Journal of Applied Sciences and Nanotechnology

Journal homepage: jasn.uotechnology.edu.iq

Phage-Antibiotic Synergy: Enhancing Efficacy Against Multidrug-Resistant Bacteria

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A R T I C L E I N F O

Article history:
Received: October, 27, 2024
Accepted: February, 09, 2025
Available online: March, 10, 2025

Keywords:
Phage therapy,
Antibiotic resistance,
Phage-antibiotic synergy,
Bacterial pathogens

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A B S T R A C T

The increasing number of bacteria that are resistant to antibiotics poses a major challenge to public health. Therefore, research into new treatment methods is essential to overcome these challenges. Phage treatment is a promising technique to increase the efficacy of antimicrobials against resistant microbes. This review documents the ability of bacteriophages to interact with conventional antibiotics due to their specificity and adaptability to target and control bacterial populations, especially when used in combination with antibiotics. Combination therapy can deliver the lowest effective concentration of antibiotics while reducing toxicity. Research has confirmed that the synergy of phages and antibiotics can disrupt biofilms and delay the development of resistance, which could prolong the efficacy of existing antibiotics. In addition, this study highlights challenges such as stability, delivery and clearance and underlines the importance of developing optimized phage-antibiotic combinations. It also discusses new technologies such as phagemids and CRISPR-mediated phage, including the modification of phage genetic material to improve their ability to target specific bacterial infections. The phages are modified to recognize bacterial surface markers or contain sequences that improve their effectiveness against resistant strains. The modifications offer new methods to improve this integrated therapy. Understanding the optimal conditions for the interaction between phages and antibiotics is crucial for the transition of research from the preclinical to the clinical phase. The synergy of phages and antibiotics is a promising strategy to fight infections that are resistant to antibiotics. Therefore, further research is needed to use them in the clinic.

https://doi.org/10.53293/jasn.2025.7535.1327, Department of Applied Sciences, University of Technology - Iraq.

1. Introduction

Public health has serious concerns about the rising number of antibiotic-resistant bacteria, as numerous antibiotics are rapidly losing their efficacy [1]. Therefore, exploring new treatment methods is essential to overcome these

challenges. This review documented the capability of bacteriophage to interact with conventional antibiotics as they have the potential to provide a precise and customized treatment response [2]. Phage therapy's potential is derived from its capacity to adapt to bacterial populations, whether they are pathogenic bacteria that induce diseases or environmental microbes that impact ecosystems. This study examines its potential to suppress two critical challenges that confront modern antibiotics: resistance evolution and evolutionary rescue. The study recommends a transition from a singular selective approach provided by current phage treatments to a multi-stage selective approach that combines phage therapy with traditional antibiotic treatment. The advantages of this combined therapy approach have been proved by two distinct laboratory experiments that have explored a method for treating bacterial biofilms to optimize their evolved adaptation to current antibiotics. Simple theoretical evolutionary models have further supported the results and are now prepared for applications in individualized treatment strategies for patients [3]. The integration of phage therapy with nanotechnology, specifically through the use of various nanoparticles (NPs) or microparticles (MPs), presents a promising approach to enhance the clinical efficacy of phage therapy [4]. This encompasses the development of rapid bacterial identification devices and platforms designed to improve phage bioavailability, inhibit their inactivation, offer protection against neutralizing antibodies (Abs), weaken their swift clearance by the reticuloendothelial system, enhance stability during prolonged storage, and facilitate targeted delivery and controlled phage release [5]. The rapid spread of bacterial resistance to the available antibiotics required an urgent demand for novel antimicrobial strategies. In this regard, phage therapy experiencing renewed interest, with successful treatment for multi-drug-resistant infections worldwide [1, 6] Several studies have highlighted the beneficial effects of combining phages with antibiotics. However, the majority employ bacteriophages for either pre- or co-treatment with antibiotics. The presented study described a ready-to-use protocol for a high-throughput application of phages to test synergism with antibiotics [7]. The main objective of this study is to investigate the potential of phage-antibiotic synergy (PAS) as a method to address antibiotic-resistant infections caused by bacteria. This involves studying the processes through which bacteriophages interact with antibiotics to improve antimicrobial effectiveness, diminish toxicity, and address issues such as biofilm formation and the development of resistance.

1.1. Phage Therapy and Delivery Challenges

The diversity of known phages alongside the simplicity of isolating novel phages with their specificity of targeting bacterial pathogens shed light on wide possibilities for phage therapy. These different uses present some challenges related to phage preparation, delivery and administration [8]. Among many possible applications of phage therapy is employing a simple method of phage preparations to enhance the efficacy of conventional antibiotics. Phages and antibiotics are combined in this method, resulting in a synergistic effect that allows for a significant reduction in the traditional minimum effective concentration of each agent [9]. Furthermore, this combination has the potential to improve the stability of phages, even in the presence of sub-inhibitory concentrations of antibiotics. This stabilization may lead to a prolonged treatment period and allow the oral administration of phages, which was previously impossible [7]. As with any application of biological agents to combat disease, numerous challenges must be addressed before the full potential of phage therapy can be realized. Issues such as phage stability, delivery, and clearance often pose greater obstacles compared to traditional antibiotics (**Fig. 1**) [10]. Phages are biological agents, that are inherently less stable and more challenging to deliver, store, and administer compared to the chemical compounds that are used in the drugs of traditional antibiotic therapy. Phages may be less stable than antibiotics due to many reasons including phages are potentially subject to spontaneous mutation and recombination, and delicate biological agents as well as, they can be eradicated by exposure to sunshine, desiccation, and other environmental factors such as extreme pH values, whether acidic or alkaline, which can disturb the structural integrity of phages, as an intense heat, which denatures proteins, or freezing and thawing cycles to which antibiotics typically exhibit resistance [11]. Phages can serve as engineered nanocarriers for the precise administration of therapeutic drugs and diagnostic reporter molecules, representing an innovative facet of nanotechnology in drug delivery systems [12]. One application of phages is the utilization of phage-based nanocarriers for antibiotic delivery. In this context, Vaks' group exhibited an antibody on the pIII minor coat protein of the f1 phage and chemically coupled it with chloramphenicol as a specific antibiotic. The conjugation occurred between the amine group of neomycin-chloramphenicol and the carboxyl group on the phage surface [13].

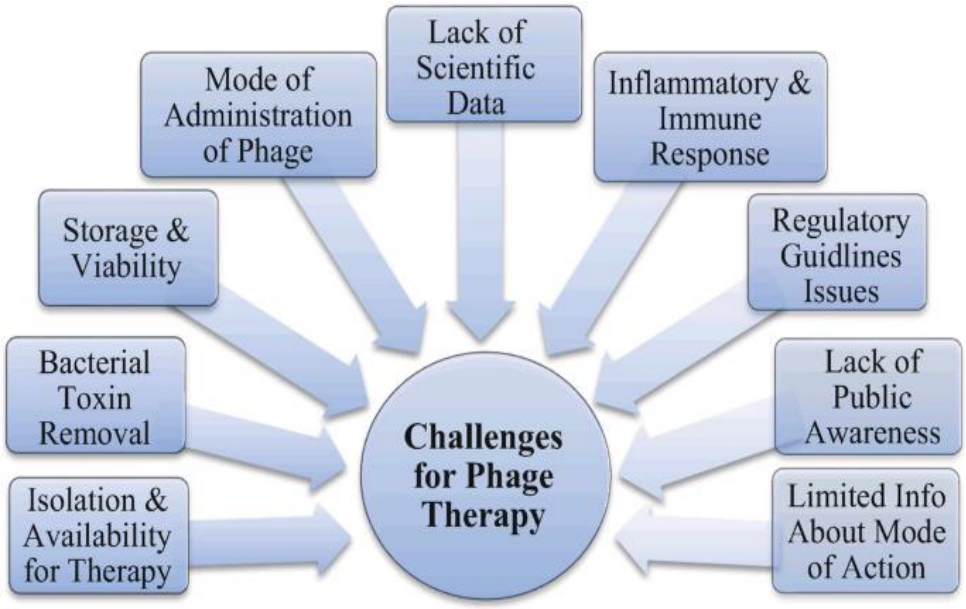

Figure 1: Difficulties and Key Challenges in the Use of Phage Therapy [14].

2. Understanding Antibiotic Resistance

The term "antibiotic resistance" refers to antibiotic-bacterium combinations, as it describes the mechanism of resistance exhibited by bacteria when exposed to antibiotics that would typically impair their viability or reduce their virulence [15]. It is essential to recognize that bacteria do not develop "resistance" while in the laboratory, on media, or in nature; determinants of resistance are only identified in the presence of the antibiotic they counteract. In addition, throughout the emergence of multiple classes of antibiotics from the end of the 19th century to the present, hundreds of resistance determinants have been detected across a wide range of bacterial species, highlighting the specificity of the term "antibiotic resistance." [16]. However, distinctly different antibiotics may share resistance determinants even though they target quite different cellular sites and mechanisms, such as RNA, ribosomes, enzymes of the cell wall, cytoplasmic membrane, and others [17]. For deeper understanding, the classical scope of basic and clinical microbiology extends to encompass the complex domains of molecular genetics, gene expression, and the biochemistry underlying the biology of bacterial resistance [18]. To elucidate "the mechanism" during the process of post-antibiotic recovery in resistant bacteria, it is essential to adopt a comprehensive and holistic perspective [19]. During the cell lysis, where both resistant and non-resistant bacterial cells are affected, an intriguing phenomenon emerges. Phages, the viral agents of infection, seize the opportunity to invade the vast array of available bacteria in an unrestrained and unhindered manner. This phenomenon is supported by a comprehensive survey that encompasses numerous cases involving resistance against casual bacterial infections caused by viruses [20]. Furthermore, a selected group of resistant bacteria, struggling to persist under laboratory conditions, face the challenges of contending with three distinct phages. Remarkably, this combination of phages exhibits an astonishing capability to effectively eliminate both the resilient cells, and the overall population of phages multiplies exponentially [21].

2.1. Mechanisms of Antibiotic Resistance

Antibiotics are chemicals that can specifically kill or inhibit bacteria. Soil bacteria produce most antibiotics and show toxicity to only microorganisms, enabling antibiotic use to show medical benefits by saving the lives of individuals infected with bacteria that would otherwise cause widespread disease and mortality. The misuse of antibiotics has led to the generation of antibiotic-resistant bacteria. Antibiotic resistance results from genetic mutations and other evolutionary processes that enable certain strains of bacteria to survive despite the presence of the drug [22]. In the 140 years since the first introduction of antibiotics, an increasing number of bacteria have developed resistance to effective medicines. Penicillin, which was produced from 1940 onwards, was initially effective in treating virtually all staphylococcal, streptococcal, and meningococcal infections, ultimately leading to many previously fatal diseases, such as pneumonia, scarlet fever, diphtheria, rheumatic fever, and syphilis, were essentially removed from the list of life-threatening illnesses [23]. Fifty years later, less than 10% of

Staphylococcus aureus, the most omnipresent bacterial infection, was resistant to penicillin, leading to the widespread use of methicillin, which was more effective in treating resistant strains. Nevertheless, after 10 years, more than half the staphylococcal strains in hospital patients showed moderate to high levels of methicillin resistance [24]. Four members of the penicillin group of antibiotics such as penicillin, ampicillin, amoxicillin, and methicillin are now useless as staphylococcal treatment. The first generation of antibiotics demonstrated effectiveness against many gram-negative microorganisms, leading along with the introduction of penicillin and streptomycin, with the discovery of vaccines for various diseases and antibiotics that targeted the only important species of bacteria, would soon render microbes powerless in their ability to harm mankind. However, within a few years, there was recognition that the population of penicillin-producing mould contained a few mutants capable of producing penicillin acylase. This enzyme reacts with penicillin to hydrolyze the molecular bond at its β-lactam ring, thus destroying the drug's capacity to inhibit bacterial growth, as shown in Table 1 [25].

Table 1: Antimicrobial classifications according to their mode of action [26].

Mechanism of Action	Antimicrobial Groups
Inhibit Cell Wall Synthesis	β-Lactams, Carbapenems, Cephalosporins, Monobactams, Penicillins, Glycopeptides
Depolarize Cell Membrane	Lipopeptides
Inhibit Protein Synthesis	Bind to 30S Ribosomal Subunit, Aminoglycosides Tetracyclines, Bind to 50S Ribosomal Subunit, Chloramphenicol, Lincosamides, Macrolides, Oxazolidinones, Streptogramins
Inhibit Nucleic Acid Synthesis	Quinolones, Fluoroquinolones
Inhibit Metabolic Pathways	Sulfonamides, Trimethoprim

2.2. Global Impact of Antibiotic Resistance

Nobel Prize-winning economist Milton Friedman once wrote, "Nothing is so permanent as a temporary government program." The purpose of this essay is to demonstrate that nothing is so permanent as a temporary and widely criticized lifesaving drug often proves to have a lasting impact whether people understand that antibiotics can save lives. Despite proving the benefits of antibiotics, underestimation of these advantages has been neglected. Their consistent misapplication contributes to worsening the problem of antibiotic-resistant bacteria [27]. Antibiotic resistance has a profound and widespread impact globally, influencing numerous aspects of society, including healthcare and the economy (Table 2). The issue is closely tied to the field of antimicrobial medications, an industry heavily dependent on and closely tracking advancements and investments in antibiotic research [28]. Nevertheless, the market for conventional antibiotics has been progressively declining, which creating significant challenges for healthcare professionals in the treatment of bacterial infections; one of the most concerning consequences of this decline is the diminishing availability of effective treatment options., which increases antibiotic resistance, leading to more challenging to combat bacterial infections effectively [29]. These limitations raise serious concerns, as it has an impact on vertebrate populations in addition to human health. When animals are infected with resistant bacteria the risk of these strains spreading to humans and causing infections increases substantially [30]. Additionally, the problem is exacerbated by the use of antimicrobial medicines in animals. Antibiotics are sometimes administrated prophylactically to maintain their overall health, even in the absence of bacterial infections. Unfortunately, this practice has resulted in further complications including that the animals with frequent exposure to these drugs are rapidly developing multi-resistant bacteria. The implications of this phenomenon are alarming, emphasizing the imperative necessity for comprehensive strategies to address antibiotic resistance in both humans and animals [31]. In conclusion, the global consequences of antibiotic resistance are vast and far-reaching. The urgency to act quickly and concerted work to address this pressing issue is highlighted by the limited treatment options, the increasing risk of human infections due to resistant bacteria in animals, and the diminishing market for traditional antibiotics. It is essential to prioritise and control the misuse of antimicrobial medications, public awareness, and research to prevent its further escalation [32].

Table 2: Antibiotic resistance in *S. aureus*, *K. pneumoniae*, and non-typhoidal salmonella (NTS) across the six World Health Organization (WHO) regions. The data originate from the WHO [33].

WHO regions		***S. aureus*** **resistance to methicillin (MRSA)**	**NTS resistance to fluoroquinolones**	***K. pneumoniae*** **resistance to third-generation cephalosporins**	***K. pneumoniae*** **resistance to carbapenems**
Africa region (47 countries)	Countries with national data	9 (19.1%)	9 (19.1%)	13 (27.6%)	4 (8.5%)
	Range (%)	0–100	0–35	8–77	0–4
	Country with lowest/highest proportion	Lesotho/Guinea-Bissau	Central African Republic/Mauritania	Namibia/South Africa	Central African Republic/South Africa
Region of the Americas (47 countries)	Countries with national data	15 (31.9%)	13 (27.6%)	17 (36%)	17 (36.2%)
	Range (%)	21–90	0–96	4–71	0–11
	Country with lowest/highest proportion	Canada/Chile	Several countries/Peru	Canada/Peru	Canada-Dominican Republic/United States of America
Eastern Mediterranean region (23 countries)	Countries with national data	4 (17.4%)	4 (17.4%)	4 (17.4%)	4 (17.4%)
	Range (%)	10–53	2–49	22–50	0–54
	Country with lowest/highest proportion	Bahrain/Iran	Oman/Jordan	Oman/Bahrain	Oman/Iran
European region (53 countries)	Countries with national data	36 (67.9%)	29 (50.9%)	33 (62.3%)	31 (58.5%)
	Range (%)	0.3–55	0–21	2–82	0–68
	Country with lowest/highest proportion	Norway/Portugal	Several countriesa/Finland nd	Sweden/Georgia	Several countriesa/Greece
South-east Asia region (11 countries)	Countries with national data	3 (27.3%)	2 (18.1%)	4 (36.4%)	4 (36.4%)
	Range (%)	10–26	0.2–4	34–81	0–8
	Country with lowest/highest proportion	Bhutan/Myanmar	Thailand/Nepal	Bhutan/Sri Lanka	Bhutan/Myanmar

3. Phage Therapy: A Historical Perspective

The first studies using viruses as antimicrobials started in the late 1800s, following the discoveries made by Ernest Hankin in 1896 and Frederick Twort in 1915. Two years later it has been reported that a filtrate from *Staphylococcus cultures* was capable of lysing bacterial cells, following the characteristics of viruses, Felix d'Herelle reported similar results for Shigella in dysentery patients. Twort's isolation was lost, and a commercial phage therapy industry was established, which reported minimal if any, adverse reactions. Research on these "natural antibiotics" significantly contributed to our understanding of life and showed that genes were made of DNA by injecting the molecule through the bacterial envelope. However, reports by some physicians of failed treatments, sometimes adverse effects, and the growing enthusiasm for new chemical antibiotics caused the gradual decline of phage therapy as a medical intervention in Western countries [34]. The first established therapeutic use of bacteriophages occurred in 1919 when a filtered preparation of Shigella-specific phages was applied to dysentery patients. Subsequent research studied the effects of using bacteriophages to treat cholera, postoperative abdominal infections, and urogenital bacterial complications, as shown in Table 3. Eastern European

countries achieved significant clinical successes in using bacteriophages, later known as phages, to treat various bacterial infections, including those caused by a variety of Gram-negative and Gram-positive pathogens. Bacteriophages were used to treat bacterial infections of the skin, gastrointestinal, urinary, and reproductive systems, septicemia, osteomyelitis, peritonitis, and burn and wound infections, as well as lung infections during the pre-antibiotic era and beyond [35]. Phages are obligatory intracellular parasites of hosts with varied life cycles (**Fig. 2**). The life cycles encompass lytic, lysogenic, and pseudolysogenic phases [36]. During the lytic cycle, the phage initiates the synthesis of new viral progeny immediately post-infection and then releases them by lysing the host cell. During the lysogenic cycle, the phage genome, referred to as a prophage, replicates alongside the host DNA, either integrated into the host's chromosome or existing in a free, plasmid-like form, establishing a prolonged stable cohabitation with the host [37]. Prophages transition from the lysogenic stage to the lytic cycle, culminating in a virion release under stress conditions [38]. A recent study described phage hunting as the biological equivalent of early drug discovery. The first commercial phage product, known as Pyophage, is now being assembled and packaged for the treatment of Staph. aureus, *E. coli*, and *P. aeruginosa* pathogens Bacteriophages are not only highly effective antibacterial agents for managing various bacterial infections but also stand out as promising anti-infective agents because of their unique potential to be self-replicating agents and bactericidal effectors that transfer antibiotic resistance-embedding enzymes across broad host ranges. That is, the phage-encoded enzymes can selectively target and eleminate antibiotic-resistant pathogens without affecting the surrounding commensal bacteria [39].

Table 3: Bacteriophage Therapy: historical development and controversies [40].

Year/Period	Event/Development	Significance
Late 20th Century–Present	Antibiotic resistance revives phage therapy.	Reconsidering phage therapy for multidrug-resistant bacterial infections.
1970s	Phage treatment withdrew from major drug markets.	Phage therapy was marginalized by economic and logistical issues, favouring antibiotics.
Cold War Era	Phage therapy was marginalised in the West due to its Soviet affiliation.	Political biases called phage therapy "un-American."
Post-World War II	Antibiotics and pharmaceutical supremacy have reduced phage therapy.	Western medicine abandoned phage therapy because antibiotics became easier, broad-spectrum, and widely available.
1945	Phages are viruses, according to a third AMA report with visual confirmation.	Changed phage understanding, but antibiotics hindered practical applicability.
1941	Second AMA report supporting the lytic enzyme theory.	Two AMA studies support lytic enzyme theory.
Late 1930s	Electron microscope view of bacteriophages.	Verified phages' viral nature and supported d'Herelle's theories visually.
1934	First AMA report on bacteriophage therapy.	Phage therapy outcomes were confusing and contradictory, indicating early standardisation issues.
1920s–1930s	Debates about phage biology (viral vs. lytic enzyme).	Disputing scientific theories, d'Herelle favoured viral nature and others enzyme theory.
1919	Initial phage-based avian typhosis prevention experiments.	Proven phage use for bacterial infection control.
1917	Dysentery fluid tests found bacteriophages.	Recognized bacteriophages as viruses and suggested in vivo antibacterial application.

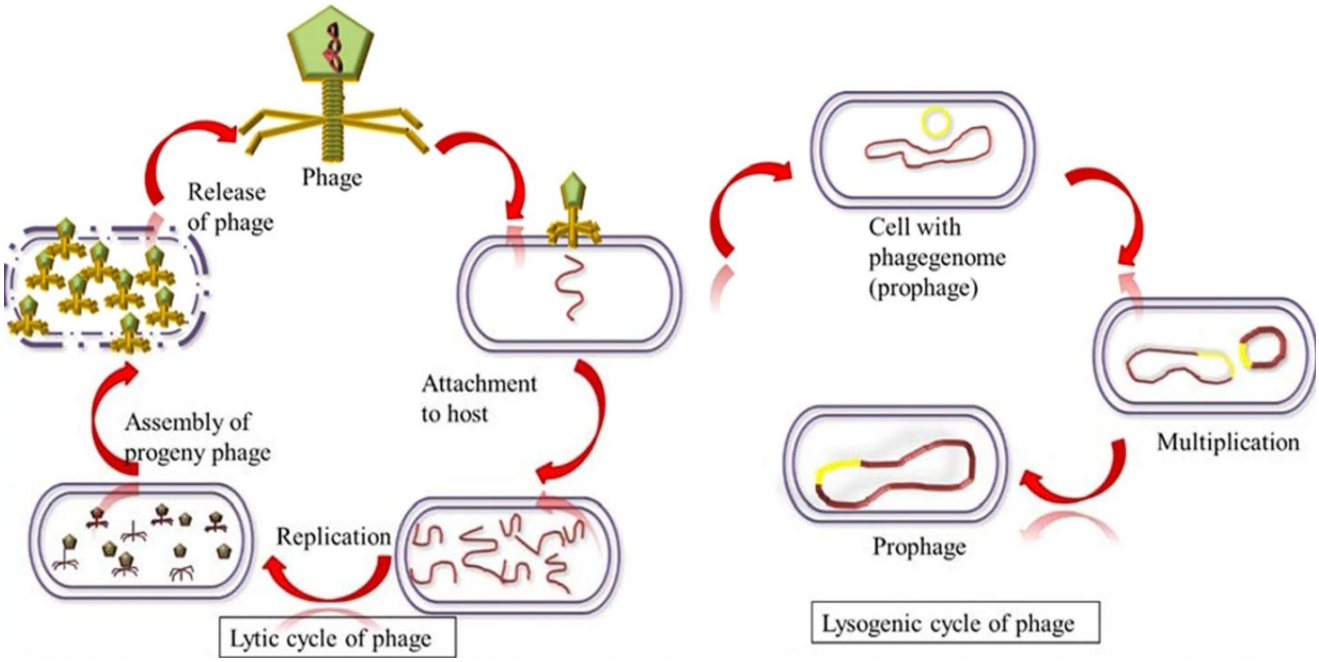

Figure 2: Life cycle of phages [41].

Since their discovery in 1917, bacteriophages have been used to control bacterial disease. Phages have been used to treat urinary tract infections, as well as other skin, mouth, and intestinally-related infections [42].

4. The Potential of Phage-Antibiotic Synergy

Antibiotic treatment is often highly toxic to the human microbiome, eradicating many species and populations of harmless commensal microbes. This disruption can increase vulnerability to infections caused by multi-drug-resistant bacteria. In contrast, bacteriophages are gaining attention as narrow-spectrum antimicrobial agents due to their ability to rapidly and precisely target specific bacterial hosts, offering a promising alternative to mitigate these issues and reduce overall antibiotic dependence. However, phage monotherapy is currently being applied in compassionate care, in a phase I/II study and other experiments, it is not without risk or challenges. One significant observation is that phage resistance in multi-drug resistant bacteria regularly occurs as a fitness-neutral mutation which arises in cultures exposed to phages [43].

To improve the efficacy of orally administered phages and provide early containment and protection againts antibiotic-resistant bacterial populations in multi-drug resistant *E. coli* associated with necrotizing enterocolitis in premature neonates, the use of phage-antibiotic synergy has been explored [44]. Phage-antibiotic synergy is the enhancement of phage-infected bacterial clearance when host bacteria are exposed to conventional antimicrobials. It is relatively simple to demonstrate synergy, as a decrease in the phage-independent colony-forming units of particular *E. coli* strains can be observed using Guardsman LB agar in the presence of sublethal doses of tandoori and doxycycline [45]. However, in part, the mechanisms behind this phage-mediated increase in these antibiotic clearance rates have been poorly understood, some evidence suggests that this effect may be linked to the activation of stress pathways. Moreover, most in vitro studies on phage-antibiotic synergy have been based on a limited number of commonly used antibiotics and host ranges. More researchers are trying to expand the number of known synergistic pairings of clinically relevant antibiotics and lytic phages as well as exploring their expanding host ranges [46]. The term "phage-antibiotic synergy" refers to the integration of phage treatment and traditional antibiotics to enhance their respective effects. This can include mutual potentiation, where the presence of one agent boosts the effectiveness of the other, resulting in higher efficacy against bacteria when combined. Other synergies can occur, such as phage-antibiotic resistance antagonism, where neither agent is effective alone, but becomes effective when combined, or using phages to boost the activity of existing antibiotics [47]. Different mechanisms for phage-antibiotic synergy have been proposed to explain phage-antibiotic synergy, such as the activation of the SOS response in bacteria or phages promoting biofilm disruption, antibiotic penetration, or

resuscitating dormant persisted cells (**Fig. 3**). These mechanisms have been categorized into various groups according to the bacterial features enforced by phages: phages weaken the bacterial wall/membrane; phages impair bacterial DNA repair systems; phages are prevalent only in nutrient-rich conditions; or phages detach bacteria from biofilms. Additionally, it has been discovered that phages can also exert their antibacterial effects by modulating bacterial immune responses through the upregulation of antimicrobial peptides or downregulation of virulence factors [48]. These immunomodulatory properties of phages enhance the overall effectiveness of phage-antibiotic synergy and provide further insight into the complex interaction between phages and bacteria. Moreover, recent studies have revealed that phages can act as vectors for direct antibiotic delivery to target bacterial cells, thus increasing the local concentration of antibiotics and improving their efficacy. This novel strategy holds great promise for the development of more targeted and efficient antibacterial therapies [49].

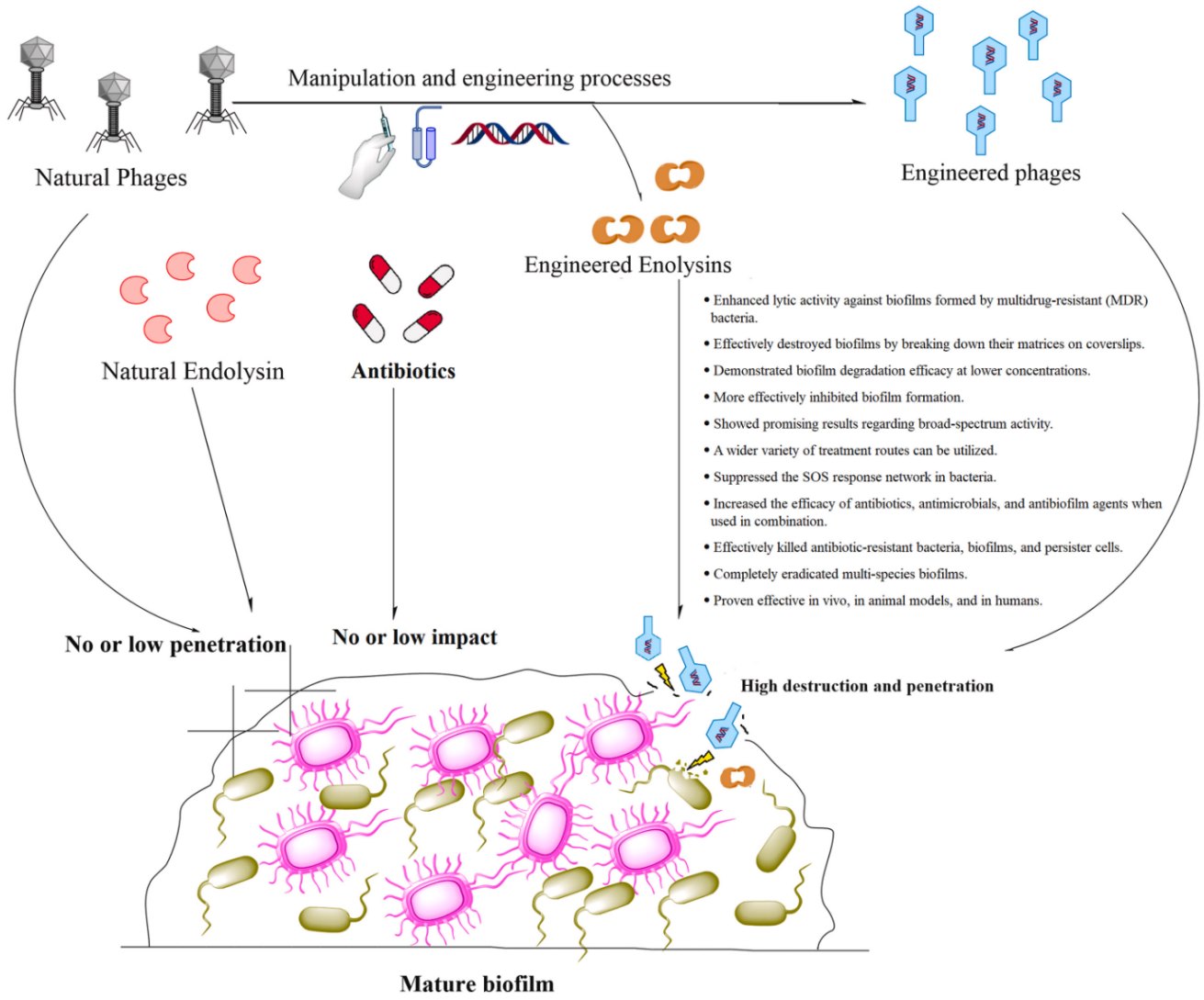

Figure 3: Phage-Antibiotic Synergy in Combating Biofilm Formation [50].

5. Experimental Evidence and Case Studies

In addition to the promising in silico evidence that phage therapy may be enhanced significantly through combination with small concentrations of antibiotics and several interesting observations of the bacterial killing capabilities of combinations, many researchers have tried to investigate the physiological interactions between phage and antibiotics to quantitatively understand the specific conditions under which synergy is likely to occur in practice (**Fig. 4**) [21]. Combined in vitro and silico study of Functionalized Lysine and piperacillin, carbenicillin, and medium concentration of chloramphenicol, the data generated in this study on the minimal antibiotic

concentration required to suppress wild-type bacterial growth in the absence of phage can generally be compared with the results of aspiration of the three species at the beginning of two resting cycles. Additionally, some data suggest the potential for slight enhancement of the bacterial killing capabilities of Functionalized Lysine and piperacillin small concentrations of carbenicillin are included. The observed increase in the degradation rate dynamics of ceftriaxone studied alongside Functionalized Lysine and piperacillin may relate to this potential as well. Overall, the increase in sensitivity of phage to concentrations of pH was hypothesized in the first place and studied more intensively to understand which parameters led to an enhanced phage replication and, consequently, enhanced degradation of the wall [51].

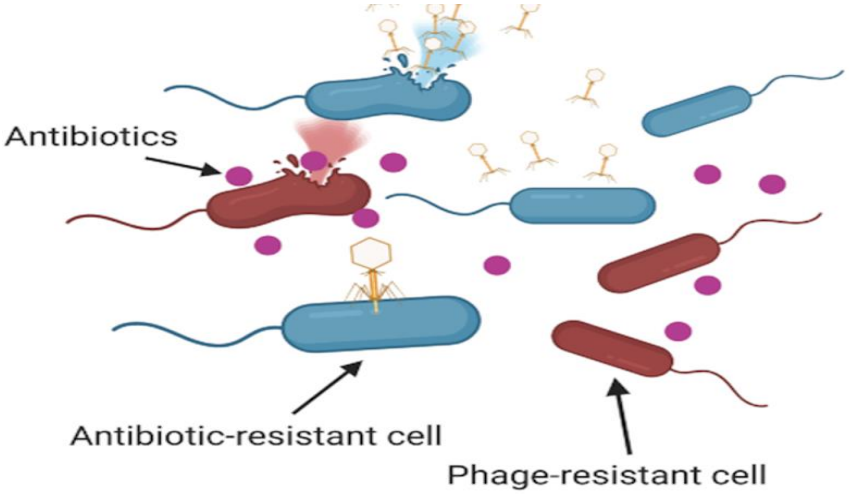

Figure 4: Phage-antibiotic combination Therapy, a dual strategy to combat antibiotic and phage resistance in bacterial populations [52].

It has been hypothesized that applying bacteriophages to environments with either increased or depleted antibiotic levels to attenuate the emergence of antibiotic resistance and potentiate depleted antibiotics; however, data addressing this question in intact populations remains limited. By contrast, there is a substantial amount of data regarding bacteriophage contributions to antibiotic incremental effect in vitro environments where growing cultures of phage-insensitive bacteria are treated with low concentrations of antibiotics (Table 4). These studies demonstrate that bacteriophages can potentiate beta-lactam antibiotics at sub-inhibitory concentrations to sterilize environments whose high nutrient densities exert selective pressures that favour the growth of mutant phenotypes belonging to various genera. The observed potentiation occurs in several mechanisms; reductions in bacterial growth rates and bacterial density, secretion of public goods that synergize with beta-lactam inhibitors, and increased inner membrane permeability that sensitizes bacteria to antibiotics by allowing increased access rates of antibiotics to their targets [53]. More recent studies have proposed that anti-phage systems abate the proton motive force of bacteria infected by double-stranded DNA bacteriophages to elevate the influx gene expression levels of genes that code for the secretion of toxins and for outer membrane proteins that allow the influx of low molecular weight antibiotics. The results documented that mid-exponentially growing phage immunotypes of Salmonella behaved synergistically in the presence of sub-inhibitory concentrations of the beta-lactam monobactam. The observed synergy was implacable until an 80,000-fold dilution of phage (dilutions of less than 80,000-fold supported filamentous growth of bacterial cells) [54].

Table 4: Overview of recently documented case reports of phage treatment.

Year	Target	Results	Causes of Failure	Reference
2016	*E. coli*/*Proteus* (children's diarrhea)	Poor clinical efficacy of oral phages	Not enough phage spread; not enough *E. coli* titers; too much *Streptococcus* growth	[55]
2019	*P.aeruginosa*/*E.coli* (burn infections)	The trial was terminated due to insufficient efficacy.	Lower the amount of phage in preparations	[56]
2021	*Staphylococcus, Streptococcus, E. coli, P. aeruginosa Proteus* (urinary tract infections)	Success rates are about the same as with a dummy (bladder irrigation).	Bacterial load reduction by bladder irrigation with placebo is comparable to phage treatments.	[57]

6. Optimizing Phage-Antibiotic Combinations

Administration of phage and antibiotics in combination can result in one of three outcomes: immediate antagonism, mutual tolerance, or short-term synergism based on phage-mediated antibiotic tolerance change, as shown in **Fig 5.** To maximize the chance of achieving synergism, which may optimize the clinical use of both agents for therapies, phage selection based on specific phenotype changes, when combined with a particular antibiotic, is essential. Against Pseudomonas aeruginosa, pairing phages with antibiotics did not show a complete shift to suppression. Unlike previous studies, combining LPS phages with a broad host range phage did not suppress synergy, which raises important questions about the best approach to resolve this issue [58]. Phage-resistant bacterial evolution during an untreated infection can last from days to decades but frequently restricts the success of phage therapy. Synergistic application of phages and antibiotics can suppress bacteria-evolved phage resistance in the bacterial population under selection, suggesting phage therapy and most types of antibiotics could be applied over a longer period as a sequential strategy to control bacterial pathogenicity. In this case, phage persistence in the environment would improve the effectiveness of the last phage treatment and represent a minimal environmental threat. In contrast to a phage-alone application, human and animal contact with phage-resistant bacteria would also be minimized if such a treatment could prevent resistance to phages and antibiotics [59].

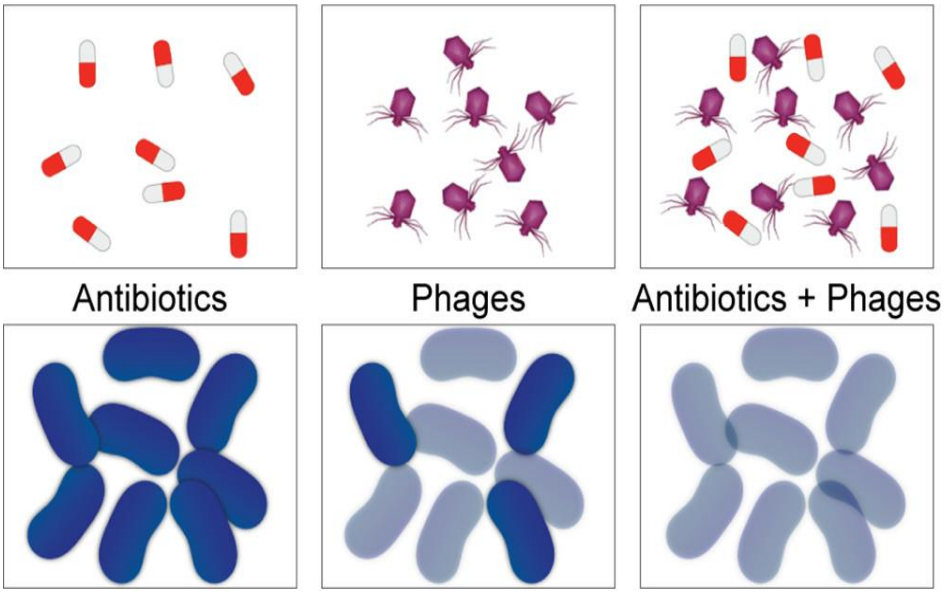

Figure 5: Short-term synergism based on phage-mediated antibiotic tolerance [60].

The use of bacteriophage-antibiotic synergy to fight resistant bacterial infections holds potential as a successful alternative strategy when phage therapy alone lacks efficacy. It is well known that resistant bacteria are not only present in medical environments but also in natural reservoirs, which is one of the challenges for bacteriophage-based therapy. This, combined with the potential for personalized medicine via phage therapy, makes it expedient to develop new feasible combinatorial therapies with classic antibiotics (Table 5) [61].

Before starting phage-antibiotic synergy studies, it is important to select potent phages as well as an effective antibiotic that works in conjunction. Before selecting phages from the environment and using them to combat resistant bacteria via phage treatment, it is necessary to investigate the principal characteristics of the isolated phages. First, the isolated phages should have a broad host range, high titer, and long shelf life. Second, analysis of the genome should reveal that the phages have a high level of genetic and morphological stability and no harmful genes. These analyses are critical, as using a lytic phage creates the risk of deleterious effects on the host bacteria. Third, the isolated phages should not affect or mutate the bacterial host to have a bacterial growth-supporting effect, which is a common phage characteristic [62].

Table 5: A summary of the documented synergistic action of antibiotics and bacteriophages.

Case	Antibiotic (Dosages)	Bacteriophage (Dosages)	Therapy	Period	Route of Administration	Single Phage/Phage Cocktail	Microorganisms of Interest
[63]	Colistin (150 mg every 24 h)	Phage (10^8 PFU/mL)	Sequentially (phage initially, followed by colistin after 8 hours)	Two weeks	Colistin administered intravenously and a local delivery system phage that has not been specifically named	phage that has been purified	MDR *P. aeruginosa*
[64]	Ofloxacin (30 and 60 ng/mL), gentamicin, and ampicillin (5 μg/mL)	Viruses called lexA3 that have been modified (108 and 109 PFU/mL)	Simultaneous	1–6 h	Unspecified	Not specified	*E. coli* infections
[65]	Rifampicin (0.6 mg/L)	Phage SAP-26 (10^8 PFU/mL)	Simultaneous	2–24 h	Unspecified	Phage SAP-26	*S. aureus* biofilms
[66]	Gentamicin (100 × MIC)	Phage SA5 (10^7 PFU/mL)	Unspecified	24 h	Unspecified	Phage SA5	*S. aureus*
[67]	Meropenem (128 and 256 mg/L)	KARL-1 bacteriophage	Unspecified	24 h	Unspecified	Phage KARL-1	MDR *A. baumannii*

7. Future Directions and Emerging Technologies

While phage-antibiotic synergy is widely acknowledged as a tremendously promising technique to combat bacterial infections effectively, much of the current work conducted in this field remains predominantly preclinical. Therefore, it becomes imperative to conduct extensive in vivo studies, as well as phase I/II clinical trials, to advance and establish this form of therapy as a viable treatment option. Rigorous testing will be answering key to critical questions about combination therapies, such as the optimal timing, duration, and appropriate combinations of phages and antibiotics for treating specific infections [68]. Furthermore, comprehensive mechanistic studies that explore the fundamental physiology underlying the activity of these combination treatments are essential for advancing their effectiveness and understanding. Gaining a deeper understanding of

how these combinations manifest their therapeutic effects will undoubtedly contribute to their further refinement and optimization. Although the concurrent use of phages and antibiotics offers numerous advantages and demonstrates the potential to mitigate the development of resistance, it is equally crucial to acknowledge the inherent limitations of these treatments. To strengthen the efforts in the ongoing battle against resistance, the pursuit of more advanced technologies appears both necessary and worthwhile [69]. Emerging technologies, such as phagemids, combinatorial cassette exchange, or leveraging T7 display and manipulating phages using CRISPR, hold incredible promise in combating stubborn and challenging infections [57, 58]. These innovative approaches can potentially overcome the barriers posed by traditional therapies, paving the way for novel solutions. As we strive to alleviate the burdens imposed by infectious diseases [69]. It is abundantly clear that leveraging newer technologies and embracing the synergistic combinations of various tools will ultimately prove pivotal in our pursuit of effective treatment strategies [70].

8. Conclusion and Implications for Clinical Practice

In conclusion, our literature assessment indicates that phages, when used as supplements to antibiotics, can enhance and/or extend the antibacterial efficacy of antibiotics amidst rising bacterial resistance. The impact of phage and antibiotic combinations on mammalian cells, particularly in humans, remains unpredictable. Still, previous animal studies give reason for optimism that the combination of phages and antibiotics might be safe as well as effective for eradicating infectious bacteria in humans. Phage-based drug development has significant potential for enhancing therapeutic approaches in various medical domains. Ongoing study, invention, as well as cooperative effort are crucial for fully exploiting the promise of phages in combating bacterial infections and other complicated diseases.

Conflict of Interest

The authors declare that they have no conflict of interest.

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