract

Phage therapy is emerging as a promising alternative to combat the global threat of antibiotic resistance. It uses bacteriophage, viruses that specifically infect and destroy bacteria, especially naturally occurring lytic phages that break down bacterial cells at the infection site. To broaden their antibacterial spectrum, phages are often combined into "phage cocktails." However, due to issues like limited host range and bacterial resistance to phages, genetically engineered phages have been developed to enhance efficacy and safety. In addition to whole phages, scientists have identified powerful enzymes called endolysins, derived from phages, which can degrade the bacterial cell wall—particularly effective against Gram-positive bacteria—and are being explored as stand-alone antibacterial agents. Combination therapies, pairing phages with antibiotics, have also shown promising results in improving infection control and reducing resistance development. Despite many advantages, such as high specificity, low side effects, self-amplification, and cost-effectiveness, several challenges remain, including immune responses, stability issues, delivery limitations, and regulatory concerns. Nonetheless, growing research and renewed clinical interest highlight the strong potential of phage therapy as a safe and effective approach to treat multi-drug-resistant infections, provided that further optimization and rigorous testing are carried out.

Keywords: Antibiotic, antimicrobial resistance, MDR, phages, Phage therapy

الملخص

يشكّل العلاج بالبكتيريوفاج أحد الحلول الواعدة لمواجهة مشكلة مقاومة المضادات الحيوية، وهي من أكبر التحديات الصحية في العالم اليوم. يعتمد هذا العلاج على استخدام فيروسات تسمى "الفاجات"، والتي تهاجم البكتيريا بشكل محدد وتدمرها من خلال آلية التحلل. تُستخدم غالبًا فاجات حالّة طبيعية، ويمكن مزج عدة أنواع منها في ما يُعرف بـ"كوكتيلات الفاجات" لتوسيع نطاق التأثير ضد أنواع متعددة من البكتيريا. وقد أدّى ظهور مقاومة بعض البكتيريا حتى للفاجات إلى تطوير فاجات معدّلة وراثيًا لزيادة فعاليتها وأمان استخدامها. إضافة إلى الفاجات، اكتشف العلماء إنزيمات مدمّرة مشتقة منها تُعرف بالـ"اندولايزينات"، يمكنها تفكيك جدار الخلية البكتيرية وتُعد واعدة كعوامل مضادة للميكروبات، خاصة ضد البكتيريا موجبة الجرام. كما أظهرت العلاجات المزدوجة، التي تجمع بين الفاجات والمضادات الحيوية، نتائج جيدة في تعزيز القضاء على العدوى والحد من تطوّر المقاومة. رغم مزايا هذا النوع من العلاج، مثل الدقة في الاستهداف، وانخفاض الآثار الجانبية، والتكلفة المقبولة، إلا أن تحديات عديدة ما تزال قائمة، مثل التفاعل مع الجهاز المناعي، صعوبة التوصيل، والحواجز التنظيمية والتصنيعية. ومع ذلك، فإن الاهتمام المتجدد بالبحث والتجريب يعزز الآمال في أن يصبح العلاج بالفاجات خيارًا فعّالًا وآمنًا لمكافحة العدوى البكتيرية المقاومة للأدوية في المستقبل القريب.

الكلمات المفتاحية: المضادات الحيوية، مقاومة المضادات الحيوية، الجراثيم المقاومة المتعددة (MDR) ،الفاجات، العلاج بالفاجات.

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Introduction

Antimicrobial resistance (AMR) is now one of the top three public health threats, according to the World Health Organization (WHO). In 2019, about 1.27 million deaths were directly caused by AMR, and nearly 5 million deaths were linked to drug-resistant infections. This number could rise to 10 million deaths per year by 2050, even more than cancer (Salam et *al.*, 2023). Most of these deaths happen in low- and middle-income countries, mainly caused by six types of bacteria already listed as priority pathogens by WHO (Strathdee et *al.*, 2023). Antibiotic resistance happens when bacteria change in a way that makes antibiotics no longer work against them (Durbas and Machnik, 2022).

Because antibiotic resistance is spreading fast, scientists are working hard to find new treatments. One promising option is bacteriophage (phage) therapy (Fowoyo, 2024). Bacteriophages, or phages, are viruses that infect and kill bacteria. They recognize specific proteins on the surface of bacterial cells. Most phages have a head and a tail and contain double-stranded DNA (Lin et *al.*, 2017; Cieplak et *al.*, 2018; Noor et *al.*, 2024).

Phages are important in nature and are very specific, meaning they usually attack only one species or strain of bacteria. This helps protect the normal microbiome, unlike antibiotics which can harm good bacteria too. Phages can follow two life cycles: the lytic cycle, where they destroy the bacteria, and the lysogenic cycle, where they hide inside the bacteria for a while (Ferry et *al.*, 2021; Anastassopoulou et *al.*, 2024).

Phages have many advantages: they multiply inside the body, have fewer side effects, are cheap to produce, can be used for people allergic to antibiotics, and can be given in different ways (Diallo and Dublanchet, 2022).

Phages were first discovered in the early 1900s. Frederick Twort noticed them in 1915, but it was Félix d'Hérelle who properly described them in 1917 at Pasteur institute in Paris (Kortright et *al.*, 2019; Noor et *al.*, 2024). D'Hérelle proved they were viruses by isolating phages that killed bacteria like *E. coli* and *Salmonella*

(Dublanchet et *al.*, 2008). He later teamed up with Georges Eliava to create a center focused on phage therapy (Ferry et *al.*, 2021; Durbas and Machnik, 2022).

At first, phage therapy showed promise, but results were inconsistent. Poor understanding of phages and commercialization problems made it hard to succeed (Lin et *al.*, 2017). Then, during World War II, the discovery of antibiotics, which were easier to make and use, pushed phages into the background (Cisek et *al.*, 2017; Brives and Pourraz, 2020). Still, phages offer a special advantage because they are much targeted. One challenge is that each phage usually kills only certain bacteria, so doctors must find the right phage for each infection (Curtright and Abedon, 2011).

Today, as antibiotic resistance grows worse, phage therapy is getting attention again. Scientists are exploring its use in human and animal health, farming, and food safety (Brives and Pourraz, 2020; Durbas and Machnik, 2022).

1. Antimicrobial resistance (AMR)

Antimicrobial resistance (AMR) is a serious global health problem. It happens when antibiotics are used too much or in the wrong way in people, animals, and farming. This causes some microorganisms to become strong enough to resist treatment. AMR started after penicillin was discovered, and now many bacteria have become resistant to several drugs. This makes it harder for doctors to treat infections. The problem is growing and is called a "Silent Pandemic" because it spreads quietly but could cause more deaths than other diseases by 2050. AMR affects both people and animals. It spreads when resistant germs pass on their resistance genes (Ahmed et *al.*, 2024). These microorganisms use different mechanisms to protect themselves from antibiotics, as illustrated in the figure below (Fig. 1).

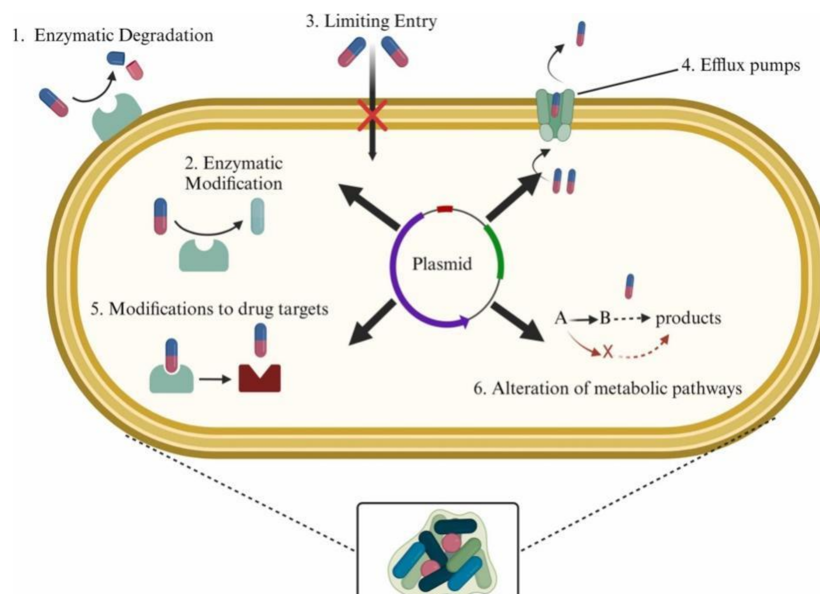

Figure 1 . Mechanisms of antimicrobial resistance in bacteria (Ahmed et *al.*, 2024).

1.1.Efflux pumps

Efflux pumps are sophisticated bacterial mechanisms that require energy and are positioned on the cytoplasmic membrane. They have the ability to remove harmful compounds, including medications, from the cell, lowering the concentration of the drug inside the cell and reducing its effectiveness. Efflux pumps are found in many types of bacteria and can provide resistance to a wide range of antibiotics (Kapoor et *al.*, 2017; Belay et *al.*, 2024).

1.2. Modification of target molecule

Bacteria can develop resistance to antibiotics by modifying the structures that these drugs typically target. For example, changes in the bacterial cell wall can reduce the number of binding sites for antibiotics like beta-lactams, which normally act on the cell wall. Similarly, mutations in bacterial DNA can alter the structure of ribosomes thereby preventing antibiotics such as tetracyclines and macrolides from binding effectively. These structural changes, whether through target substitution, bypass mechanisms, enzyme alterations, or genetic mutations, decrease the drug's ability to act, leading to antimicrobial resistance (Belay et *al.*, 2024).

1.3. Drug inactivation

Antibiotics entering the cell can be hydrolyzed or modified by the inactivating enzymes produced by bacteria, such as antibiotic hydrolases or inactivating enzymes, to make them inactive before they reach the target location. Numerous enzymes in bacteria, including N- acetyltransferase, O-phosphotransferase, and O-adenosyltransferase, can change the structure of aminoglycoside antibiotics by acetylating, phosphorylating, or adenylating them, respectively. Bacteria produce a variety of inactivating enzymes, including aminoglycoside inactivating enzymes, chloramphenicol acetyltransferase, and β-lactamase (Zhang and Cheng, 2022).

1.4.Target site modification

One common resistance mechanism is target site modification, in which bacteria change the molecular structures that antibiotics normally bind to, making the medications less effective. This can happen as a result of enzymatic changes, genetic mutations, or horizontal gene transfer that acquires resistance genes (Elshobary et *al.*, 2025).

1.5. Modifications of the antibiotic molecule

One of the main ways bacteria protect themselves from antibiotics is by making special enzymes that either change the antibiotic or break it apart, so it can't work anymore. Some bacteria produce enzymes that add small chemical groups to the antibiotic, which stops it from attaching to its target. Others make enzymes that destroy the antibiotic completely. For example, in the case of β-lactam antibiotics, certain enzymes called β-lactamases break a key bond in the antibiotic's structure,

making it useless (Munita and Arias, 2016).

1.6. Permeability reduction

Porin channels or outer membrane porins (Omps) are necessary to promote the passage of hydrophilic substances through the lipid bilayer, which is primarily composed of proteins and lipopolysaccharides in Gram-negative bacteria. Specific porins (such as OmpF, OmpC, and OmpE) are produced by each type of bacteria, and one of the causes of bacterial resistance is the loss or destruction of one or more Omps. By altering the characteristics and amount of porin to lower the bacterial membrane permeability following antibiotic exposure, acquired drug resistance can be created (Zhang and Cheng, 2022).

2. Bacteriophages

Bacteriophages, or phages, are viruses that specifically infect bacteria. They are the most abundant biological entities on earth and exhibit significant diversity in shape, size, and genome organization. All phages consist of a nucleic acid genome enclosed in a protein capsid, which protects the genetic material and facilitates its delivery into bacterial cells. Though some resemble organisms with "heads" and "tails," phages are non-motile and rely on Brownian motion to encounter their bacterial targets.

Phages infect only specific bacterial species or even strains, using either a lytic or lysogenic replication cycle (Fig. 2):

- **In the lytic cycle,** a bacteriophage binds to a susceptible bacterial host and injects its genetic material into the cytoplasm and utilizes the host's ribosomes to manufacture its proteins. The host's resources are redirected toward producing new viral genomes and assembling them into complete phage particles. Eventually, the infected bacterium is destroyed either through active mechanisms or passive degradation releasing numerous newly formed phages to infect other bacterial cells.

- **In the lysogenic cycle**, phage DNA integrates into the host genome (as a prophage) or persists episomally, replicating without killing the host. Environmental changes can trigger prophages to enter the lytic cycle (Cui et *al.*, 2024).

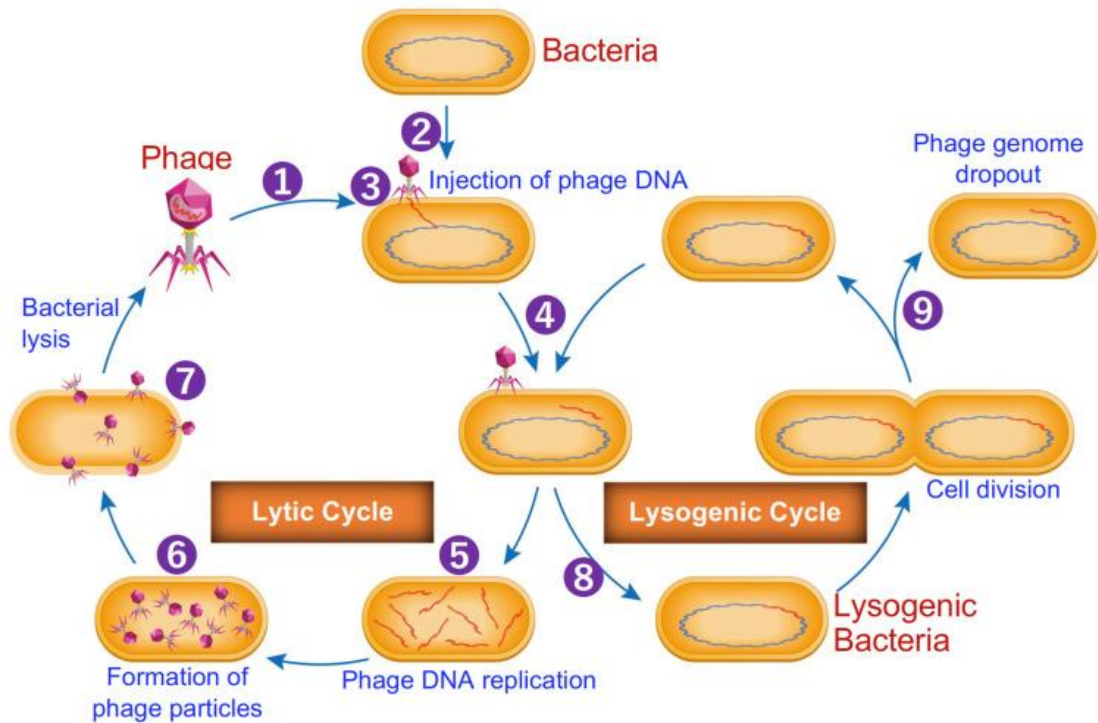

Figure 2. Lytic and lysogenic replication cycle of bacteriophages (Cui et *al.*, 2024).

3. Phage therapy

Several alternatives are being explored to tackle antibiotic resistance, including probiotics, antimicrobial peptides, essential oils, new antibiotics, and better use of existing ones. One promising approach is phage therapy.

3.1. Definition

Phage treatment is considered a biocontrol strategy that uses bacteriophages to eliminate or limit the growth of bacterial infections in humans, thereby helping to prevent, treat, or cure the associated diseases. Phage therapy is one of the earliest, if not the first, known attempts to use viruses for practical purposes in human medicine. It involves the use of natural or genetically modified bacteriophages to treat bacterial infections, both internally and externally (Marintcheva, 2017).

3.2 . A brief history of phage therapy

The use of bacteriophages to treat bacterial infections has experienced a fluctuating history from its promising beginnings in the early 20th century to a period of decline with the rise of antibiotics, and now a renewed interest driven by the global challenge of antibiotic resistance (Fig.3). Its historical trajectory can be traced through several key milestones and turning points (Brives and Pourraz, 2020):

- Bacteriophages were first described by Frederick Twort in 1915 and later isolated for therapeutic use by Félix d'Hérelle in 1917 at the Pasteur Institute in Paris.

- D'Hérelle used phages to successfully treat bacterial infections, even though their nature was not yet fully understood. He collaborated with George Eliava, leading to the creation of a phage therapy center in Tbilisi, Georgia.

- Phage therapy initially spread rapidly across Europe and the United States, with pharmaceutical companies producing phages. However, it declined in the West from the 1940s due to critical scientific reviews, unclear mechanisms of action, and the rise of sulfonamides and antibiotics.

- In contrast, phage therapy persisted and developed in the Soviet Union, especially in Georgia, Poland, and Russia, where access to antibiotics was more limited.

- The success of antibiotics, particularly penicillin, drastically reduced infectious diseases and shaped medical expectations, causing phage therapy to fall into obscurity in much of the world.

- In the late 1990s and early 2000s, phage-based biotechnologies began to reemerge in fields such as human and animal health, food safety, and agriculture. Notably, phage products have been approved for use against *Listeria* and for protecting crops.

- Despite widespread skepticism, phage therapy continued to be used sporadically in France and Belgium in cases where antibiotics failed.

- The past 15 years have seen resurgence in clinical and laboratory research on phage therapy, with notable treatment successes, such as the recovery of Tom Patterson in the U.S. and a cystic fibrosis patient in the U.K.

- Although some large-scale trials like Phagoburn have had inconclusive results, clinical interest and the number of scientific publications on phage therapy have increased significantly.

- This renewed attention is largely driven by the growing threat of antibiotic-resistant bacteria ("superbugs") and the realization that phages offer fundamentally different.

3.3. Types of phage therapy

Phage therapy encompasses several strategies depending on how bacteriophages act against bacteria. These strategies can be broadly divided into five categories: conventional phage therapy, genetically modified phage therapy, enzyme-based therapy utilizing phage-derived enzymes, protein-based therapy using phage-derived proteins, and combination therapy involving phages and other antimicrobial agents.

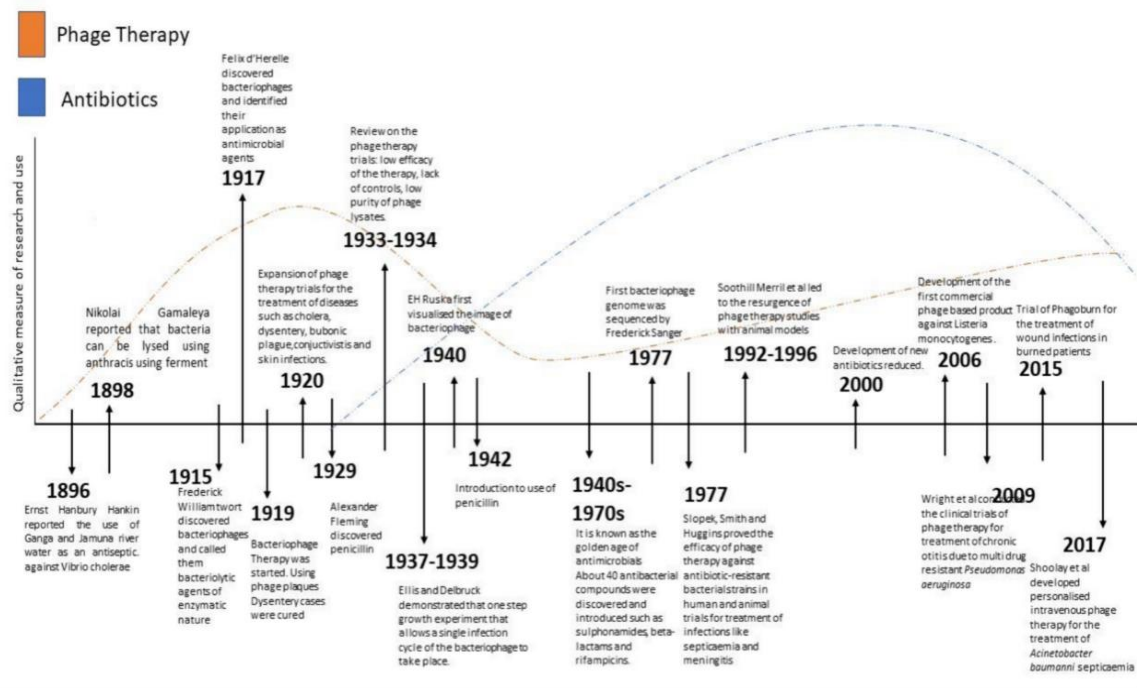

Figure 3. The major milestones and historical events that have shaped the development and application of both bacteriophages and antibiotics over time (Kapoor et *al.*, 2024).

3.3.1. Conventional phage therapy

Traditionally, phage therapy employs naturally occurring lytic phages to infect and lyse bacteria at the site of infection (Viertel et *al.*, 2014). The main objective of this approach is to eliminate the bacterial pathogen responsible for acute or chronic infections. Lytic phages are preferred over temperate phages because they do not integrate their genome into the host bacterium, thereby minimizing the risk of transferring virulence or antibiotic resistance genes (Viertel et *al.*, 2014; Kapoor et *al.*, 2024). To enhance efficacy and broaden the spectrum of action, lytic phages are often combined into "phage cocktails" mixtures of multiple phages that have demonstrated *in vitro* activity against the target pathogen (Lin et *al.*, 2017).

However, subsequent studies have shown that not all phages replicate in the same way, and that lytic and lysogenic phages differ significantly in their replication cycles. For phage therapy to be widely adopted, several challenges must be addressed, including the development of phage resistance, narrow host range, limited immune system response to phages, difficulties in phage delivery, potential systemic side effects, and manufacturing challenges (Sulakvelidze et *al.*, 2001; Kapoor et *al.*, 2024).

3.3.2. Modified phage therapy

Modifications to bacteriophages have been explored and shown to be an effective alternative to traditional phage therapy in order to address its limitations. The challenges associated with lytic phage-based therapy have led to growing interest in genetically modified phages (Kapoor et *al.*, 2024). Researchers are investigating more controlled phage systems due to the potential side effects of lytic phages, safety concerns related to the use of self-replicating live microorganisms, and the inconsistent outcomes observed in treating bacterial infections. Through techniques such as directed mutation, recombination, artificial *in vivo* selection, chimeric phages, and other rational design strategies, additional features have been introduced into phage genomes to enhance their therapeutic potential. These novel, engineered phages have been shown to effectively overcome the limitations of earlier phage therapy approaches (Moradpour et *al.*, 2011).

3.3.3. Phage-derived enzyme therapy

Among the most exciting developments in phage therapy is the discovery of lytic enzymes encoded by phages, which share functional similarities with the antimicrobial enzyme lysozyme found in eukaryotes. The majority of phage species utilize two primary classes of proteins during the process of lysing their bacterial hosts. One of these is holin, a transmembrane protein, while the other is a hydrolase known as endolysin (or lysin), which acts on the peptidoglycan cell wall (Fig.4). Unlike holins, which cannot lyse bacterial cells on their own, phage lysins can independently cause lysis, leading to significant interest in their potential as antimicrobial agents (Lin et *al*., 2017).

3.3.3.1. Holins

They are typically small, hydrophobic proteins that assemble in the bacterial cytoplasmic membrane and create lethal pores at the appropriate time, facilitating the escape of endolysin to the peptidoglycan layer of the cell wall (Oliveira et *al.*, 2018).

3.3.3.2. Endolysins

Endolysins also referred to as phage lysins, which are enzymatic proteins encoded by phages that are primarily responsible for the breakdown of the peptidoglycan (PG) layer of the host bacterium from within during the concluding phase of their lytic replication cycle. Endolysins derived from phages targeting Gram-positive bacteria generally possess a modular domain architecture that includes an N-terminal enzymatically active domain (EAD) and a C-terminal cell wall binding domain (CBD), linked by a short connector. Nevertheless, the relative positioning and interaction of these domains are not consistently maintained across all endolysins (Oliveira et *al.*, 2018). Endolysins can be classified based on their source, separating them into those from Gram-positive or Gram-negative specific phages. Another categorization hinges on the cleavage site of endolysins and divides these proteins into four main classifications:

(i) glycosidases (including lysozyme and N-acetyl-β-D-glycosaminidase) that target the polysaccharide backbone of PG;

(ii) lytic transglycosylases that disrupt the connection between N-acetylmuramyl and N-acetylglucosaminyl residues of PG, aside from muramidases, forming the N-acetyl-

1,6-anhydro-muramyl moiety;

(iii) N-acetylmuramoyl-L-alanine amidases, which break the amide bond between N-acetylmuramic acid and the L-alanine within the stem peptide; and

(iv) endopeptidases capable of cleaving either the stem peptide or the cross-links of PG (which include L-alanoyl-D-glutamate endopeptidases, D-glutamyl-m-DAP endopeptidases, interpeptide bridge-specific endopeptidases, D-alanoyl-m-DAP endopeptidases, glycylglycine endopeptidases, D-alanoyl-glycine endopeptidases, D-glutamyl-L-lysine endopeptidases, and D-alanoyl-L-alanine endopeptidases) (Drulis-Kawa *et al.*, 2015).

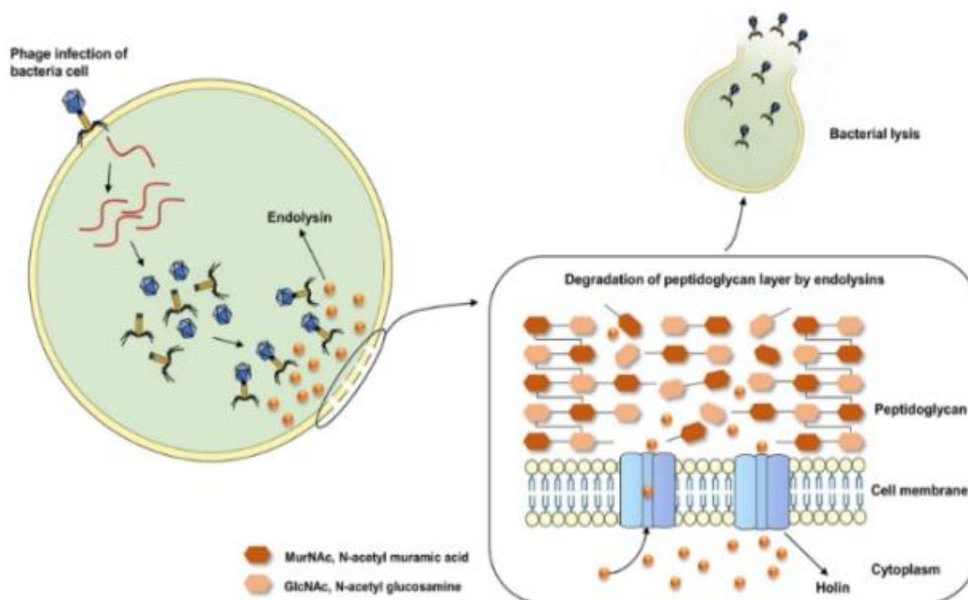

Figure 4. Mechanisms of action of holins and endolysins in Gram-positive bacteria (Liu et *al.*, 2023).

3.3.4. Phage derived proteins

Numerous proteins derived from phages have been discovered (Ferry *et al.*, 2021). The scientific community has shown particular interest in phage depolymerases due to their role in phage adsorption and bacterial degradation (Topka-Bielecka et *al.*, 2021). Additionally, phages often employ enzymes known as phage lytic proteins to terminate their bacterial hosts. Unlike antibiotics, which can act broadly against various bacteria, phage lysins comprising a diverse group of unrelated antimicrobial peptides (AMPs) hydrolyze the bacterial cell wall with high specificity,

functioning like guided missiles to eliminate their targets (Nandi et *al.*, 2022).

3.3.4.1. Bacteriophage Depolymerases

Enzymes that degrade polysaccharides, also known as polysaccharide depolymerases, are proteins typically associated with the virion (Drulis-Kawa *et al.*, 2015). Most of these enzymes are encoded within or adjacent to the structural gene region of a phage genome (e.g., tail fibers and base plates). These phage encoded enzymes are specifically designed to recognize, bind to, and break down the polysaccharide components of bacterial cell walls. This degradation is crucial for exposing the phage receptors necessary for effective infection of the host bacterium (Topka-Bielecka et *al.*, 2021). However, these enzymes alone cannot induce bacterial cell lysis (Anastassopoulou et *al.*, 2024).

For phages, polysaccharides often serve as physical barriers that hinder access to cell surface receptors required for adsorption. Polysaccharides are classified based on their morphological localization as intracellular polysaccharides, structural polysaccharides (e.g., lipopolysaccharides or LPS), and extracellular polysaccharides (EPS). EPS are the most abundant and are located at the outermost layer of the bacterial cell envelope. Depending on the bacterial species, EPS may either be tightly bound to the cell surface as capsular polysaccharides or released in a loose, slime-like form.

Polysaccharide depolymerases have not been shown to be either bacteriolytic or bacteriostatic. Their therapeutic potential mainly lies in their ability to strip away extracellular polysaccharides, which many bacterial pathogens use to facilitate host colonization (e.g., biofilm formation), enhance virulence, and protect against host immune responses, antimicrobial agents, and phage attack (Roach and Donovan, 2015).

3.3.4.2. Virion-associated peptidoglycan hydrolases (VAPGH)

Unlike endolysins, which act during the later stages of phage gene expression in the lytic cycle, virion-associated peptidoglycan hydrolases (VAPGHs) function during the initial phase. These phage-lytic proteins are promising candidates for combating drug-resistant bacterial infections, as they can target both Gram-positive and Gram-negative bacteria. VAPGHs primarily assist in releasing newly formed phage progeny from the host bacterial cell by degrading the peptidoglycan-based cell

wall. Their activity is part of a coordinated process involving other phage-encoded proteins, such as holins and endolysins. Unlike endolysins, VAPGHs possess highly conserved domains but lack a cell wall-binding domain (CBD) (Mtimka et *al.*, 2024).

3.3.5. Phage combination with antibiotics

Phage therapy, when combined with antibiotics, can help reduce the emergence of phage-resistant bacterial subpopulations (Uchechukwu and Shonekan, 2024). The co-administration of phages and antibiotics has demonstrated synergistic effects in various treatment regimens (Oechslin, 2018; Nikolich and Filippov, 2020; Pires et *al.*, 2020; Uchechukwu and Shonekan, 2024). This phenomenon, known as phage-antibiotic synergy (PAS), has been observed in both Gram-positive and Gram-negative bacteria.

One mechanism proposed for PAS involves delayed lysis and a significant increase in phage burst size, triggered by bacterial filamentation and reduced holin production due to antibiotic-induced stress. This results in more effective killing of planktonic bacterial populations, as demonstrated by Pires et *al.* (2020). Additionally, Nikolich and Filippov (2020) highlighted the effectiveness of PAS in disrupting bacterial biofilms *in vitro*.

However, not all phage-antibiotic combinations produce synergistic effects (Pires et *al.*, 2020). In fact, high concentrations of antibiotics, particularly those inhibiting protein synthesis, can impair phage replication. Nonetheless, even in the absence of synergy, the combined use of phages and antibiotics may still reduce or prevent the development of resistant bacterial strains. Although numerous studies have explored phage-antibiotic combinations, relatively few have systematically examined the bacterial responses to these therapies (Pires et *al.*, 2020).

4. Phage resistance and pharmacology

4.1. Mechanisms of bacteriophage resistance

Bacteria can develop resistance to phages, which can limit the effectiveness of phage therapy (Ly-Chatain et *al*., 2014; Pires et *al*., 2020). They become resistant to phage treatment through spontaneous mutations, the acquisition of restriction–modification (RM) systems, adaptive immunity *via* the clustered regularly interspaced short palindromic repeat-associated (CRISPR-Cas) system (Hibstu et *al*., 2022).

4.1.1. Modification or mutation of surface receptors

Phages bind to bacteria by attaching to specific receptors located on the bacterial cell wall. Bacteria can modify the structure or amount of these receptors to hinder phage binding and prevent further infection (Egido et *al*., 2022; Sawa et *al*., 2024).

4.1.2. Bacterial restriction-modification systems

Restriction–modification (RM) systems, commonly referred to as primitive immune systems in bacteria, are widespread. They serve crucial roles as defense mechanisms against phage genomes (Hibstu *et al.*, 2022). These systems consist of two opposing enzymatic functions: a restriction endonuclease (REase) and a methyltransferase (MTase) (Azam and Tanji, 2019; Hibstu *et al.*, 2022).

The defensive mechanism of bacterial RM systems involves recognizing the methylation state of invading phage genomes. Methylated sequences are deemed self, while those on the foreign phage genome that lack methylation are classified as non-self and are cleaved by the REase. The function of the REase is to identify and cut non-self-nucleic acid sequences at defined locations, whereas the MTase protects the bacterial genome by adding methyl groups to the same specific nucleic acid sequences, allowing the system to distinguish self from non-self DNA (Hibstu *et al.*, 2022).

4.1.3. Clustered regularly interspaced short palindromic repeats and associated proteins (CRISPR-Cas)

Clustered Regularly Interspaced Short Palindromic Repeats and Associated Proteins (CRISPR-Cas) systems are currently the only known form of adaptive immunity in prokaryotes These systems are found in numerous bacterial genomes and, occasionally, in plasmids.A CRISPR locus within a bacterial genome comprises a CRISPR array and a *cas* gene operon**.** The CRISPR array consists of repeating sequences and segments of foreign origin, referred to as spacers**,** which serve as the immunological memory of the system. The *cas* operon includes all the genes necessary for producing the Cas proteins that constitute the immune machinery.

Bacteria possessing the CRISPR locus have the unique ability to modify their genomes by inserting small fragments of foreign DNA (spacers) into the CRISPR

array. After transcription of the CRISPR loci into CRISPR RNAs (crRNAs), the spacer sequence guides the Cas nuclease protein to cleave the corresponding nucleic acids that enter the cell (Edigo et *al*., 2018; Azam and Tanji, 2019; Chen et *al*., 2019).

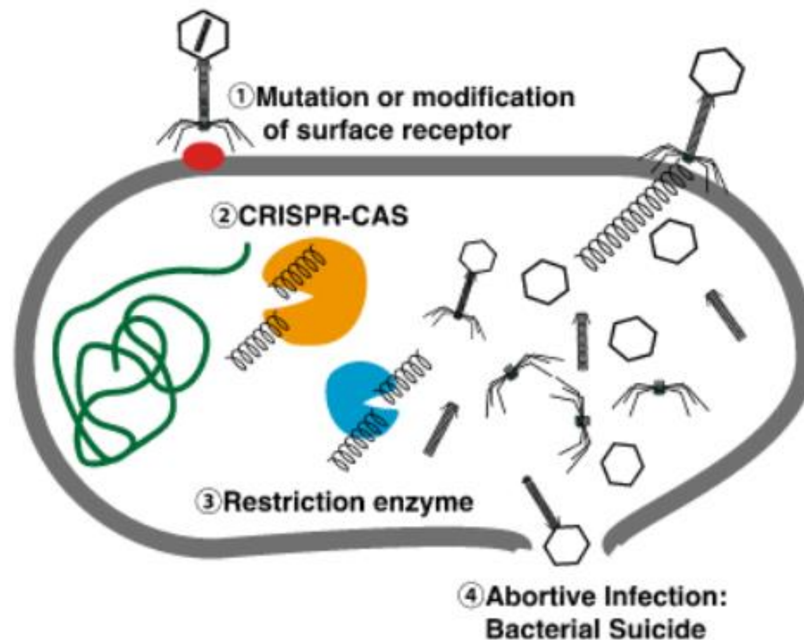

Figure 5. Bacterial mechanisms actions (Sawa et *al*., 2024).

4.2. Innovative strategies in phage therapy

One of the main drawbacks of phage therapy is the rapid emergence of bacterial mutants that are resistant to phages during treatment. In this context, addressing these phage defense mechanisms could enhance the therapeutic effectiveness of phages in clinical environments.

4.2.1. Selecting highly effective phages

According to Oromí-Bosch et *al*. (2023) using phages that propagate efficiently can help diminish bacterial populations. Certain characteristics of the lytic phage replication cycle that may be advantageous for maximizing the effectiveness of phage treatment include adsorption dynamics, latent periods, and burst sizes. A phage that binds strongly to one or more receptors on the bacterial surface effectively ensures quick and permanent adsorption to susceptible cells. The latent period, which refers to the duration between phage adsorption and the release of new phages through cell lysis, should be minimized to optimize the number of infection cycles within a given time. Larger burst sizes are favorable as they lead to the production of

thousands of new phage particles from a single bacterial infection, thereby increasing the chances of overpowering the target bacterial population with a limited number of phages. Lastly, the stability of the phage (for instance, preventing particle degradation and/or clumping) is essential for the long-term storage of phages and may improve the durability of the virus when administered to patients (Oromí-Bosch et *al*., 2023).

4.2.2. Engineered phages

Phage engineering serves as a crucial and effective strategy to enhance the success rate of phage therapy. Significant effort has been devoted to expanding the host range of phages through genetic modifications, addressing a primary limitation in the therapeutic use of phages (Pires et *al*., 2020; Bleriot et *al*., 2024).
This approach could considerably reduce the time required to identify effective phages for specific bacterial infections and facilitate the selection of a few well-characterized phages to serve as scaffolds for developing customized variants. Moreover, it may reduce variability between treatments and improve the transition to clinical applications (Bleriot *et al*., 2024).

4.2.3. Phage cocktails

The most common approach to enhance the success of phage therapy involves using mixtures of different viruses, known as phage cocktails. In addition to making it harder for bacteria to develop resistance (Jurado et *al*., 2022), phage cocktails offer a broader range of effectiveness against various pathogens (Curtright and Abedon, 2011; Oromí-Bosch et *al*., 2023; Uchechukwu and Shonekan, 2024; Palma and Qi, 2024), making them suitable for situations in which the infecting strain is unidentified or multiple pathogens are involved (Uchechukwu and Shonekan, 2024).

Phage cocktails have emerged as the predominant and most frequently utilized strategy in phage therapy. These mixtures typically consist of around 20–30 phages targeting diverse bacterial species and are routinely updated to maintain efficacy against newly emerging epidemiological strains. Historically, the design of phage cocktails has focused on expanding host range; however, this may not sufficiently meet clinical needs. A key challenge in cocktail design is the careful selection of phages to prevent treatment failure due to the evolution of bacterial resistance to one

phage, which could result in cross-resistance to others within the mix (Oromí-Bosch et *al.*, 2023).

4.3. Phage pharmacology

Pharmacology is generally divided into two primary components: pharmacodynamics and pharmacokinetics. Pharmacodynamics refers to the examination of how drugs affect the body, with effects that can be positive (such as supporting or restoring health) or negative (like causing harmful side effects). In contrast, pharmacokinetics focuses on how the body influences the drug, typically examined through four key processes: absorption, distribution, metabolism, and excretion.

4.3.1. Phage therapy pharmacokinetics

Phage pharmacokinetics, which refers to a drug’s capacity to attain adequate concentrations in the vicinity of targeted tissues, has been examined more thoroughly alongside pharmacodynamics (Abedon and Thomas-Abedon, 2010; Qadir et *al.*, 2015; Danis-Wlodarczyk et *al.*, 2021; Gangwar et *al.*, 2022). This process includes absorption, distribution, metabolism, and excretion (Qadir et *al.*, 2015; Danis-Wlodarczyk et *al.*, 2021; Palma and Qi, 2024). Absorption moves the drug into the bloodstream, distribution carries it to tissues, metabolism alters it into its active form, and excretion removes it from the body. These aspects can either enhance or diminish drug concentrations. The pharmacokinetics influence drug delivery methods, which depend on the target tissue, drug sensitivity to enzymes, immunity, pH, intake schedule, and patient convenience (Qadir et *al.*, 2015).

4.3.2. Phage therapy pharmacodynamics

Phage therapy's pharmacodynamics focus on the beneficial effects of phages, which depend on sufficient drug concentrations in targeted tissues associated with specific bacteria. Effective phage therapy can be achieved through passive treatment, which involves the direct application of phages, or active treatment, which combines phage application with *in situ* amplification. (Danis-Wlodarczyk et *al.*, 2021). The success of such therapy is influenced by the initial dosage, timing of administration, and the virulence of the phages used (Noor et *al.*, 2024). However, obstacles,

including challenges with tissue penetration, retention, and bacterial resistance, can limit effectiveness (Dąbrowska and Abedon, 2019).

Key pharmacodynamic considerations include safe phage solution administration, determining the killing titer, and the multiplicity of infection, which are essential for ensuring adequate phage numbers are present to effectively eliminate the bacteria-causing infection.

4.4. Comparison of therapeutic characteristics of bacteriophages and antibiotics

Antibiotics remain irreplaceable in certain clinical situations, such as infections caused by intracellular pathogens or those affecting parenchymatous organs. Conversely, bacteriophages are particularly valuable in other cases, especially in infections involving multidrug-resistant bacteria where antibiotics are less effective. Moreover, the concurrent use of phages and antibiotics is recommended, as phages reduce the bacterial load, allowing antibiotics to act more effectively. This dual pressure weakens the bacteria, lowering the probability of resistance development. Additionally, the use of bacteriophages may lead to a reduced reliance on antibiotics, which is significant considering that antibiotic overuse is a key driver of bacterial resistance.

By integrating phage therapy, we can potentially slow the emergence of antibiotic-resistant strains. The benefit of combining antibiotics and bacteriophages lies not only in their synergistic interactions but also in their complementary mechanisms of action in different clinical scenarios.

Therefore, antibiotics and bacteriophages should not be viewed as competitors, but rather as complementary tools in the fight against bacterial infections. Phage therapy should be considered a valuable strategy to address the growing challenge of antimicrobial resistance (Ravat et *al*., 2015).

Table I. Comparison between phages and antibiotics (Furfaro et *al.*, 2018)

	Bactériophages	Antibiotics
BACTERICIDAL AGENTS	• Virulent phages cause cell lysis (bactericidal). • Bioengineering of phages can produce bacteriostatic results.	Induce a static state (bacteriostatic) or cause cell death (bactericidal).
DOSAGE	• Dose escalation through release of exponential phage numbers caused by host-dependent replication	• Dose is dependent on absorption, concentration, metabolism and excretion of the agent.
TOXICITY	• Generally considered as inherently non-toxic due to nucleic acid and protein composition. • Risk of lysed cell remnants that may lead to severe reactions in therapeutic use unless phages are highly purified.	• Range of toxicity levels which can vary according to the drug depending on contraindications such as pregnancy.
MICROBIOTA DISRUPTION	• Host specificity of phages results in minimal to no disruption of the normal microbiota.	Broad-spectrum antibiotics are well known for disrupting the overall microbiota and causing a dysbiotic state. • Narrow spectrum antibiotics reduce this impact, but still target many species.
RESISTANCE	• The specificity of phages for their hosts means that if resistance does emerge only a select bacterial population will be affected. • Phage-resistant mutants are often less virulent as phage receptors are commonly associated with pathogenicity. • Cocktail formulations reduce resistance emergence and may be used to treat antibiotic-resistant pathogens.	• Broader range of activity means that a substantial proportion of bacterial species are affected and the potential for widespread resistance to emerge is greater. • Mutations resulting in resistance to one antibiotic may cause cross-resistance to other agents.
DISCOVERY	• Phage discovery is generally rapid and relatively easy due to their ubiquitous nature. Antibiotics	Antibiotic discovery is expensive and complicated by drug design and development in addition to assessment of potential toxicity. • Minimal new discoveries.

5. Therapeutic applications of phage therapy

5.1. Urogenital tract infections

Urinary tract infections (UTIs) are inflammations of the urothelium caused by uropathogens, which may be community-acquired or hospital-acquired. *Escherichia coli* is the primary pathogen, responsible for approximately 80% of cases. The effectiveness of traditional antibiotics has significantly declined due to the increasing prevalence of antimicrobial resistance (AMR), largely driven by overuse and unregulated antibiotic consumption (Fowoyo, 2024). This threat has renewed interest in bacteriophages as alternative treatment options (Al-Anany et *al.*, 2023; Fowoyo, 2024). Phages can be utilized to treat various infections of the urogenital system either systemically, such as through direct injection into the bladder, or via topical application (Abedon et al., 2011).

5.2. Phage therapy for gastrointestinal infections

Gastrointestinal infections, commonly known as bacterial gastroenteritis, pose a significant global health challenge affecting the stomach and intestines, often resulting in diarrhea. Most cases of gastrointestinal infections are mild and typically resolve on their own within a few days. However, for certain demographic groups, such as the elderly, young children, and individuals with chronic conditions or weakened immune systems, diarrheal diseases can lead to severe dehydration, necessitating medical intervention. Phage therapy presents a promising alternative to antibiotics for addressing these infections (Palma and Qi, 2024). Phages are being evaluated against *Clostridium difficile* colitis and the elimination of adherent invasive *Escherichia coli* (AIEC) in Crohn's disease. Phages have mostly been investigated as prospective tools in gastroenterology for infectious disorders, such as cholera. Assessing the safety and effectiveness of the intended phages is the most crucial step in the therapeutic development of phages. Research in this area is now conducted using appropriate in vitro systems or in vivo animal models (Gutiérrez and Domingo-Calap, 2020).

5.3. Musculoskeletal infections

Numerous clinical applications of phage therapy for musculoskeletal infections have been documented. Treatment has been utilized for infections related to periprosthetic joints, spinal issues, trauma, and craniectomy. Phage therapy has been administered both in vitro and directly to address musculoskeletal infections (Kapoor et *al*., 2024). According to Kapoor *et al*. (2024), a case report was published describing the use of phage therapy to treat four orthopedic infections using standardized treatment protocols involving the local administration of phages. The phages were administered intraoperatively and then postoperatively three times a day for ten consecutive days. During phage therapy, all patients also received antibiotics, and their clinical status was monitored daily. No recurrence of infection was observed during the follow-up period, which lasted between 8 and 16 months.

5.4. Respiratory infections

Phage therapy has demonstrated both preclinical and clinical effectiveness against various bacterial pathogens, indicating its potential against pulmonary pathogens as well (Wang et *al*., 2021). Inhaled phage therapy has a long history of

use, particularly in Eastern European nations such as Georgia, Russia, and Poland (Chang et *al*., 2018; Wang et *al*., 2021). Studies dating back to 1936 have primarily focused on administering phages through inhalation to combat a variety of pulmonary pathogens, including *E. coli*, *Klebsiella*, Streptococci, Staphylococci, and *Pseudomonas*. Many studies have reported efficacy rates as high as 80-100%, although some resulted in treatment failures due to insufficient understanding of phage specificity, quality assurance, and stability challenges. Recent advancements in phage therapy and improvements in inhalation and aerosolization methods have contributed to bridging these gaps in knowledge concerning pulmonary phage therapy (Wang et *al*., 2021).

6. Advantages and limitations of phage therapy

6.1. Advantages

6.1.1. Automatic “dosing.”

We call this automatic "dosing" because the phages themselves assist in selecting the phage dose, even though the dependence on relatively high bacterial concentrations is a limitation. Phage proliferation is possible (Loc-Carrillo and Abedon, 2011; Mishra et *al*., 2024), especially in situations where hosts are present during the bacterial-killing process (Fig.6).

6.1.2. The inherent toxicity is low.

Since proteins and nucleic acids make up the majority of their structure, phages are inherently harmless. There is, however, less evidence that doing so while treating phages poses a risk. Phage-immune interactions can result in adverse immunological responses. To avoid allergic reactions to bacterial components such endotoxins found in crude phage lysates, certain phage therapy regimens could call for high-purity phage preparations. Like antibiotics, phages can destroy bacteria *in situ* by damaging their cell walls, but they can also liberate portions of the bacterium (Mishra et *al*., 2024).

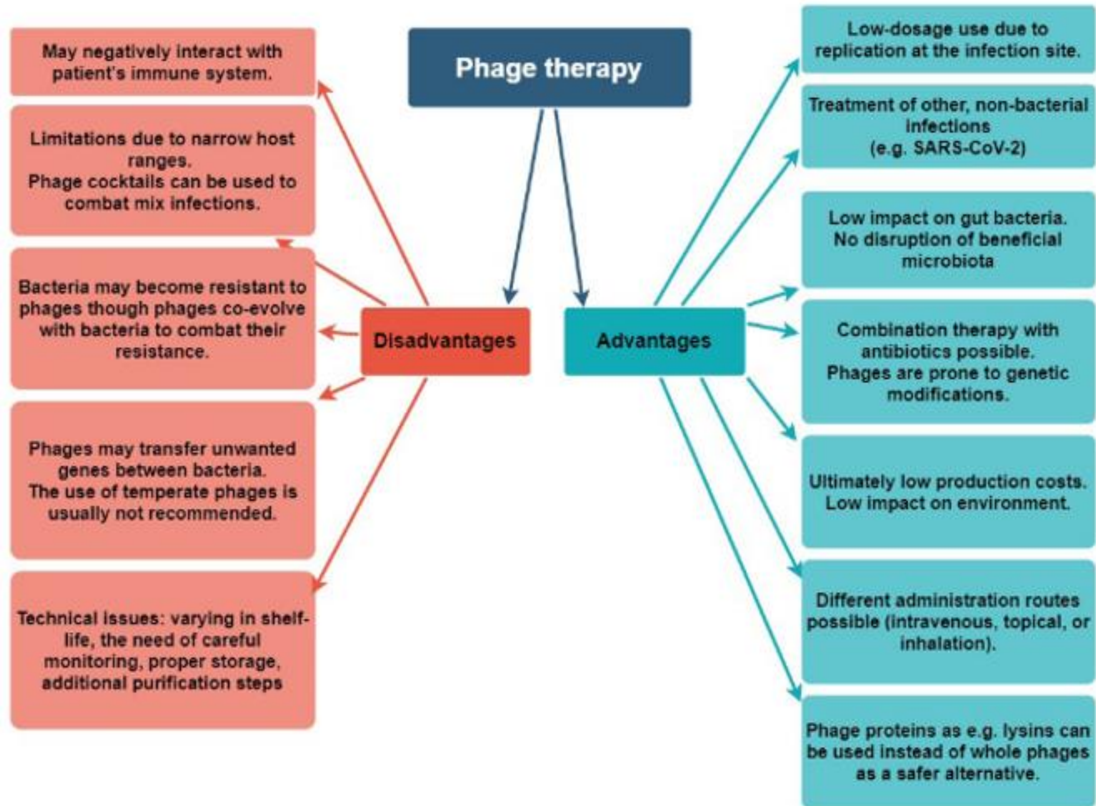

Figure 6. Disadvantages and advantages of phage therapy (Durbas and Machnik, 2022).

6.1.3. Capacity for low-dose use

Given the right bacterial density, the phage's density rises *in situ*, so lowering the quantity of phage required to attain its efficacy can save treatment expenses. Because phages produce density when they actively eliminate germs and do not remain in the body for an extended amount of time, their use in low concentrations can aid in promoting product safety. There is, however, little proof that utilizing pure phage preparations or greater phage dosages could result in more adverse consequences than using lower phage doses. For safety reasons, it is only advantageous to avoid phage treatments at greater doses if doing so poses a danger (Loc-Carrillo and Abedon, 2011).

6.1.4. Possible transfer of phages between subjects

This is basically cross-infection of phages from treated subjects or environments to untreated subjects, which may be helpful in some agricultural applications (Loc-Carrillo and Abedon, 2011).

6.1.5. Biofilm clearance

Biofilms, which are complex colonies of bacteria that attach to surfaces and are coated in a protective matrix, provide a substantial treatment issue due to their increased antibiotic resistance. Some phages have demonstrated a distinct capacity to infiltrate and dissolve biofilms, providing a possible therapy for infections linked with medical equipment and chronic wounds (Cui et *al*., 2024).

6.2. Potential disadvantages

The main drawback of phage therapy is the need to find a suitable lytic phage with high virulence and a wide range of bacterial hosts (i.e., species and strains) in order to tailor its suitability for different patients suffering from infections caused by different bacterial strains. Additionally, phage-resistant bacteria can occasionally produce anti-phage antibodies, which can occasionally interfere with the treatment that is being administered (Zalewska-Piątek, 2023; Cui *et al*., 2024). A limiting effect of neutralizing antibodies has been observed in a patient with bronchiectasis who had refractory *Mycobacterium abscessus* lung disease (Zalewska-Piątek, 2023).

Phage stability is a crucial topic, as phages can be sensitive to environmental variables such as temperature and pH, affecting their viability and efficacy. Phage treatment has extra challenges in obtaining regulatory approval. Because of their distinct characteristics, selectivity, and capacity for evolution, phages require a different regulatory framework than conventional medications. Gaining regulatory approval and guaranteeing the security and effectiveness of phage-based therapies depend on the establishment of standardized procedures for phage characterization, manufacture, and clinical testing (Cui *et al*., 2024).

7. Current research and clinical trials

7.1. Recent development in phage therapy

Numerous studies have confirmed the effectiveness of phage therapy in treating bacterial infections. For instance, Fathima and Archer (2021) demonstrated that bacteriophages serve as a powerful therapeutic option against multidrug-resistant (MDR) and biofilm-forming pathogens. Another research conducted by Wei et *al.* (2019) showed that bacteriophages had a significant impact on inhibiting bacteria in both *in vivo* and *in vitro* studies.

7.1.1. Efficacy

A study by Wang *et al*. (2021) highlighted that inhaled phage therapy could revolutionize the prevention and treatment of bacterial respiratory infections, especially those caused by antibiotic-resistant bacteria. The outcomes of various studies support the notion that inhaled phage therapy is a safe and effective antibacterial treatment with no reported adverse events. Additionally, research by Sithu Shein *et al.* (2024) illustrated the success of phage therapy in adult patients with cystic fibrosis suffering from chronic pulmonary infections caused by *P. aeruginosa*.

7.1.2. Safety

Research by Schooley *et al*. (2017) indicated that there were no adverse effects associated with phage administration in either intracavitary or intravenous treatments for patients with disseminated resistant *Acinetobacter baumannii* infections. Furthermore, studies involving cystic fibrosis patients with chronic pulmonary *P. aeruginosa* infections confirmed the safety and tolerance of “AP-PA02,” an inhaled phage cocktail that reported no side effects. Incidences of side effects from phage treatment have been exceedingly rare across several medical conditions, including cystic fibrosis, diabetic foot ulcers, periprosthetic hip joint infections, and urinary tract infections, suggesting that phage therapy has a favorable safety profile across a variety of patient groups and stages of illness (Ibrahim *et al*., 2025). Moreover, phage products can contain harmful products, such as endotoxin, during their formulation process and that can be tackled by various purification methods. Bacteriophages, due to their high specificity, have a minimal impact on the normal gut microflora, since they usually infect only a few strains of a bacterial species. The potential of

bacteriophages to encode virulence factors or transduce unwanted genes can be, in a large scale, avoided by avoiding the usage of temperate phages (Kakasis and Panitsa, 2019).

7.1.3. Ongoing clinical trials and experts accomplishment

Sahoo and Meshram (2024) are exploring and investigating the synergistic effects of combining phages with antibiotics to tackle resistant infections. As of March 2023, there are 45 clinical trials listed on clinicaltrials.gov, marking a significant rise in interest and availability in recent years for phage therapy as a legitimate treatment option. Ongoing studies include multicenter trials assessing the safety and effectiveness of phage therapies for different infections, particularly those due to antibiotic-resistant bacteria.

7.1.3.1 Studies on *Mycobacterium abscessus*

According to Petrovic Fabijan et *al.* (2023), a breakthrough study by Dedrick and colleagues in 2019 described the first published clinical application of genetically modified phages to treat a clinically disseminated infection caused by the notoriously antibiotic-resistant *Mycobacterium abscessus*. For this case study, one naturally obligately lytic phage (phage Muddy) and two temperate phages (BP and ZoeJ) were identified that could effectively kill the clinical isolate, starting from a library of over 1,800 phages.

7.1.3.2. COVID-19 and carbapenem-resistant *Acinetobacter baumannii* (CRAB) Infections

Wu *et al.* (2021) made a significant discovery by enrolling four hospitalized patients with lung infections caused by carbapenem-resistant *Acinetobacter baumannii* (CRAB) and critical COVID-19 into compassionate phage therapy. The treatment involved two consecutive doses of 10^9 plaque-forming units (PFU) of phages. If antibiotic treatment was unable to eliminate CRAB infections, all patients in a COVID-19- specific intensive care unit (ICU) who tested positive for CRAB in sputum samples or bronchoalveolar lavage fluid were eligible to be included in the trial. Treatment with a pre- optimized 2-phage cocktail was linked to lower CRAB burdens, even though phage susceptibility testing showed the same profile of CRAB

strains from these patients.

7.1.3. 3. Complex wound and bone infections

In 2025, Paskhalova et *al.* conducted a study at the National Medical Research Center for Surgery's Department of Wounds and Wound Infections that included 70 adult patients with complex skin, soft tissue, and bone infections of different etiologies and localizations. In accordance with the study design, surgical debridement was necessary for purulent-necrotic wounds of soft tissues and bones of varying origin and localization in all of the patients included in the analysis. According to the results, Pyobacteriophage complex liquid can be used to treat purulent-necrotic wounds of different etiologies and localizations when multiple antibiotic resistance is present, as well as when systemic antibacterial therapy is not appropriate. Combining general and local phage therapy through a variety of delivery methods-specifically, vacuum devices with the potential to instill-is the most successful.

7.1.4. Future directions of phages as a human medicine

WHO (2025) states that, similar to many customized medications, phages presently face difficulties in adhering to rules intended for traditional medications made in a single formulation. Before phages are generally accessible for therapeutic use in humans, more proof from clinical trials and surveillance is required. Given their biological nature and the requirement for individualized treatment approaches, the route to regulatory approval for phage therapy must be addressed. Regulators should think about establishing manufacturing and clinical trial requirements for phages that take into account their distinct qualities and patient- specificity.

Table II. Preclinical and clinical trials (Subramanian, 2024).

Condition	Causative agent	Used bacteriophage	Route of administration	Study model	Study outcome
Preclinical studies					
Vibrio parahaemolyticus infection	Multiple-antibiotic–resistant *Vibrio parahaemolyticus*	Phage pVp-1	Intraperitoneal and oral	Mice	92% (intraperitoneal) and 84% (oral) reduction of mortality were observed in BP-treated mice through
Bacteremia	Imipenem-resistant *P. aeruginos*	Phage Ø9882	Intraperitoneal	Mice	100% survival in the treatment group
Sepsis	*Pseudomonas aeruginosa*	Phage strain KPP10	Oral	Mice	66.7% reduction of mortality in the bacteriophage (BP)-treated group
Ileocecitis	*Clostridium difficile*	C. difficile specific phage CD140	Oral	Hamster	Most of the BP treated hamsters survived, while all the control animal died within 96 h after challenge
Bacteremia	β-lactamase producing *Escherichia coli*	Phage Ø9882	Intraperitonea	Mice	100% survival in the treatment group
Meningitis and Sepsis	*Escherichia coli*	EC200PP (a lytic phage)	Intraperitoneal or subcutaneou	Rat pups	50 and 100% reduction of mortality in case of sepsis and meningitis, respectively
Wound infection	*Staphylococcus aureus*	Phage LS2a	Subcutaneous	Rabbit	Administration of BP and S. aureus prevented staphylococcal Infection
Preclinical trials					
Wound infections	*Pseudomonas aeruginosa*	Anti-*Pseudomonas aeruginosa* bacteriophages (PP1131)	Topical	Human	BP treatment reduced bacterial burden in burn wounds
Typhoid	*Salmonella typhi*	typhoid bacteriophages	Oral	Human	Five-fold reduction of typhoid cases in the treated group
Diabetic foot ulcer	MDR *S. aureus*	Staphylococcal phage Sb-1	Topical	Human	All six treated patients recovered following BP applications
Dysentery	*Shigella dysenteriae*	Cholera bacteriophage	Oral	Human	All treated individuals recovered after 24 h following a single Administration
Chronic Otitis	Antibiotic-resistant *P. aeruginosa*	Biophage-PA	Oral	Human	Significantly lower level of P. aeruginosa was reported in the BP-treated group, along with no adverse event
Cholera	*Vibrio cholera*	Cholera Bacteriophage	Oral	Human	93% survival rate was observed in the BP-treated group versus 37% survival rate in the control group

Discussion

Recent research has repeatedly shown the safety and effectiveness of phage therapy in treating bacterial infections, especially those caused by antibiotic-resistant strains. For example, a study on wound infections indicated that phage therapy, when used in conjunction with antibiotics, was more effective at eradicating multidrug-resistant *Pseudomonas aeruginosa* than either treatment on its own (Palma and Qi, 2024). Moreover, phage therapy has proven successful in addressing lung and pleural infections, with high recovery rates noted when it is combined with antibiotics (Sulakvelidze et *al.*, 2001).

The progression of phage therapy offers a promising strategy for combating the escalating issue of antibiotic-resistant bacterial infections. Its distinctive capability to specifically target and eliminate pathogenic bacteria while preserving the commensal microbiota distinguishes it from conventional antibiotics (Ibrahim et *al.*, 2025). In addition, phage treatments have been effective in eradicating biofilms formed by *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* on medical device surfaces. These results are particularly significant in relation to persistent infections linked to implanted medical devices like catheters, lenses, and prostheses, which frequently experience biofilm formation. (Lin et *al.*, 2017).

Despite nearly a century of research on bacteriophages, information regarding the treatment of bacterial infections remains incomplete. This uncertainty was one factor that led to the temporary discontinuation of phage therapy. However, with the recent in-depth exploration of phage therapy, advancements in related technologies, and the wealth of data gathered, the widespread implementation of phage therapy has now become feasible. Although phage therapy has significant potential, it also presents unavoidable limitations such as a narrow host range, immune clearance by the host, and the potential emergence of bacterial strains resistant to phages (Lin et *al.*, 2017). Furthermore, there are additional considerations regarding the impact of phages on the indigenous microbiota, a subject that has yet to be fully investigated.

Moving forward, substantial technological advancements may address the challenges surrounding the patenting of phage preparations. This could enhance the specificity and availability of phage therapy. Phages might also be tailored using

genome editing methods (such as sequencing, CRISPR/Cas-based phage engineering, homologous recombination, and phage genomic DNA assembly) to create preparations that target only antibiotic-resistant bacteria without disturbing the patient's commensal microflora (Zalewska-Piątek, 2023). According to Anomaly et *al*. (2020), governments should provide funding for clinical trials—such as "challenge studies" where patients with otherwise untreatable infections receive a phage cocktail. They should also support the development of effective diagnostics, as these will not only enhance patient health but also extend the effectiveness of existing antibiotics.

Conclusion

Phage therapy has emerged as a viable alternative to conventional antibiotics in response to the growing threat of MDR pathogens, which are predicted to cause more deaths from resistant infections than from cancer by 2050. Advances in biotechnology have broadened the scope of phage therapy, including the use of purified lytic protein and genetically engineered phages, increasing its effectiveness against multi-drug resistant bacterial infections. However, successful implementation requires addressing issues like phage resistance development, optimizing delivery methods, and evaluating combination therapies with exciting antimicrobials. Current research and clinical trials demonstrate the potential of phage therapy in treating a variety of infections, but more studies are required to ensure safety, efficacy, and practical applications.

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Annexes

Ministry of Higher Education and Scientific Research
M'Hamed Bougara University, Boumerdes
Faculty of Sciences
Laboratory of Bioinformatics, Applied Microbiology and Biomolecules
(BMAB)

CERTIFICATE

---- OF PARTICIPATION----

This certificate is proudly presented to:

Mr RAGOUB Abdelhalim

For participating in the National Day of Applied Microbiology in Service of Industry (JNMASI'25), Held on May 08th, 2025 at University of Boumerdes (UMBB, Algeria), by a Poster communication entitled:

Le future pour la lutte contre les infections bactériennes multi résistance.

Co-author: GHANEM Billal

The President of the JNMASI'25

Chairwoman of scientific committee

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Faculté des Science de la Nature et de la Vie, et des Sciences de la Terre
Université de Ghardaïa

2ème Séminaire National sur les Substances Bioactives (SBio)
16 Avril 2025

ATTESTATION DE PARTICIPATION

ce certificat est décerné à

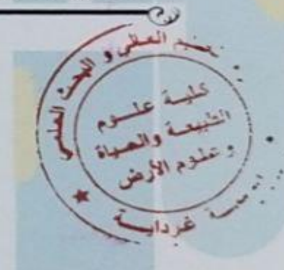
RAGOUB Abdelhalim

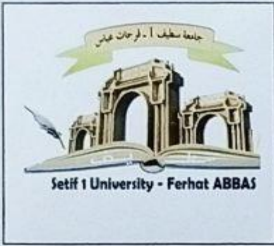
Le comité scientifique du 2ème séminaire national sur les Substances Bioactives (SBio, 2025) tenu à l'Université de Ghardaia le 16 Avril 2025, atteste par la présente son participation par une communication affichée, intitulée: «Phagothérapie : Une nouvelle approche thérapeutique contre les infections bactériennes».

Co-auteurs: GHANEM Billal

Présidente du séminarie

Doyen de la Faculté SNV-ST





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1st International Seminar on the Interest of Chemistry in the Development of Medical Sciences (SIICDSM2025) Sétif from 04 to 09/05/2025

CERTIFICATE OF PARTICIPATION

The president of the organizing committee of the '**1st International Seminar on the Interest of Chemistry in the Development of Medical Sciences (SIICDSM2025) Sétif from 04 to 09/05/2025'**

Certifies that :

Mr: RAGOUB ABDELHALIM

Has successfully participated in this event with a poster communication entitled:

" Bactériophage et leur application"

Co-authors: Ghanem Billal

President of scientific committee
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Abstract

Phage therapy is emerging as a promising alternative to combat the global threat of antibiotic resistance. It uses bacteriophage, viruses that specifically infect and destroy bacteria, especially naturally occurring lytic phages that break down bacterial cells at the infection site. To broaden their antibacterial spectrum, phages are often combined into "phage cocktails." However, due to issues like limited host range and bacterial resistance to phages, genetically engineered phages have been developed to enhance efficacy and safety. In addition to whole phages, scientists have identified powerful enzymes called endolysins, derived from phages, which can degrade the bacterial cell wall—particularly effective against Gram-positive bacteria—and are being explored as stand-alone antibacterial agents. Combination therapies, pairing phages with antibiotics, have also shown promising results in improving infection control and reducing resistance development. Despite many advantages, such as high specificity, low side effects, self-amplification, and cost-effectiveness, several challenges remain, including immune responses, stability issues, delivery limitations, and regulatory concerns. Nonetheless, growing research and renewed clinical interest highlight the strong potential of phage therapy as a safe and effective approach to treat multi-drug-resistant infections, provided that further optimization and rigorous testing are carried out.

Keywords: Antibiotic, antimicrobial resistance, MDR, phages, Phage therapy

الملخص

يشكّل العلاج بالبكتيريوفاج أحد الحلول الواعدة لمواجهة مشكلة مقاومة المضادات الحيوية، وهي من أكبر التحديات الصحية في العالم اليوم. يعتمد هذا العلاج على استخدام فيروسات تسمى "الفاجات"، والتي تهاجم البكتيريا بشكل محدد وتدمرها من خلال آلية التحلل. تُستخدم غالبًا فاجات حالّة طبيعية، ويمكن مزج عدة أنواع منها في ما يُعرف بـ"كوكتيلات الفاجات" لتوسيع نطاق التأثير ضد أنواع متعددة من البكتيريا. وقد أدّى ظهور مقاومة بعض البكتيريا حتى للفاجات إلى تطوير فاجات معدّلة وراثيًا لزيادة فعاليتها وأمان استخدامها. إضافة إلى الفاجات، اكتشف العلماء إنزيمات مدمّرة مشتقة منها تُعرف بالـ"اندولايزينات"، يمكنها تفكيك جدار الخلية البكتيرية وتُعد واعدة كعوامل مضادة للميكروبات، خاصة ضد البكتيريا موجبة الجرام. كما أظهرت العلاجات المزدوجة، التي تجمع بين الفاجات والمضادات الحيوية، نتائج جيدة في تعزيز القضاء على العدوى والحد من تطوّر المقاومة. رغم مزايا هذا النوع من العلاج، مثل الدقة في الاستهداف، وانخفاض الآثار الجانبية، والتكلفة المقبولة، إلا أن تحديات عديدة ما تزال قائمة، مثل التفاعل مع الجهاز المناعي، صعوبة التوصيل، والحواجز التنظيمية والتصنيعية. ومع ذلك، فإن الاهتمام المتجدد بالبحث والتجريب يعزز الآمال في أن يصبح العلاج بالفاجات خيارًا فعّالًا وآمنًا لمكافحة العدوى البكتيرية المقاومة للأدوية في المستقبل القريب.

الكلمات المفتاحية: المضادات الحيوية، مقاومة المضادات الحيوية، الجراثيم المقاومة المتعددة (MDR) ،الفاجات، العلاج بالفاجات.