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Review article

A review on phage therapy

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SUMMARY

The world's health is currently under threat from antibiotic resistance, as more and more infections become practically impossible to treat or extremely difficult to manage. As a result of this circumstance, there are now more illnesses, deaths, and financial obligations. One intriguing treatment option among the many approaches to combat antibiotic resistance is the use of bacteriophages, viruses that infect and kill bacteria. Despite its initiation almost a century ago, phage therapy faced a setback following the successful introduction of antibiotics. However, in the current landscape marked by rising antibiotic resistance, phage therapy is experiencing resurgence. This review provides an overview of phage therapy starting with its historical origins dating back to the early 20th century, it discusses the mechanism of phage action, results of human clinical trials, also addressing the significant barriers hindering the use of phages in clinical settings. Finally, this review discusses future directions and opportunities for integrating phage therapy into clinical practice.

Keywords: antibiotic resistance, phage therapy, bacteriophage, clinical trials

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INTRODUCTION

The discovery and implementation of antibiotics in clinical practice in 1928 marked a ground breaking advancement in medicine, saving millions of lives by effectively treating severe infections [1]. However, the unforeseen consequences of widespread antibiotic use have led to the development of multiple resistance mechanisms in bacteria, leading to the decline of the golden age of antibiotics [2]. In the post-antibiotic world that we now inhabit, even seemingly little wounds or illnesses can have potentially fatal consequences (WHO, 2014). More than 2.8 million antibiotic-resistant illnesses and over 35,000 fatalities are reported to occur in the US each year [3].

The diminishing efficacy of antibiotics has paved the way for the rise of resistant pathogens, posing a substantial threat to public health. Infections caused by multi-drug-resistant (MDR) bacteria, both in environmental and healthcare settings, have become a major concern [4,5]. The worldwide burden of antimicrobial resistance (AMR) was evaluated in a thorough analysis carried out in 2022 using data from 2019 that included literature studies, hospital systems, surveillance systems, and other sources. In all, 471 million individual records or isolates and 7585 study-location years were analysed. About 4.95 million deaths were attributed to bacterial AMR, with Sub-Saharan Africa having the greatest mortality rate and Australasia having the lowest. 1.5 million resistance-related fatalities were caused by respiratory illnesses [6]

Research shows that in 2019, 929,000,000 deaths attributable to antimicrobial resistance (AMR) and 3.57 million deaths related to AMR were caused by the priority pathogens identified by the World Health Organization (WHO), which included *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* [4,6]

The rising prevalence of multidrug-resistant (MDR) organisms necessitates the exploration of innovative non-antibiotic therapeutic approaches. A current focus involves the extensive development and utilization of bacteriophages as a novel yet age-old antibacterial treatment option [7]. In the current era, a century after their discovery, there is a renewed interest in phage therapy, utilizing bacteriophages as novel therapeutic agents against MDR infections. Bacteriophages are viruses, and as obligate cell parasites they are capable of infecting and replicating within bacterial cells. They are abundant and ubiquitous entities, serving as tools in molecular biology, gene recombination, horizontal gene transfer, and contributing to microbial physiology, population dynamics, and therapeutics [8]. Phage treatment uses lytic and designer phages, phage proteins, or phages in conjunction with antibiotics to eradicate harmful bacterial strains without altering the treated patient's normal microbiota. Phages reproduce at the site of infection and do not require read ministration when they replicate on the target bacteria. Phage titers decrease when bacteria are eradicated, and the patient's urine is the last place where phages are removed from their body [9]. Phage treatment has been successfully used in Georgia and Poland, although there are still barriers to its implementation in Western nations, mainly related to regulatory concerns [10]. Clinical trials for phage treatment are now being conducted in the US and Europe as part of ongoing attempts to increase its accessibility [10]. This study examines the situation of phage treatment today, emphasizing the main obstacles and upcoming prospects facing the discipline.

2. A Concise History of Bacteriophage Therapy

The initial observations of potential bactericidal activity linked to bacteriophages date back to 1896, were documented by Ernest Hankin's in the Ganges and Jumna rivers in India[11]. However, the clarity of these early findings remains subject to interpretation. Frederick Twort was the first to propose the involvement of a virus in this observed antibacterial activity in 1915 [12]. Twort, hindered by funding constraints, was unable to substantiate his hypothesis. The definitive discovery and nomenclature of bacteriophages occurred later, in 1917, by

Felix d'Herelle at the Institut Pasteur in Paris [13].

After drinking the phage therapy himself to confirm safety, d'Herelle began testing phage therapies on human patients suffering from acute dysentery at the Hospital des Enfants-Malades in Paris in 1917. Subsequent reports indicated initial success, with four patients exhibiting symptoms of recovery in less than a day. In 1921, Bruynoghe and Maisin conducted the first clinical study in France. They injected phages into surrounding skin lesions caused by staphylococcal infections, and the infection subsided in a matter of 24 to 48 hours [14].

Phage therapy began to spread in the 1920s, when thousands of people in India received treatment for a variety of illnesses, including cholera and the bubonic plague, using phage formulations. Phage therapies were brought to market by the 1930s and the beginning of the 1940s. At L'Oréal in Paris, D'Herelle started manufacturing five phage treatments, while the Eli Lilly Company manufactured seven phage therapeutics in the US. However, when antibiotic medications were more widely used, technological difficulties forced the US to terminate the programme. Phage treatment developed further in Poland, Russia, Georgia, and the former Soviet Union, but in the West, due to a dearth of studies that met contemporary clinical research criteria, skepticism was widespread.

Phage treatment has received a second look in the West as a result of the controlled animal trials that were published in English-Language Publications in the 1980s. and its promise has been recognized by a new generation of scientists. Phage products were approved in the United States to control food processing-related bacterial contamination. Some Western European nations have authorized the use of cannabis for medicinal purposes in recent years, notably Belgium and France. Phage therapies are being developed in the US by many organizations and are pending FDA clearance. As a result of the FDA's recent approval of "expanded access" experimental phage treatments in the United States, phage therapeutics have received media attention that has highlighted their safety and potential. This has generated enthusiasm and encouraged physicians to consider using phage treatments in conjunction with standard antibiotics [15,16].

3. Life cycle of a bacteriophage

Recent research on the life cycle of bacteriophages has cast doubt on the idea that viruses are inanimate objects. A novel small-molecule communication mechanism governing lysis–lysogeny choices in a temperate phage was seen in a research by Erez et al., which contradicted earlier theories on viral behavior [17]. A distinct method for compartmentalizing viral replication in phages is suggested by the discovery of a nucleus-like structure generated during the replication of phage 201F2-1 in *Pseudomonas chlororaphis*. These results suggest that viruses and bacteria and fungus, which are parasitic creatures that depend on hosts to complete their life cycles, may be related.

Microscopic phages have complex and varied structures when seen using transmission electron microscopy. Tailed phages are members of the order Caudovirales and have two key characteristics: a variable-sized tail and a capsid that contains genetic material in the form of DNA or RNA

The lytic cycle and the lysogenic cycle are the two unique life cycles that bacteriophages go through. Phages insert their genetic material into the cell after

initially attaching to certain receptors on the surface of the bacterial host during the lytic cycle. The phage genetic material is replicated and new phages are created by the host cell providing the building blocks and enzymes needed for this process. The host cell lyses as a result of phage-encoded proteins like holin and endolysin, which occur inside the cell. Small proteins called holins accumulate in the host's cytoplasmic membrane, making it possible for the endolysin to rupture the peptidoglycan and release the offspring phages. Although lytic phages are unique to certain bacterial species and have a limited host range, their capacity to generate vast numbers of offspring is a significant benefit in phage treatment. By employing a phage cocktail—a collection of phages—this restriction can be overcome [18].

However, temperate phages in the lysogenic cycle do not lyse the host cell right away. Rather, at some locations, their genome merges with the host chromosome. Prophage is the term for this phage DNA that is present in the host genome. The prophage is reproduced along with the bacterial genome in the host cell known as a lysogen. Though certain phage populations may implant their genome into the host chromosome, remaining dormant or changing the host's phenotype, using temperate phages in phage treatment poses some obstacles. As long as the bacteria are not under stress or in an unfavourable environment, the lysogenic cycle can continue forever. Bacterial SOS reactions, which are frequently brought on by antibiotic therapy, oxidative stress, or DNA damage, cause prophage production [18].

Recent findings involving phages that infect *Bacillus* species demonstrate their need on tiny chemicals known as "arbitrium" for communication and the execution of lysis–lysogeny choices. This physiologically relevant communication system elaborates on how a solitary phage prefers the activation of the lytic cycle when it comes into contact with a large bacterial population, taking advantage of the quantity of hosts. Progeny phages find it more advantageous to go latent and develop lysogeny when host numbers decline. To ascertain whether additional bacteriophages use comparable communication peptides or whether there is cross-talk between various bacteriophage species, more investigation of these results is imperative [17].

4. Current state (Experience in clinical trials)

Phage therapy for various illnesses first emerged in the early 1920s. But difficulties emerged in the 1930s as a result of uneven outcomes in phage trials, along with worries about safety and effectiveness resulting from problems with controls and improper characterization, manufacturing, and purification of phage preparations [19].

As such, phage treatment continued mostly in a few Eastern European nations where research provided strong support for its effectiveness in treating certain illnesses with few side effects. However, the lack of validation consistent with evidence-based medicine (clinical trials) adds to the reluctance of Western nations' regulatory bodies and physicians to embrace phage treatment [20]. Unambiguous effectiveness results from randomised controlled clinical studies are required to establish phage treatment as a feasible substitute for antibiotics [21]. As a result, a greater number of clinical studies have been carried out recently, albeit not all of them have been completed [20].

Wright reported on a phase I/II clinical trial that was approved by the Central

Office for Research Ethics Committees (COREC) and the UK Medicines and Healthcare Products Regulatory Agency (MHRA) for its randomization, double-blind methodology, and placebo control. In order to assess the safety and effectiveness of a phage preparation made up of six phages for treating otitis caused by antibiotic-resistant *Pseudomonas aeruginosa*, this experiment was conducted on 24 patients who had chronic otitis. On day 42 of the experiment, patients receiving phage therapy showed considerable improvements in clinical markers such ulceration, inflammation, type and quantity of discharge, and odour. However, only three of the 12 patients getting phage therapy appeared to be healed, and there were no significant side effects reported [22]. Some scientist conducted a second randomised, double-blind controlled study in 2009 that was more concerned with the safety than the effectiveness of a phage cocktail intended to treat venous leg ulcers (VLUs) by targeting *P. aeruginosa, Staphylococcus aureus,* and *Escherichia coli* [23]. In the first phage therapy experiment ever carried out in the US, 42 patients with VLU were recruited and treated topically for 12 weeks with either the phage cocktail or a saline solution (control) before beginning a 24-week follow-up phase.

Phage therapy was administered without any negative consequences; nevertheless, there were no significant differences in the frequency and pace of healing between the phage-treated group and the control group. The absence of evaluating the phages for infectivity on the bacteria causing venous leg ulcers (VLU) is the reason for this lack of divergence. According to the authors, a phase II efficacy study with a larger sample size and wounds infected with bacteria sensitive to the phage cocktail should be used to assess the effectiveness of the phage preparation [23].

Twenty-seven burn wound infection patients were enrolled in this multinational randomised controlled phase I/II clinical study from French and Belgian hospitals. They received either normal treatment (1% sulfadiazine silver emulsion cream) or phage therapy, which involved a mix of 12 lytic phages, at random. Both therapies were applied topically for seven days, and then the subjects were observed for an additional fourteen days. In comparison to the control group (normal care), the phage cocktail group showed relatively slower progress in reducing the bacterial load in burn wounds. Positively, the phage-treated group did not experience any negative side effects. Participants received significantly less phages than originally expected due to a considerable decline in phage titre during GMP manufacture, which was blamed for the phage cocktail's reduced effectiveness. It is noteworthy that the phage cocktail sensitivity of wound bacteria was not evaluated before to treatment, and in situations where phage therapy was unsuccessful, bacterial investigation afterwards indicated resistance to low phage dosages [24].

A phase I/II experiment was carried out by Nestlé (Switzerland) in association with the International Centre for Diarrheal Disease Research, Bangladesh, at the Dhaka Hospital [25]. In order to determine the safety and effectiveness of giving oral T4-like phage mixture or a placebo to children hospitalised with acute bacterial diarrhoea, researchers undertook a randomised, double-blind, placebo-controlled experiment between 2009 and 2011. Although the oral coliphages entered the gut, no phage replication was shown to provide any advantageous outcomes [25].

Higher oral phage dosages were required, and the phage cocktail's limited host range coverage—some strains were not infected—was blamed for the inability to enhance diarrheal outcomes. Without protective measures like encapsulating or neutralising stomach acid before delivery, oral phage application lowers the amount of phages that enter the intestine to levels too low to provide a discernible therapeutic impact [25].

It was later shown that acute bacterial diarrhoea was not primarily caused by *E. coli*. It was not possible to enhance the diarrheal outcomes even with an effective *E. coli* phage therapy. The clinical study by some scientists [23] highlight how important it is to determine the etiologic agent(s) causing illness and test for phage susceptibility prior to therapy. Phage therapy clinical trials must be carefully planned to prevent problems that might affect the way the medication is administered.

A clinical experiment was carried out with the goal of evaluating the safety, tolerability, and initial effectiveness of a phage cocktail consisting of three lytic phages administered intravenously to patients suffering from resistant chronic rhinosinusitis (CRS) brought on by *S. aureus* [26]. Only individuals with a clinical isolate that was susceptible to the phage cocktail were taken into consideration for this phase I, open-label clinical study. During the course of the nine patients' 14-day therapy, the twice-daily intranasal irrigation of phages was safe and well-tolerated; no significant side effects were noted. The authors emphasised the need for a randomised clinical study to establish the ideal dosing regimen and prove the phage cocktail's efficiency, even if the preliminary efficacy data were encouraging—two of the nine patients reported infection eradication [26]. Phage banks with well-characterized phages are essential to provide global access, as evidenced by the rising interest in phage treatment among doctors and patients and the corresponding rise in phage requests globally.

5. Current Challenges and Limitations in Phage Therapy

5.1 Quality and safety requirements

The safety of phage preparations is crucial to the effectiveness of phage treatment, which poses hurdles in terms of formulation and production. Large-scale phage manufacturing carried out in accordance with regulatory authorities' authorised Good Manufacturing Practices (GMP) is necessary for widespread medicinal use [27]. Specific rules for phage manufacture have not yet been created, despite the fact that phage production must adhere to strict pharmaceutical laws that guarantee high-quality standards [28]. A team of phage researchers has developed standards for the quality and safety of sustainable phage treatment products in order to close this gap. Phages that encode virulence factors, antibiotic resistance, or lysogeny must be excluded, among other conditions. This limitation, however, could make phage treatment less effective against other bacteria, such as Clostridium difficile, for which strictly virulent phages are unknown. Endotoxins and other contaminants must be kept to a minimum in phage preparations, but current purification techniques haven't produced the best results yet [29]. Since phages are living organisms, it is essential to create reliable, GMP-compliant manufacturing methods in order to avoid variation in phage preparations [28].

5.2 Stability of phage preparations

Phage preparations must be stable for them to be approved as medicines by regulatory

bodies and for therapy to be successful. A viable phage candidate for therapy has to have a long shelf life to ensure activity without a notable phage titre reduction during processing and long-term storage, since these drops might affect the effectiveness of treatment [24, 30]., A number of tactics have been devised to improve phage stability such as spray-drying, freeze-drying, extrusion dripping methods, emulsion, and polymerization procedures [30]. But phage kinds and formulations (liquids, gels, and powders) differ in terms of stability [31].

Encapsulation on other matrix, such as liposomes, alginate, cellulose, or other polymers, is an alternate method to extend the shelf life of phage storage [30]. Phage encapsulation has medicinal uses in addition to extending shelf life. phages must be shielded from severe circumstances seen in the human body, such as low pH or immune system clearance mechanisms to avoid phage inactivation energy [30, 32]. Studies have demonstrated varying impacts on the capacity of phages to clear illnesses, despite the immune system, in particular phage-specific antibodies, playing a role in phage clearance [32].

5.3 Efficacy of Phages Against Biofilms

Bacteria are mostly found in biofilm formations, which are surfaces covered in a self-produced matrix and adhered to by bacterial populations, both in natural habitats and within the human body [33]. Bacterial survival and persistence are greatly enhanced by biofilms, which also boost antibiotic tolerance. Although the dynamics of phage-bacteria interactions have been well investigated in planktonic cultures, there are extra difficulties and complexity associated with their unique dynamics in biofilms.

A matrix made mostly of proteins, lipids, polysaccharides, and extracellular DNA surrounds bacteria inside biofilms [34]. Through a number of hypothesised mechanisms, this matrix greatly affects a phage's capacity to destroy a biofilm. Phage diffusion is impeded and live cells inside the biofilm are kept from being infected by the matrix, which can also serve as a physical barrier to adsorb phages [35]. Phages have developed ways to overcome these constraints in response, and many of them encode depolymerases that break down capsular polysaccharides and give access to the surfaces of bacterial cells. Using a phage cocktail containing a variety of depolymerases may improve therapeutic efficiency since depolymerases are specific to distinct polysaccharides [36].The spatial organization of biofilms is crucial for phage infection, impacting nutrient availability, bacterial motility, metabolic state, and gene expression. Diffusion limitations and the proximity of cells affect phage movement through the biofilm, potentially leading to multiple phages infecting the same host cell and reducing progeny phages. However, local infection may disrupt the biofilm structure, facilitating its dispersal and removal [36].

5.4 Evolution of Bacterial Resistance to Phages

One major worry with phage treatment is the possibility of bacteriophage-insensitive mutants (BIMs) emerging and endangering the effectiveness of the therapy. Phage-resistant mutants arise often and are almost always present, according to recent research that have looked closely at bacterial resistance to phages [21]. In order to counteract phage evasion, bacteria use a variety of mechanisms. These mechanisms include: (i) blocking phage adsorption by modifying or losing bacterial receptors; (ii) preventing phage DNA entry through

superinfection exclusion systems; (iii) breaking down phage DNA using restriction-modification (R-M) systems, related systems (BREX, DISARM.), or CRISPR-Cas systems; (iv) using abortive infection systems to prevent phage replication, transcription, or translation; and (v) using cyclic oligonucleotide-based antiphage signalling systems [37].

5.5 Regulatory Framework of Phage Therapy

Phages are governed by pharmaceutical law and need marketing clearance for industrially made medical goods since they are categorized as biological substances by regulatory bodies [38]. For a phage product to be marketed, the regulatory frameworks of the United States and the European Union need evidence of safety, effectiveness, and quality that satisfies Good Manufacturing Practice (GMP) requirements [39, 40]. GMP compliance is a major financial burden and a major obstacle for hospitals and non-profit phage treatment centres. The law also specifies requirements for phages, such as lytic activity, potency testing, control over contaminants, and the lack of prophages and antibiotic resistance in the bacteria employed in manufacture. Although this stringent control works well for industrial fixed-composition phage cocktails, it is insufficient for patient-specific, customised phage cocktails that are not meant for widespread dissemination [40].

The goal of discussions between regulatory bodies and phage sponsors is to create more acceptable rules for customised phage therapy. The European Union permits special cases, including medicinal and herbal formulas, as well as non-routinely prepared advanced therapy medicinal products (ATMPs) used in hospitals under the supervision of medical practitioners within the same Member State [39, 40]. Exemptions for compassionate use are allowed for medications that are conducting clinical studies or the application procedure for a marketing licence [40].

Member states of the European Union are creating national solutions in response to the inadequate regulatory framework that currently exists. By establishing a national law for the magistral creation of customised phage medications, Belgium led the way in the development of phage treatment legislation [41]. According to this rule, a chemist will prepare a personalised medication based on a doctor's prescription after conducting laboratory tests and publishing a monograph evaluating the phage's quality. Despite being used in Belgium, this strategy gives the chemist and prescriber a lot of responsibility [42].

It is predicted that more clinical proof of phage treatment's efficacy and regulatory developments, as well as changes to the laws as they are now, will lead to the widespread use of phage therapy and other individually prescribed medications.

6. Strategies used for Phage Therapeutics

6.1 Approaches carried out for Phage Therapeutic Design

Phage therapy employs a variety of general strategies, such as: (a) fixed mixes or cocktails made up of multiple phage components; these can be purchased as pre-made or off-the-shelf solutions; (b) cocktails of phages that are periodically modified to include activity against additional circulating clones of the target bacterium or bacteria; these are referred to as modifiable; (c) customised phage therapy using a phage bank and precision techniques; these are referred to as sur-mesure; and (d) the use of *in vitro* adapted and genetically engineered phage therapeutics [15, 43].

Creating a preset mixture of phages to address the diversity within a single bacterial pathogen or many bacterial diseases is the method known as the "fixed phage cocktail." The purpose of this strategy is to create a phage preparation that may be used as a readily accessible antibacterial solution for prevention as well as therapy [31, 43]. This strategy is currently being extensively explored in the US and Western Europe since it fits in well with the current regulatory frameworks for the clinical development of antibiotics. The capacity to withstand the challenge of growing host resistance and the width of the lytic spectrum (host range) are important factors to consider in this strategy.

The fixed cocktail strategy is enhanced by the adjustable cocktail approach, which includes the phage components being added or removed on a regular basis. This helps to handle newly developing pathogen clones that might provide difficulties and broadens the mixture's host range. This approach makes it easier to create flexible off-the-shelf phage therapies that can be updated over time to successfully combat shifting epidemiologic circumstances and evolving bacterial resistance. Georgia has been utilising the changeable cocktail technique in phage therapy programmes for many years, going back to the Soviet period.

Products like pyophage and intestiphage, which are still in use in Georgia and Russia today, are prime examples of the complex phage products that have been produced and are constantly being updated. These medicines are frequently made to target numerous infections. The composition of these products is revealed by metagenomic research to be complex phage mixes [44].

6.2 Construction and Designing Fixed Phage Cocktails for Off-The-Shelf Use

The identification of possible therapeutic phages, assessment of their lytic capacity, comprehensive phenotypic and genomic investigation, and evaluation of therapeutic efficacy are critical phases in the development of phage therapies. The first step in the procedure is the assembly of therapeutic phage collections that are suited to the particular development approach being used. A set cocktail strategy requires a more restricted collection that satisfies predetermined criteria, but a personalized approach requires huge libraries to satisfy the needs of each particular patient [31,48]. Relatively simple techniques are used to isolate novel phages from a variety of sources, such as untreated urban and hospital waste, ambient waterways, or soil samples. In order to ensure broad application for the fixed cocktail technique, thorough characterization of phage lytic spectra requires testing against several panels of clinical isolates of the target bacteria. On the other hand, the personalised approach centres on the unique bacterial strain that the patient is experiencing and for which the therapy is intended [31, 48].

Several authors have explained the strategic design of fixed phage cocktails, which aims to provide optimal host range breadth, effectiveness, and durability [8, 45]. The phages with overlapping host ranges, safe genomic characteristics, phages that infect the same host using different receptors, phages that use receptors incurring a high fitness cost for the host, minimizing resistance rates, phages with anti-biofilm activity, phages that synergize with treatment antibiotics, phages that are non-immunogenic, and (i) phages suitable for manufacturing and guaranteeing long-term stability are all

included in this design. The combined effect of these factors enhances the phage therapeutic product's safety and effectiveness [45].

Given the wide range of resistance mechanisms evolved during millions of years of coevolution, the main challenge in creating phage cocktails that are consistently successful is minimizing resistance within the bacterial host. Resistance is a given when using phage treatment [44] , but it may be overcome by carefully and logically designing the cocktail [45].

Fixing phage cocktails for various kinds of bacterial pathogens necessitates consideration of the pathogen's genetic-phenotypic variety as well as the traits of the lytic phages that infect it. For example, it is well known that when it comes to Staphylococcus aureus, only a small number of phage components—or even a single phage, as demonstrated by Eliava's Staphylococcal Phage therapeutic—can be chosen to create therapeutic cocktails that successfully address a sizable diversity of global strains of the pathogen [46].

A phage cocktail composition that makes sense must take into account the significant genetic variety that the target pathogen exhibits. Concurrently, it is essential to create the cocktail in a way that inhibits cross-resistance. This means making sure that host mutants that are immune to one phage in the cocktail still have a vulnerability to the other elements. Using phage components that use different host receptors for infection is a calculated strategy in the selection of phages for a fixed cocktail that aims to improve product durability in the face of host resistance [31]. This approach protects against any alterations in a single receptor that would hinder infection because the host can still be successfully infected by other phages in the cocktail.

6.3 Degradation of Biofilm and Killing of Bacteria in Biofilms by Phages

One common issue in modern orthopaedic and surgical treatments is the presence of biofilm-forming bacteria, such as P. aeruginosa and *S. aureus*, colonising wounds, surgically implanted components, or catheters. Bacteria use biofilm development as a way to evade the immune system and increase their resistance to antibiotics. Biofilms are communities of microorganisms that are grown on biotic or abiotic surfaces. They are made up of bacterial cells embedded in a matrix made of proteins, polysaccharides, teichoic acids, and extracellular DNA, which is based on extracellular polymeric substances (EPS) [47].

A major obstacle to treating infections linked to biofilm development is the resistance of bacterial biofilms to chemical antibiotic treatment. This can hinder wound healing and result in chronic wounds that may need amputation or have lethal consequences. Numerous methods have been devised to control the development of biofilms in wound infections due to the challenges that biofilm formation brings. These methods concentrate on making biofilm-bound bacteria planktonic, which increases sensitivity to traditional antibiotic therapy, as opposed to eradicating the bacteria within the biofilm. It is possible to eradicate bacteria from biofilms by using various antibiotics that interfere with vital biological functions or by using endolysins that attack the bacterium's outer membrane [47].

Using lytic phages is a potential method of treating biofilm-associated illnesses that are resistant to antibiotics, including those caused by *S. aureus* or *P. aeruginosa*. A

number of labs, including ours, have shown how some phages may effectively infect and lyse not just planktonic but also biofilm forms of host bacteria. Phages must reproduce inside their hosts within the biofilm matrix in order to be effective against biofilms. They must also discharge their progeny into the surrounding environment for dispersal. This means that phage candidates that are effective against biofilms must both show a strong burst size upon infecting biofilm bacteria and disrupt the biofilm's EPS matrix. Nevertheless, further research and explanation are needed to fully understand the interactions and ideal qualities needed for phage anti-biofilm function. Bacteriophages have been found to be effective in suppressing P. aeruginosa and other bacterial biofilms in a number of recent papers [48].

6.4 Phage Synergism with Antibiotics

Antibiotics will remain the main therapeutic standard for treating bacterial infections, notwithstanding the growing problems caused by antimicrobial resistance and multidrug-resistant (MDR) illnesses. A crucial focus in current drug development involves the exploration of new antimicrobials that can synergize with antibiotics. Phages and antibiotics used in treatment regimens have shown synergy that provide significant prospects. Making use of these synergies improves the effectiveness of antibiotics and makes it easier to incorporate workable combination medicines into the therapeutic toolbox. Consequently, an important goal in medication development is to understand how phages and antibiotics interact [49].

Both Gram-positive and Gram-negative bacteria have been shown to exhibit the phage-antibiotic synergy (PAS) phenomena. It has been demonstrated that sub-lethal doses of several antibiotic classes have a favourable effect on phage plaque size and propagation efficiency [50]. A significant increase in phage burst size and a delay in bacterial lysis are two recently discovered mechanisms of phage-antibiotic synergy (PAS). This result is explained by the overgrowth and filamentation of bacterial cells in response to antibiotics acting as stressors, together with a relative deficiency of holin availability [50].

A lytic phage that targets *P. aeruginosa* by using its receptor, an outer membrane porin, a part of a multidrug efflux system, was selected as a more focused strategy. In order to avoid the phage, this selection forces the host to adapt towards greater drug sensitivity[48]. The goal of this novel approach is to increase the effectiveness of traditional antibiotics by re-sensitizing multidrug-resistant (MDR) microorganisms to them. Combination therapy using antibiotics and certain phages can improve sensitivity. The fact that not all phage-antibiotic combinations work well together emphasizes how important it is to take possible interference into account when designing and implementing combined antibiotic-phage therapy [7].

7. Conclusion and Future prospects

The future prospects of phage therapy are promising, with several key areas of development and innovation on the horizon. Firstly, the advancements in phage engineering techniques, including synthetic biology approaches and CRISPR-based technologies, are poised to enhance phage specificity, stability, and efficacy. These developments may lead to the creation of tailored phage cocktails capable of targeting multidrug- resistant bacterial strains with precision

Furthermore, the integration of phage therapy into mainstream medical practice

is anticipated to expand, supported by the growing body of clinical evidence demonstrating its safety and efficacy. As regulatory agencies establish clear guidelines for phage therapy, including standardized manufacturing processes and quality control measures, its adoption in clinical settings is likely to accelerate.

Moreover, the exploration of phage therapy in niche applications, such as biofilm-associated infections, chronic disease, and veterinary medicine, offers new avenues for therapeutic intervention. Research efforts focused on understanding the interplay between phages, bacteria, and the host immune system will further elucidate the mechanisms underlying phage mediated bacterial clearance and pave way for optimised treatment strategies.

Overall, the future of phage therapy is characterized by innovation, collaboration, and interdisciplinary research, with the potential to revolutionize the treatment of bacterial infections and mitigate the global threat of antibiotic resistance. However, continued investment in research, infrastructure, and education is essential to realize the full therapeutic potential of phages and ensure their responsible use in clinical practice.

REFERENCES

1. Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., ... & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance*, 1645-1658.

2. Malik, B., & Bhattacharyya, S. (2019). Antibiotic drug-resistance as a complex system driven by socio-economic growth and antibiotic misuse. *Scientific Reports*, *9*(1), 9788.

3. CDC (United States Centers for Disease Control and Prevention) (2019). Antibiotic Resistance Threats in the United States, 2019; 2019 AR Threats Report; Department of Health and Human Services, CDC: Atlanta, GA, USA, pp. 1–118.

4. Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., ... & Zorzet, A. (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases*, *18*(3), 318-327.

5. WHO (World Health Organization). Antimicrobial Resistance (2019).

6. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Lancet* 2022, 399, 629–655. [CrossRef] [PubMed]

7. Abedon, S. T. (2019). Phage-antibiotic combination treatments: antagonistic impacts of antibiotics on the pharmacodynamics of phage therapy?. *Antibiotics*, *8*(4), 182.

8. Fernández, L., Gutiérrez, D., García, P., & Rodríguez, A. (2019). The perfect bacteriophage for therapeutic applications—a quick guide. *Antibiotics*, *8*(3), 126.

9. Strathdee, S. A., Hatfull, G. F., Mutalik, V. K., & Schooley, R. T. (2023). Phage therapy: From biological mechanisms to future directions. *Cell*, *186*(1), 17-31.

10. Fauconnier, A. (2019). Phage therapy regulation: from night to dawn. *Viruses*, *11*(4), 352.

11. Hankin, E. (1896). L'action bactericide des eaux de la Jumna et du Gange sur le vibrion du cholera. *Ann Inst Pasteur*, *10*, 511.

12. Twort, F. W. (1961). An investigation on the nature of ultra-microscopic viruses. *Acta Kravsi*.
13. D'Herelle, F (1917). Sur unMicrobe Invisible Antagoniste des Bacilles Dysente'riques. C. R. Acad. *Sci*. 165, 373–375.

14. Brunoghe, R., & Maisin, J. (1921). Essais de therapeutique au moyen du bacteriophage du staphylocoque. *CR Soc. Biol*, *85*, 1120-1121.

15. Schooley, R. T., Biswas, B., Gill, J. J., Hernandez-Morales, A., Lancaster, J., Lessor, L., ... & Hamilton, T. (2017). Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection. *Antimicrobial agents and chemotherapy*, *61*(10), 10-1128.

16. Aslam, S., Courtwright, A. M., Koval, C., Lehman, S. M., Morales, S., Furr, C. L. L., ... & Schooley, R. T. (2019). Early clinical experience of bacteriophage therapy in 3 lung transplant recipients. *American Journal of Transplantation*, *19*(9), 2631-2639.

17. Erez, Z., Steinberger-Levy, I., Shamir, M., Doron, S., Stokar-Avihail, A., Peleg, Y., ... & Sorek, R. (2017). Communication between viruses guides lysis–lysogeny decisions. *Nature*, *541*(7638), 488-493.

18. Penadés, J. R., Chen, J., Quiles-Puchalt, N., Carpena, N., & Novick, R. P. (2015). Bacteriophage-mediated spread of bacterial virulence genes. *Current opinion in microbiology*, *23*, 171-178.
19. Gordillo Altamirano, F. L., & Barr, J. J. (2019). Phage therapy in the postantibiotic era. *Clinical microbiology reviews*, *32*(2), 10-1128.

20. Expert Round Table on Acceptance and Re-Implementation of Bacteriophage Therapy, Sybesma, W., Rohde, C., Bardy, P., Pirnay, J. P., Cooper, I., ... & Kurtböke, D. İ. (2018). Silk route to the acceptance and re-implementation of bacteriophage therapy—part II. *Antibiotics*, *7*(2), 35.

21. McCallin, S., & Oechslin, F. (2019). Bacterial resistance to phage and its impact on clinical therapy. *Phage therapy: a practical approach*, 59-88.

22. Wright, A., Hawkins, C. H., Änggård, E. E., & Harper, D. R. (2009). A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. *Clinical otolaryngology*, *34*(4), 349-357.

23. Rhoads, D. D., Wolcott, R. D., Kuskowski, M. A., Wolcott, B. M., Ward, L. S., & Sulakvelidze, A. (2009). Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. *Journal of Wound Care*, *18*(6), 237-243.

24. Jault, P., Leclerc, T., Jennes, S., Pirnay, J. P., Que, Y. A., Resch, G., ... & Gabard, J. (2019). Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *The Lancet Infectious Diseases*, *19*(1), 35-45.

25. Sarker, S. A., Sultana, S., Reuteler, G., Moine, D., Descombes, P., Charton, F., ... & Brüssow, H. (2016). Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomized trial in children from Bangladesh. *Ebio Medicine*, *4*, 124-137.

26. Ooi, M. L., Drilling, A. J., Morales, S., Fong, S., Moraitis, S., Macias-Valle, L., ... & Wormald, P. J. (2019). Safety and tolerability of bacteriophage therapy for chronic rhinosinusitis due to Staphylococcus aureus. *JAMA Otolaryngology–Head & Neck Surgery*, *145*(8), 723-729.

27. Regulski, K., Champion-Arnaud, P., & Gabard, J. (2021). Bacteriophage manufacturing: From early twentieth-century processes to current GMP. *Bacteriophages: Biology, Technology, Therapy*, 699-729.

28. Mutti, M., & Corsini, L. (2019). Robust approaches for the production of active ingredient and drug product for human phage therapy. *Frontiers in Microbiology*, *10*, 465742.

29. Hietala, V., Horsma-Heikkinen, J., Carron, A., Skurnik, M., & Kiljunen, S. (2019). The removal of endo-and enterotoxins from bacteriophage preparations. *Frontiers in Microbiology*, *10*, 464722.

30. Malik, D. J., Sokolov, I. J., Vinner, G. K., Mancuso, F., Cinquerrui, S., Vladisavljevic, G. T., ... & Kirpichnikova, A. (2017). Formulation, stabilisation and encapsulation of bacteriophage for phage therapy. *Advances in Colloid and Interface Science*, *249*, 100-133.

31. Merabishvili, M., Pirnay, J. P., & De Vos, D. (2018). Guidelines to compose an ideal bacteriophage cocktail. *Bacteriophage Therapy: From Lab to Clinical Practice*, 99-110.

32. Dąbrowska, K. (2019). Phage therapy: What factors shape phage pharmacokinetics and bioavailability? Systematic and critical review. *Medicinal Research Reviews*, *39*(5), 2000-2025.

33. Hobley, L., Harkins, C., MacPhee, C. E., & Stanley-Wall, N. R. (2015). Giving structure to the biofilm matrix: an overview of individual strategies and emerging common themes. *FEMS Microbiology Reviews*, *39*(5), 649-669.

34. Seviour, T., Derlon, N., Dueholm, M. S., Flemming, H. C., Girbal-Neuhauser, E., Horn, H., ... & Lin, Y. (2019). Extracellular polymeric substances of biofilms: Suffering from an identity crisis. *Water Research*, *151*, 1-7.

35. Dunsing, V., Irmscher, T., Barbirz, S., & Chiantia, S. (2019). Purely polysaccharide-based biofilm matrix provides size-selective diffusion barriers for nanoparticles and bacteriophages. *Biomacromolecules*, *20*(10), 3842-3854.

36. Taylor, B. P., Penington, C. J., & Weitz, J. S. (2017). Emergence of increased frequency and severity of multiple infections by viruses due to spatial clustering of hosts. *Physical biology*, *13*(6), 066014.

37. Bernheim, A., & Sorek, R. (2020). The pan-immune system of bacteria: antiviral defence as a community resource. *Nature Reviews Microbiology*, *18*(2), 113-119.

38. Reindel R, Fiore CR. Phage therapy: considerations and challenges for development. *Clin Infect Dis* 2017; 64:1589–90.

39. Directive 2001 /83/EC. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community role relating to medicinal

products for human use (2001) *Official Journal* L311, p. 67, 2001.

40. Pelfrene, E., Sebris, Z., & Cavaleri, M. (2019). Developing Phages into Medicines for Europe. *Phage Therapy: A Practical Approach*, 351-361.

41. Pirnay, J. P., De Vos, D., Verbeken, G., Merabishvili, M., Chanishvili, N., Vaneechoutte, M., ... & Adamia, R. (2011). The phage therapy paradigm: prêt-à-porter or sur-mesure?. *Pharmaceutical Research*, *28*, 934-937.

42. Fauconnier, A. (2017). Regulating phage therapy: the biological master file concept could help to overcome regulatory challenge of personalized medicines. *EMBO Reports*, *18*(2), 198-200.

43. Chen, Y., Batra, H., Dong, J., Chen, C., Rao, V. B., & Tao, P. (2019). Genetic engineering of bacteriophages against infectious diseases. *Frontiers in Microbiology*, *10*, 455781.

44. Oechslin, F. (2018). Resistance development to bacteriophages occurring during bacteriophage therapy. *Viruses*, *10*(7), 351.

45. Rohde, C., Resch, G., Pirnay, J. P., Blasdel, B. G., Debarbieux, L., Gelman, D., ... & Chanishvili, N. (2018). Expert opinion on three phage therapy related topics: bacterial phage resistance, phage training and prophages in bacterial production strains. *Viruses*, *10*(4), 178.

46. Lehman, S. M., Mearns, G., Rankin, D., Cole, R. A., Smrekar, F., Branston, S. D., & Morales, S. (2019). Design and preclinical development of a phage product for the treatment of antibiotic-resistant *Staphylococcus aureus* infections. *Viruses*, *11*(1), 88.

47. Diban, F., Di Lodovico, S., Di Fermo, P., D'Ercole, S., D'Arcangelo, S., Di Giulio, M., & Cellini, L. (2023). Biofilms in Chronic Wound Infections: Innovative Antimicrobial Approaches Using the In Vitro Lubbock Chronic Wound Biofilm Model. *International Journal of Molecular sciences*, *24*(2), 1004. https://doi.org/10.3390/ijms24021004

48. K Chan, B., & T Abedon, S. (2015). Bacteriophages and their enzymes in biofilm control. *Current Pharmaceutical Design*, *21*(1), 85-99.

49. Morrisette, T., Kebriaei, R., Lev, K. L., Morales, S., & Rybak, M. J. (2020). Bacteriophage therapeutics: a primer for clinicians on phage-antibiotic combinations. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *40*(2), 153-168.

50. Kim, M., Jo, Y., Hwang, Y. J., Hong, H. W., Hong, S. S., Park, K., & Myung, H. (2018). Phage-antibiotic synergy via delayed lysis. *Applied and environmental microbiology*, *84*(22), e02085-18.