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Review Article

Phage Therapy Approaches

Advancement of Phage Therapy Approaches in The Battle of Multi-Drug Resistance: A Review

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Abstract: One of the most significant issues faced by humanity today is antibiotic resistance. Drugs are used in such vast amounts for human health, aquatic life, and agricultural animals that harmful bacteria have developed antibiotic resistance to various antibiotics. Further, the usage of antibiotics is increasing because of situations such as increased infections and chronic diseases that need antimicrobial treatment. Since antimicrobial resistance is rising, it is necessary to take action to help reduce and eliminate infectious diseases and ensure animal and human health. Because of this, many attempts are being made to tackle multi-drug-resistant bacteria. Among the many advanced techniques that are occurring, the use of phage therapy is one such emerging procedure. The main aim of this prospective review is to identify the various new phage formulations available as a potential therapeutic intervention to combat multidrug resistance among bacteria and the objective is to identify the various reasons associated with the induction of the phenomenon of "multidrug resistance" among different bacteria, focusing on the use of phage therapy, its advantages as well as disadvantages over antibiotics as a possible therapeutic intervention. Various phage formulations, such as phage cocktails with antibiotics, nanoparticles, phage-delivering hydrogels, and many more, are emerging formulations that have successful results in fighting against multi-drug-resistant bacteria. Commercial phage solutions have helped combat antimicrobial resistance in poultry and livestock farms, improving everyone's health worldwide. As a result, this study shall serve as a source of information and understanding of the concerns mentioned above for the entirety of society and every human community.

Keywords: Antibiotic Resistance, Multidrug-Resistant Bacteria, Antimicrobial, Bacteriophage and Phage Therapy

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1. INTRODUCTION

Public health is still at risk due to the growth of multidrug-resistant bacteria (MDR) and antibiotics' decreasing efficacy in treating severe bacterial illnesses[1]. Antibiotics have proved as a significant step forward in modern medical practice since their discovery[2]. However, the appropriate treatment of various serious illnesses and disorders in the medical field is constantly compromised due to the growth of multidrug-resistant bacteria. The first instance of antibiotic resistance among bacteria was reported during the second world war[3]. Much before, in 1959, Maurois warned about the consequences of penicillin resistance among *"Staphylococcus aureus"*[4]. The facts he stated came true when multiple antibiotic-resistant strains of "*Streptococcus pneumoniae"* and "*Staphylococcus aureus"* against penicillin and methicillin, respectively, were discovered [5]. Bacteria can avoid the antimicrobial effects of antibiotics via three different strategies: resistance, tolerance, and persistence. Although key bacterial activities intolerant bacteria have been found to slow down, tolerant bacteria are metabolically more active than persistent subpopulations[6]. The "ESKAPE" pathogens, also known as "*Enterococcus faecium", "Klebsiella pneumoniae", "Staphylococcus aureus"*, "*Acinetobacter baumannii", "Enterobacter species"*, and "*Pseudomonas aeruginosa"*, are a group of multidrug-resistant organisms that cause serious healthcare-associated illnesses[7]. In recent years, *"Escherichia coli", "Neisseria gonorrhoeae",* and "*Staphylococcus aureus"* have demonstrated increased death rates due to multidrug-resistant strains of "*Klebsiella pneumoniae"*, "*Neisseria gonorrhoeae"*, and "*Staphylococcus aureus"*, according to "World Health Organization (WHO)" reports[7]. Furthermore, several published reports say approximately 2 million Americans contract antibiotic-resistant germs each year, and about 23,000 people died from exposure to them[8]. This problem is exacerbated by the lack of infection control in healthcare facilities, poor public cleanliness, the abuse of antibiotics, low educational attainment about antibacterial drugs, and the absence of supervisory authority over their use, production, and sale[2]. Several antimicrobial compounds are considered adequate for treating bacterial ailments, including compounds that block the function of efflux pumps in bacterial cells and "metalloantibiotic compounds" that are thought to enhance the efficacy of standard antibiotic medications[2]. Further, "antimicrobial peptides" induce the formation of pores in the bacterial cell membrane and disrupt the process of synthesis of DNA in bacteria[2]. Among all the therapeutic intervention methods available, phage treatment is an excellent antibiotic substitute for multidrug-resistant organisms since new techniques have been discovered to battle these diseases due to rising antimicrobial resistance. Bacteriophages or phages are viruses that may infect and multiply inside bacteria and aid in the fight against multidrug-resistant organisms. They are diverse and prevalent everywhere in nature. Phages have essential advantages over antibiotics in the case of antibiotic resistance since they can lower bacterial populations. Bacteriophage life can be divided into two types: lytic and lysogenic cycles[9-11]. The host cell ruptures during the lytic cycle, releasing many viral offspring. Here, phage replication occurs within the host bacteria, causing a bacterial rupture and cell death that results in the release of offspring virions[12]. The phage genome is combined with the host chromosome throughout the lysogenic or temperate cycle, and the prophage state is maintained until environmental cues initiate the lytic pathway. Lysogens are viral cells that have progeny. Multidrug resistance in various bacteria has been a significant challenge for civilization for a long time. Also, the phenomena of "multidrug resistance" and "phage treatment" has garnered the interest of scientists worldwide. Therefore, the authors bring to the attention of the general public, through this review, the various causes associated with the induction of multidrug resistance in diverse bacterial species, with a particular emphasis on the use of phage, by employing the different phage formulations available to combat this long-recognized problem of multidrug resistance among diverse bacterial species that coexist with humans in nature. The review will now focus on the life cycle of the bacteriophages present.

2. LIFE CYCLE AND BIOLOGY OF BACTERIOPHAGES

Bacteriophages are the specific group of viruses that can infect only bacterial cells, as bacteria have the necessary receptors to facilitate phage attachment on the bacterial surface[10]. However, the phage life cycle can be divided into two parts: the lytic cycle and the lysogenic cycle[9-11]. Phages entering the lytic cycle exploit the replication machinery of the host bacterial species to produce progeny viruses within the host cell. The process continues unless a "critical mass" is achieved, at which point, the host bacterial cell lyses, releasing the new progeny viruses. These offspring restart the lytic cycle again (Figure 1)[10]. On the other hand, in the case of the lysogenic cycle (Figure 2), the viruses incorporate their genetic material into the host bacterium. This is known as a prophage, permitting the horizontal transfer of genetic information from the parent virus to the progeny bacterial cells due to cell division[9-11]. Less frequently, the virus does not integrate its genetic material into the chromosome of the host bacterium but instead stays as a distinct plasmid inside the host bacterium, which is nonetheless passed from one bacterial generation to another, i.e., from parent bacterium to its offspring. Rarely factors involving the environment can change the life cycle of bacteriophages, from lytic to lysogenic, but only under extraordinary conditions[11].

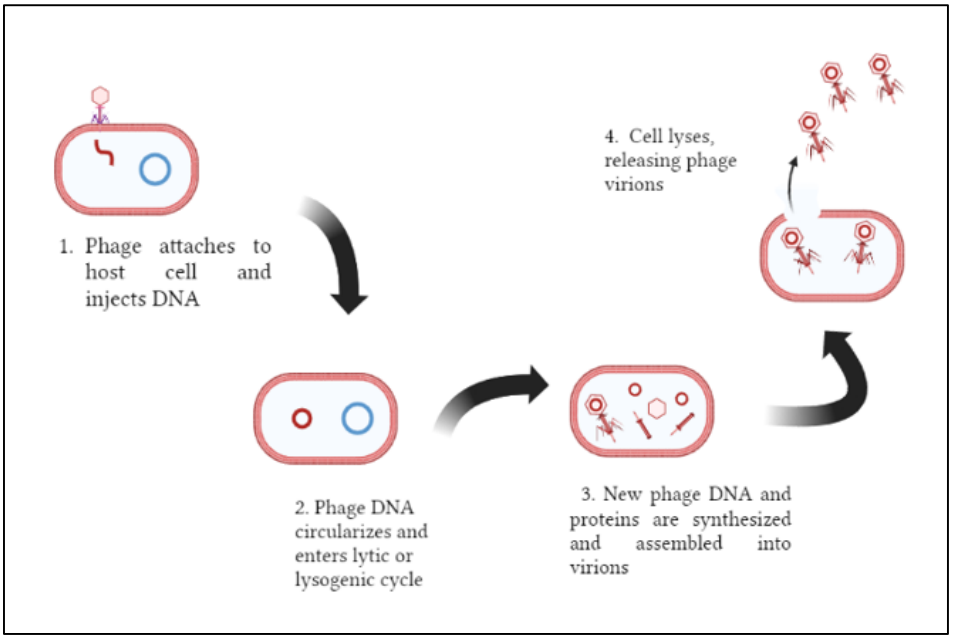

Fig 1. Lytic cycle of bacteriophage[10]

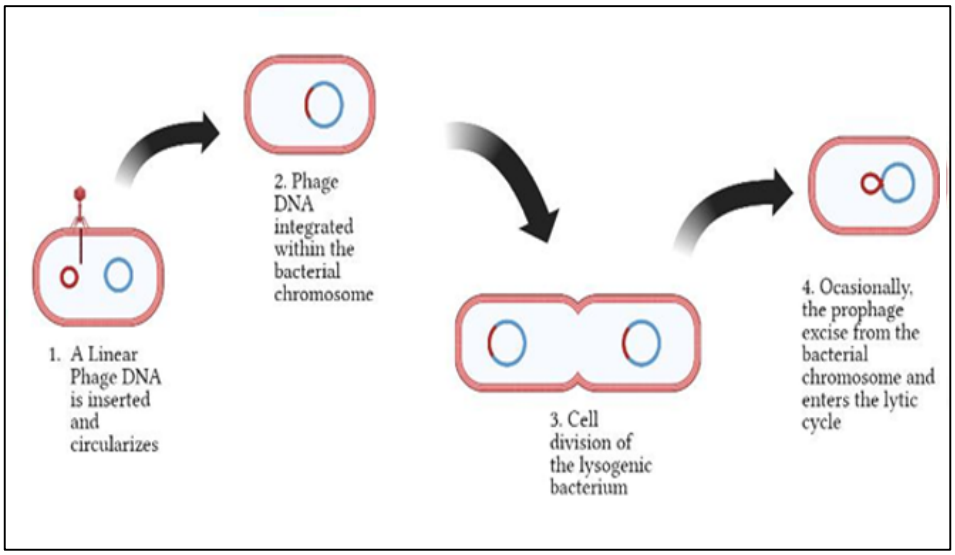

Fig 2. Lysogenic cycle of bacteriophage[11]

Now that bacteriophage biology has been covered in detail. The following primary objective is to define the steps that lead to the emergence of "drug resistance" in virulent bacteria.

3. PROCEDURE OF INDUCTION OF ANTIBIOTIC RESISTANCE IN PATHOGENS

Bacterial mutations are often at the root of increased resistance to antibiotics. Using bactericidal or bacteriostatic chemicals arbitrarily and incorrectly can function as a selective pressure that leads to these changes. Eventually, the antibiotic-resistant bacteria that have been chosen could pass their resistance gene on to other bacteria if the screening process is allowed to continue[2]. Bacteria can evolve resistance to many antibiotic drugs through distinct mechanisms. The first mechanism followed is the inactivation of the antimicrobial drug by the action of enzymes, as found in the case of enzymatic inactivation of "beta-lactamase" antibiotics[12]. Second, altering the binding sites of antimicrobial medications might potentially contribute to the spread of antibiotic resistance, as found in the case of penicillin-binding proteins in the case of methicillin-resistant "*Staphylococcus aureus*"[13]. Thirdly, bacteria are capable of acquiring many genes for enacting metabolic activities. This modification of bacterial cell membranes renders antimicrobial agents incapable of binding to their bacterial targets. Lastly decrease in drug accumulation inside the bacterial cells due to the upregulation of efflux pumps or reduction in permeability towards the antimicrobial drugs is also responsible for developing resistance to antibiotic drugs in different bacterial populations. Commonly identified efflux mediators include "major facilitator superfamily (MFS)", "resistance nodulation cell division superfamily (RND)", "ATP binding cassette transporters (ABC)", "multidrug and toxic compound extrusion superfamily (MATE)", Infectious pathogens can acquire resistance to antibiotics via above methods, rendering the medications ineffective[2,14,15]. The review will now address the therapeutic use of bacteriophages to overcome bacterial drug resistance.

4. PHAGES AS A PROMISING ALTERNATIVE THERAPY AGAINST MULTIDRUG RESISTANCE BACTERIA

Viruses that infect bacteria solely are called bacteriophages. They are the smallest and most numerous organisms in the troposphere. There is a wide variety of phages, but they all have two primary life cycles: the Lytic cycle and the Lysogenic cycle[9-11]. Phage treatment has recently emerged as a valuable method for fighting antibiotic-resistant pathogens[16]. A significant benefit of utilizing phages as a therapeutic intervention instead of antibiotics is that they can eliminate bacteria whether or not they have developed resistance to the antibiotic. For instance, phage therapy can combat drug-resistant *Acinetobacter baumanii* infection[16]. In addition, phages multiply randomly upon contact with the site of infection, which is an additional benefit of phage treatment. This is advantageous as a single dose of phage can cure difficult-to-reach infections[17] (Figure 3).

Moreover, phages are incredibly particular to their host bacteria. Therefore, phage cocktails, which comprise an assortment of phages, may be used to modulate a broad spectrum of bacteria[18]. It should be emphasized that bacteria and bacteriophages have coexisted in the environment for many years. This shall also occur when phage therapy is used as a potential therapeutic intervention since co-evolution will render the treatment adaptable[16,19]. Therefore, compared with the use of individual phages, phage cocktails have a greater tendency to reduce the development of phage-resistant pathogens[20]. It is also possible that they will interact differently with the immune system. In addition, the features of the infection, such as its size, severity, or composition of the bacterial cell, will affect how effective these treatments are. Their interaction with many plasma proteins is another area that is mainly unexplored[21].

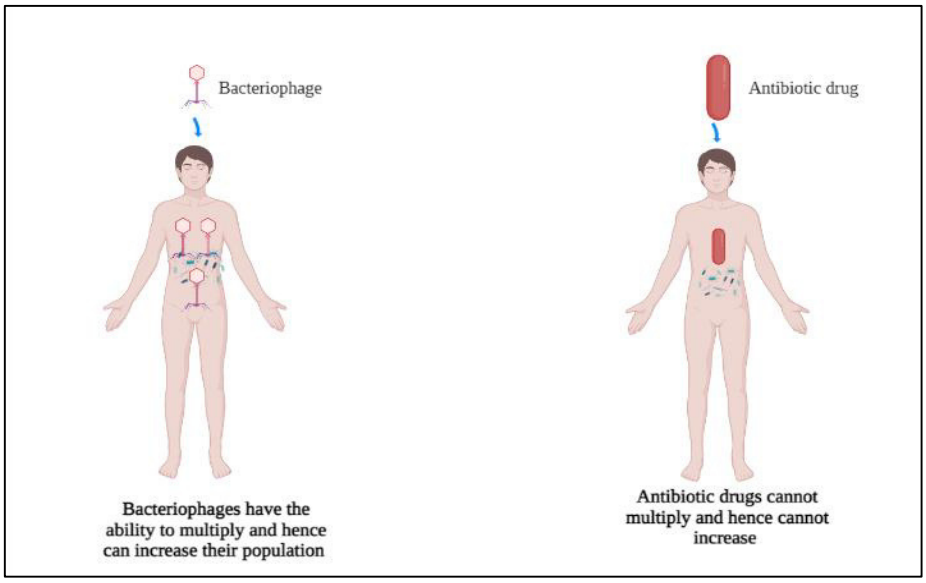

Fig 3. Bacteriophage vs Antibiotics[20,21]

The inability of phages to survive in the stomach's low acidic environment is a significant drawback of phage therapy, which hinders its use as a therapeutic intervention. Therefore, administering phages orally is not feasible. Hence, effective encapsulation of phages is crucial for overcoming this issue. According to the published literature, numerous phage encapsulation strategies with low change in phage titer are available. Encapsulating phages in silver nanoparticles ("Phage M13" encapsulation against "*Fusobacterium nucleatum*"[22]), hydrogels ("Phage T7" encapsulation against "enterotoxic *Bacteroides fragilis*"[23]), or sodium alginate beads combined with gelatin or honey ("Phage ZCEC5" encapsulation against the same bacterial pathogen[24,25]), are just a few examples. Lastly, liposomes can also be used for phage encapsulation ("PhageUAB_Phi20", "PhageUAB_Phi78", "PhageUAB_Phi87" encapsulation against "*Salmonella typhimurium*"[26]). Apart from employing phages as prospective therapeutic intervention approaches, another way to overcome antibiotic resistance is to use enzymes produced from phages that promote the lysis of bacterial cells. Some enzymes that fall within this category include Holins[20,27], Lysin[28], and Depolymerases[29]. The canonical endolysins are regarded as the most effective lysin to overcome antibiotic resistance. To offer just one illustration, tests were conducted on a mouse model infected with *Acinetobacter baumannii*. The lysine known as "LysSS" proved successful in eliminating the infection[20,27]. Another example is that depolymerase "Dp42" enhanced the overall survival and significantly decreased the bacterial burden in the liver and the lung of the treated mice infected with *Klebsiella pneumoniae*[30]. After discussing the several variables that make phage treatment a viable alternative intervention for halting the development of multidrug-resistant bacteria, this review will focus on the specific drug-resistant bacterial infections that can be treated using phage therapy.

5. RELEVANT ANTIBIOTIC-RESISTANT BACTERIAL PATHOGENS FOR WHICH PHAGE TREATMENT CAN BE APPLIED

Antibiotics are being used more often and improperly, which has led to a rise in bacterial infections that have acquired resistance to antibiotics that are usually effective against them. The following are examples of bacterial pathogens:

5.1 Staphylococcus Aureus

Staphylococcus aureus has emerged as a drug-resistant pathogen over the years. It is considered a pathogen, gram-positive, and is usually found in the nasal passages along with layers of skin[31]. *Staphylococcus aureus* has developed resistance towards "beta-lactam antibiotics" through the synthesis of the enzyme penicillinase (plasmid-mediated synthesis of the enzyme) that causes degradation of the "beta-lactam antibiotics". This led to the development of methicillin, a more advanced form of

penicillin. However, all this success became painful when the bacterium acquired the "mecA gene", encoding the penicillin-binding protein (PBP2a), making the bacterium resistant to antibiotic drugs[31]. However, the strains have also developed resistance to vancomycin, which can be attributed to the "vanA gene" transfer. These strains are notoriously challenging to identify and are often related to unsuccessful treatment. The methicillin-resistant Staphylococcus aureus has developed into a predicament that poses an increasingly severe threat since it is no longer confined to settings within hospitals but has spread into other places in the community and has since spread around the world[31].

5.2 Yersinia Pestis

The highly contagious bacteria *Y. pestis* is responsible for the pathophysiology of plaque. As gram-negative bacteria, they are unable to preserve gram staining. This kind of bacteria is classified as an "Enterobacteriaceae". The bacteria begin their destructive process by infecting the local macrophages and then making their way to the lymph nodes. The death of infected macrophages triggers the discharge of the bacteria, which then spreads to and colonizes other vital organs, eventually causing death[32].

5.3 Salmonella

Salmonella has many hosts and is effective against potent plant or animal pathogens. *Salmonella enterica* of the *Salmonella* sp. is the most common pathogen responsible for producing food poisoning, contaminating vegetables, poultry, food, nuts, etc[32]. According to the Centre for Disease Control and Prevention (CDC), fifteen per cent of Americans will contract Salmonella-related illnesses, and in the worst circumstances, these illnesses may prove fatal[33].

5.4 Listeria Monocytogenes

The bacterium is gram-positive and implicated in developing infectious disorders like influenza, fever, gastroenteritis, and most notably, "Listeriosis". Children, the elderly, and those with impaired immune systems are most vulnerable to listeriosis, a deadly illness characterized by meningitis, septicemia, and abortion[34]. It's also considered a potentially dangerous infection that may spread through food[35].

5.5 "*Escherichia Coli* O157H7"

To be specific, *E. coli* is a potentially fatal bacterium that causes various diseases, including diarrhoea or colitis. In addition, the enterohaemorrhagic ones of the bacterium are responsible for the pathogenesis of "hemolytic-uremic syndrome" or "haemorrhagic colitis" in humans. Furthermore, the strain "O157:H7" differs from the other strains of the bacterium with its ability to produce "Shiga toxin". The foodborne pathogen is spread mainly by consuming uncooked meat, raw dairy products without pasteurization, and crops tainted with bovine excrement[36].

5.6 Mycobacterium Tuberculosis

M. tuberculosis is regarded as another powerful, potent pathogen capable of infecting humans. The alveolar area of the lungs is the bacterium's target, and the macrophages there provide a haven and a fertile breeding ground. Further, antigenic peptides are released from the lymph nodes once programmed cell death has occurred, thanks to the dendritic cells that transported them there. Anti-infective defences rely on T-cell activation, which results in the formation of "T effector cells" that travel back to the lungs' passageways to continue the battle against the infection. These steps culminate in the development of granulomas that trap the infection[37]. The formation of drug-resistant strains is a challenge despite the availability of numerous drug-based treatment strategies to regulate the pathogenesis. For instance, various medications are being developed to treat drug-resistant pathogenic *M. tuberculosis* and drugs for handling *Staphylococcus aureus* resistance to methicillin[31].

6. BENEFITS AND DRAWBACKS OF PHAGE THERAPY OVER ANTIBIOTICS

There are benefits and drawbacks to using phage treatment rather than antibiotics. Here are some of the advantages and disadvantages to consider:

6.1 Specificity

Viruses are notoriously particular in killing off their prey bacteria. They can only infect bacteria with the corresponding receptor for phage antigen[38]. In certain phage families, infection is limited to a single type of bacteria, whereas in others, the phage may infect a wide variety of bacteria. For instance, no reports of alteration of other gut microbiomes were found after oral delivery of four "T4 coliphages" for treating diarrhoea triggered by *E. coli* in mouse models[39]. No different microbiota was affected by applying phages to *S. soneii*-caused infection in mouse models[40]. It is unlikely that a phage would wipe out all of the local flora or cause secondary infections because of its targeted approach. On the other hand, antibiotic use has been linked to health issues such as asthma, diabetes, and obesity[41-43], whereas such health issues are not applicable in the case of phage therapy. This characteristic of phages is not without its restrictions, though. Therefore, in the event of polymicrobial illnesses, doctors must identify which species they are treating before administering a phage or phage cocktail. Since antibiotics are often broad-spectrum antimicrobials[44], their use in these settings becomes preferable to phage treatment. Example: T4 phages were used in a 2014 study to combat E. coli found in Mexico and Bangladesh. Study results were more encouraging when phage was tested against bacterial isolates from the exact geographical locations than when the bacterial and phage isolates were cross-applied[45]. Supporting this research was a clinical experiment that gave "T4 coliphages" collected from Russia to 120 youngsters in Bangladesh infected with Escherichia coli (Enteropathogenic) for four days[46]. Unfortunately, the final results were unsatisfactory since they yielded no helpful information on the efficacy of phage treatment. Therefore, in vitro and in vivo experiments have shown that phages are very particular for their host bacteria. Thus, the phage treatment is most effective when applied to a specific host bacterium collected from the exact location.

6.2 Safety

Antibiotics have been widely used, yet their usage, misuse, and abuse have all been documented in scientific literature. Allergic reactions, either from an overdose of the medicine or from the drug's primary interaction with the body, are often considered the most prevalent side effect of antibiotic use[47,48]. Antibiotic penicillin, for instance, has been linked to various

adverse human responses, including cardiovascular system collapse and, in extreme cases, death[49]. On the other hand, bacteriophages have stayed and co-evolved with humans for a long time. Thus, they are considered safer, with no adverse effects on health or an individual's well-being. This topic has attracted the attention of researchers worldwide, and several clinical trials have been undertaken to understand the efficacy of phage therapy. The tests have administered phages orally, in topical applications, or by preparing a cocktail of different phages to combat several bacterial populations at a time[46,50,51-53,18]. However, a 2016 study showed that giving rats an oral cocktail of phages increased cytokine levels or made the intestinal wall more porous. Healthy "albino rats" were given a mixture of Salmonella and Pyobacteriophage in the study. In addition, the ratio of lactose to mannitol was monitored to determine intestinal permeability. Increases in both ratios were found, indicating a rise in intestinal permeability, as demonstrated by research[54].

6.3 Resistance Development Against Host Bacterium

Antibiotic resistance can emerge from various causes, such as the improper use of antibiotic drugs and the horizontal transfer of genes from one bacterium to another. However, bacteria can develop resistance to phages through mechanisms such as mutational changes in the surface receptors facilitating phage binding, secretion of chemical compounds to prevent phage binding to bacterial cells (such as Extracellular polymeric substances), inhibition of bacteriophage replication, and blocking the injection of phage genetic material into bacterial cells[55]. Additional research revealed that the presence of extracellular polymeric compounds generated by *Pseudomonas* sp. or "glycoconjugates" as well as "alginates" produced by *Enterobacteriaceae* prevented phage adhesion[56]. In another study, mutational alterations in the "OprM" surface receptor, which is the target receptor of Bacteriophage OMKO1, impede the binding of the same[57]. Another method contributing to antibiotic resistance among the bacterial population is phage therapy. Phage treatment is another approach that contributes to antibiotic resistance in bacterial populations. This is facilitated by lysogenic bacteriophages, which incorporate their DNA into the host genome. Consequently, horizontal transfer of drug-resistant genes can occur not just across bacterial populations but also between phages and bacteria[58]. Phages have a significant function as a reservoir of antibiotic resistance genes, as demonstrated by research in which the prevalence of resistant genes in phage DNA was significantly greater than in bacterial DNA[59]. In a later study, bacterial isolates from hospital effluents and water treatment plants were found to contain genes such as "blaTEM" (for resistance against "beta-lactam antibiotics"), "qnrS" (for resistance against "fluoroquinolones"), "sull" (for inducing resistance against "sulphonamides"), and "tetW" (for causing resistance against "tetracyclines")[60].

6.4 Cost and Administration of the Therapy Technique

The use of phage treatment to combat antibiotic-resistant bacteria is inexpensive. As long as it is not regarded as a treatment of last resort, phage therapy is economically less expensive than antibiotic administration, according to a study[61]. Furthermore, in contrast to antibiotics, bacteriophages can multiply themselves. This characteristic of phages prohibits the administration of multiple doses of bacteriophage over time. Nonetheless, the "pharmacodynamic" and "pharmacokinetic" features of the phages constitute a significant restriction to phage treatment. Therefore, this review will now concentrate on its most prominent subject: the many phage formulations available to fight this long-recognized problem of drug resistance among harmful bacteria.

7. VARIOUS PHAGE FORMULATIONS

7.1 Powder Formulations of Phage Against Pseudomonas Aeruginosa for Treating Respiratory Infection.

Pseudomonas aeruginosa significantly increases the risk of morbidities and mortality in patients suffering from cystic fibrosis, non-cystic fibrosis, bronchospasms, and sepsis[9,11,62-64]. When ciprofloxacin and phage PEV20 were combined, they had a highly synergistic effect against Pseudomonas aeruginosa, which was multidrug resistant. There were found to be two formulations: Formulation A, which contains ciprofloxacin, lactose, and "L-leucine" in a mass ratio of 1:1:1; Formulation B, which contains ciprofloxacin as well as "L-leucine" without lactose in a mass ratio of 2:1. "L-leucine" functions as a dispenser enhancer[65], increasing the fine particle fraction (FPF). Using a "Buchi spray drier (B-290, Buchi Labortechnik AG)", powder formulations of ciprofloxacin and the "phage PEV20" were created[66]. "*P. aeruginosa* FADD1-PA001" and "*P. aeruginosa* JIP865" strains were used, and "phage PEV20" doses of 108 pfu/mL and 109 pfu/mL were added. In both strains, both formulations had a potent antimicrobial-killing synergy. Significant regrowth was seen in areas treated with ciprofloxacin or PEV20. However, after 24 hours, regrowth was inhibited by the powder formulations. Spray drying of the three *Pseudomonas aeruginosa* phages, PEV20 (both myoviruses), PEV1, along with PEV2 (podovirus), with the addition of both lactose (80%), leucine (20%) as excipients, resulted in phage cocktail powder. This proved the feasibility of the work by introducing patients to a biochemically stable formulation of phage PEV20 along with ciprofloxacin that can be inhaled to reduce the pathogenesis of the bacterium and simultaneously cure the disease. Dry powder compositions of phage and a cocktail of different phages for inhalation are produced using spray drying[67-70]. The mixture slightly decreased the titer of the phages, and all of the phages were still viable when the spray-dried powder was used. Subsequently, "high-performance liquid chromatography analysis was performed (HPLC)". The mass fraction of particles with a diameter smaller than 5.0 m with the loading dose was used to calculate the fine particle fraction (FPF). No matter which phage was employed, the spray-dried phage powders with identical excipient content exhibited similar shapes and particle sizes[67]. The phage cocktail formulation with three different phages provided equivalent biological stability and physicochemical powder qualities to single phage powders[71].

7.2 Application of Phage Therapy Against Antibiotic-Resistant Pseudomonas Infection

Pseudomonas sp. has come to be seen as a harmful organism with the inbuilt power to destroy humanity. But more concerning than its pathogenic potential is its capacity for medication resistance. A wide variety of infections have been brought on by drug-resistant Pseudomonas in numerous situations, according to multiple previously published scientific literature. Additionally, using phage treatment to treat these infections has been successful in various positions. For

instance, phage therapy in conjunction with antibiotics like meropenem, ciprofloxacin, ceftazidime, and Gentamicin has helped patients suffering from urinary tract infections brought on by pathogenic *Pseudomonas aeruginosa*. Phage therapy was performed with a bacteriophage dosage of 20 ml (2×10^7 PFU) every 12 hours for ten days. The trial confirmed the effectiveness of phage therapy to treat urinary infections caused by *Pseudomonas aeruginosa* when examined at the end of the tenth day and found no trace of pathogenic *Pseudomonas aeruginosa* in urine samples[72]. In a further study, it was found that using phage therapy in conjunction with the antibiotic formulations ciprofloxacin and ceftazidime to treat an aortic transplant that had become infected with *Pseudomonas aeruginosa* during heart surgery once again produced encouraging outcomes. This time, the bacterium had developed ciprofloxacin resistance. To remove the biofilm that encourages antibiotic drug resistance, phage therapy was provided by injecting one dosage of phage (1×10^7 PFU) straight into the fistula established in the chest. The trial indicated that the patient's condition had improved[73]. Furthermore, phage therapy has once again demonstrated its effectiveness in lowering the pathogenesis of Pseudomonas putida infection generates otitis in another experiment where ear otitis was caused as a result of disease owing to Pseudomonas putida. A decrease in the bacterial community was seen in the investigation after each of the 12 subjects was given a single dosage of a phage cocktail containing 10^5PFU (below 80 per cent)[74].

7.3 Single and Cocktail Phage Therapy for "Uropathogenic Escherichia Coli (UPEC)" In Vitro Bacterial Death Test

A multidrug-resistant bacteria-targeting phage with a broad host range was to be isolated. Both MDR UPEC bacteria and the "*E. coli* ST 131" strain were targeted. To separate phages from the sewage water, they were employed. One phage and cocktail phage treatments were used in a 24-hour mortality assay to test the infectivity capacity of phages in vitro. Single and cocktail phage-killing experiments were performed using a 96-well plate and OD_{600}nm measurements over a 24-hour period. On infecting the bacteria host at various multiplicity of infection (MOI) ratios, phage infectivity for single phage treatment was achieved. All of the phages were able to demonstrate infectivity for the UPEC S79EC strain as a result of this. Even after displaying infectivity at all MOI levels[75], Phage A4 was isolated from UPEC S79EC with an average bacterial growth suppression varied from 2 to 5 hours. For the phage cocktail treatment, ten distinct phage combinations were used. Phage cocktail combinations were created by combining phages with wide and restricted host ranges, such as A1 and A2, with A3 and A5. Several UPEC strains were infected for 24 hours with an MOI of 10 for the cocktail-killing test. The most considerable average bacterial growth suppression period was demonstrated by phage cocktails[75]. According to what is known about phage therapy, it is simple to isolate infectious phage from bacterial hosts that are linked to pathogenicity or infectious diseases[76-78].

7.4 Antibiotic-Phage Combination

There is a synergy observed between antibiotics and phage. So, this phage-antibiotic synergy can work with great potential to help in bacteria killing[79-80]. In a study, there are 11 different phages that are used with a combination of 8 other antibiotics. Around 88 phage and antibiotic combinations were tested along with bacterial swelling in cocci or antibiotic-induced bacterial filamentation[81]. *P. aeruginosa* biofilm eradication was improved when the Pseudomonas-targeting "phage PEV20" was coupled with ciprofloxacin, showing the possibility for lowering the antibiotic dose required to tackle extremely refractory infections associated with biofilms[82]. It is believed that raising the antibiotic concentration in phage–antibiotic treatment will improve synergy, however, several investigations have found that when antibiotics are used in conjunction with phages, the antibiotic minimum inhibitory concentration (MIC) is reduced[82-86]. Similar to the previous work, the imipenem-resistant strain "*Klebsiella pneumonia*e K2534" and persistent strain "*Klebsiella pneumoniae* K3325" of the Gram-negative bacterium were treated with "mitomycin C", "imipenem", and the lytic "phage vB KpnM-VAC13". Except for the strain resistant to the antibiotic imipenem, which was co-treated with phage plus "mitomycin C" or "imipenem", the survival rate of the larvae increased to 50% and 75%, respectively, as compared to either antibiotic formulations or phage monotherapy. This was brought on by imipenem being hydrolyzed by resistant strains of lactamase. However, the larvae mortality rate was reduced substantially with the help of the other therapeutic measures[87].

7.5 Hydrogels for Phage Delivery

The primary prerequisites of a steady phage preparation for therapeutic applications are complete physical consistency of the formulation and assurance of phage steadiness. Hydrogels have been utilized to deliver biologics, such as phages, to the target place of interest, such as wounds and implants[88-94]. Phage hydrogels, which combine the advantages of both phages and hydrogels, have been utilized to treat and prevent multidrug-resistant bacterial infections. Multiple preclinical investigations have been conducted in vitro and in vivo [95], suggesting that hydrogels may be the optimum phage delivery method. Different hydrogel formulations are mixed with phage to combat bacteria that have developed a resistance to many drugs; some of their specifications are addressed. "PEG (polyethene glycol) hydrogels", due to their adaptive physicochemical characteristics, minimal toxicity, and constrained protein adsorption, are among the most frequently utilized synthesized hydrogels in the field of biomedicine[88]. Many formulations can be created by combining PEG hydrogels with different functional groups, such as "PEG-4-MAL (Polyethylene Glycol-4-Maleimide)" and "PEG (Polyethylene Glycol)-Polyurethane" [88]. Because of their remarkable capabilities for integrating biological elements, hydrogels are an excellent medium for the delivery of phages. Similarly, other hydrogel compositions have been employed to treat bacterial infections resistant to antibiotics. Table 1 is a discussion of certain hydrogels, phages, and the bacteria that they target.

Table 1. Different types of hydrogels and the phages used with their antimicrobial properties

Hydrogel	Bacteria	Strain	Phage	Antimicrobial activity	Reference
Alginate	*Escherichia coli*	K12 strain of *Escherichia coli* (A324)	Λ vir	Coating a bone ceramic material with 1% alginate–CaCl2 hydrogel enhances the lytic activity and permits the phages to release for longer periods of time.	88,95-97
	Enterococcus faecalis	*E. faecalis* 201 - strain	vB_EfaS_LM99 (LM99)	Planktonic bacteria were strongly suppressed (approximately 99 percent) in the presence of phage-loaded hydrogels after 24 hours of incubation.	88,95-97
Poly (ethylene glycol)-4-maleimide (PEG-4-MAL)	*Pseudomonas aeruginosa*	Collection of *Pseudomonas aeruginosa* strains	Φ Paer4, Φ paer14, Φ paer22, and Φ W2005A	*Pseudomonas aeruginosa* bacteriophage-treated cultures did not significantly vary in optical density over the course of the 6-hour treatment period and had a significantly decreased optical density at the halfway point.	35,89,95
PVA-SA (Polyvinyl Alcohol-Sodium Alginate)	*Klebsiella pneumoniae* *Staphylococcus aureus* (Resistant to Methicillin) *Pseudomonas aeruginosa*	B5055 43300 PAO1	Kpn5 MR10 PA5	When the phages were enclosed in a "PVA-SA crosslinked membrane" and cultured with the bacterial host, the activity of the phages on their surface was maintained. As a result, the host was lysed, and an obvious zone of inhibition formed around the membrane.	36, 90,95
HPMC (Hydroxypropyl Methylcellulose) Hydrogel	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa* dog-ear strain PAV237	PEV1 PEV31	"PEV31" was comparatively stable in "HPMC hydrogels" (0.4 log). "PEV1" remained stable in PEO as well as PVA hydrogels with no titer loss, although a small titer drop (0.4–0.8 log) was seen in other compositions.	37, 91,95

7.6 *Synergistic Effect of Nanoparticles and Phage*

Bacterial resistance to phages may also emerge throughout the phage treatment process[98-101]. In order to increase phage effectiveness, tolerance, and overall delivery, recent methods suggest combining phages with other bio-control agents including antibiotics[102], natural products (such as venom and propolis), as well as syntactic compounds and nanoparticles[24,103,104]. Phage and nanoparticles (NP) like AgNPs together have demonstrated very significant impacts on various multidrug-resistant bacteria. Salmonella[105,106] and other multidrug-resistant bacteria are those whose development is restricted by AgNPs. The ability of green AuNPs and phage combinations to destroy multidrug-resistant Staphylococcus aureus biofilms has also been demonstrated[107]. It was investigated whether the Salmonella "phage ZCSE2 (MK673511)" could be utilized to control Salmonella growth. In various conditions, including pH and temperature, the phage was remarkably stable[108]. It can be characterized by colour change, Zeta potential "UV-Vis spectrum", "Fourier transform infrared spectroscopy (FTIR)", and "transmission electron microscope (TEM)". In order to evaluate the antibacterial activity of AgNPs alone and in combination with "phage ZCSE2" against Salmonella, time killing curve, measurements of MIC, minimum bacterial concentration (MBC), bacterial survival, and reduction were made. The data demonstrated that the combination of AgNPs and "phage ZCSE2" significantly suppressed bacterial growth[109] in comparison to other treatments. This reflects the potential for phage applications that combine phage and nanoparticles to manage bacterial diseases. Bacteriophages, combined with diverse formulations, have demonstrated promising outcomes. These therapies show that phage therapy is an innovative and successful treatment that will soon provide an excellent replacement for antibiotics in the fight against multidrug resistance.

7.7 *Commercialized Phages*

Various phage products (as listed in Table 2) have been commercialized for food safety. In addition, phages and their derivatives are becoming more widely recognized as viable complementary approaches for use in food safety at various stages of the manufacturing process[110].

Table 2. Commercialized phage products and their effectivity

Phage product name	Microorganism	Phage	Effect	Reference
SalmoFresh™	*Salmonella enterica*	"SKML-39" "SBA-1781" "SSE-121" "STML-13-1" "SPT-1" "STML-198"	It eradicated 780 (85%) of the 916 *Salmonella* isolates.	111,112
Phageguard Listex™ P100	*Listeria monocytogenes*	"P100"	P100 insensitive Listeria monocytogenes were looked for in 486 isolates of Listeria monocytogenes from 59 diaries during a 15-year period. Immunities were noted in 5 diaries. The non-susceptible isolates found weren't just discovered at random; rather, they were connected to phage treatments.	113,114
ListShield™		"LIST-36" "LMSP-25" "LMTA-34" "LMTA-57" "LMTA-94" "LMTA-148"	Ensures a wider host range by lysing all tested strains *Listeria monocytogenes* strains	112,115-118
EcoShield PX™	*Escherichia coli* O157:H7	3 to 8 lytic phages	The use of 5×10^6 and 1×10^7 PFU/g bacteriophage in 8 different food products infected with *Escherichia coli* O157:H7 resulted in significant reductions of up to 97% in all foods.	112,119
ShigaShield™	*Shigella sonnei*	"SHSML45" "SHFML-26" "SHSML-52-1" "SHFML-11" "SHBML-50-1"	With the exception of melon, where the decrease was only around 45% at the lowest phage dosage (9×10^5 PFU/g), all phage-treated food items had significantly lower shigella levels when compared to controls.	112,120

In addition to focusing on the numerous phage formulations available to combat drug resistance among harmful bacteria, the study will now examine pertinent topics such as "Phage-based assay" and "Phage-based biosensor" to contribute to society in its battle against drug-resistant pathogens.

8. PHAGE-BASED ASSAY AND PHAGE-BASED BIOSENSOR

The utilization of phage-based assays and biosensors has expanded dramatically in recent years, greatly aiding the fight against multidrug resistance. Several types of phage-based treatments and phage-based biosensors are reviewed below.

8.1 Phage Amplification Assay

Phage amplification methods have recently advanced, removing the requirement for complex apparatus and requiring just a small number of the original target pathogens [31]. This method's primary benefit is that it enables the detection of target bacteria in a mixed bacterial sample[31], even when such bacteria are present at initially low quantities. Mycobacteriophages, or viruses that invade mycobacterial hosts, have been studied extensively as a vital tool in the diagnostic and drug susceptibility evaluation of Mycobacterium species since their discovery more than 70 years ago. Understanding their structure and function has been greatly aided by recent developments in genetic engineering [121,122]. Because bacteriophages can only reproduce within living cells, the phage amplification method allows the detection of viable mycobacterial cells between 24 to 48 hours, making it significantly more sensitive than standard culture methods and requiring no sophisticated equipment[123]. Adsorption capacity, a single-step growth curve, and lytic capability were used to characterize "Salmonella phage PBST32" in research comparing antibiotic-sensitive and -resistant *Salmonella Typhimurium* by the phage amplification test[31,124]. The phage amplification experiment was performed at 50% of the drug's minimal inhibitory concentration (MIC) [125] to detect "ST^{CIP}", which includes phage infection, phagecidal treatment, neutralization, and amplification in the presence of ciprofloxacin. Using the "PBST32" based technique in combination with antibiotic treatment, it was detected that "ST^{CIP}" could be selectively identified in combination cultures of *S. aureus,* "ST^{WT}" and *K. pneumoniae*, and also identified that the "PBST32" amplification experiment is a simple and effective tool for the quantitative and targeted recognition of antibiotic-resistant Salmonella[124].

8.2 Phage Lytic Assay

The lysis of the host bacterium and the subsequent release of intracellular components and progeny viruses into the extracellular environment are the results of lytic dispersion by bacteriophages[31,124]. Therefore, two "Yersinia pestis lytic phages (A1122 and PST)" were tested to see if they could infect and kill off a fluorescent "*Y. pestis* EV76" strain. At the same time, it was suspended in "Brain Heart Infusion (BHI)"-a rich medium or whole human blood, both miming the host environment[126]. Prior research indicated that "phage A1122" is

a universal phage that can lyse all studied strains of *Y. pestis*[127,128]. Hence it has been used by the CDC for *Y. pestis* diagnostics. Further, it has already been found to have the quickest lysing ability in investigating bacteria cultured in broth[127,128].

8.3 Phage Based Electrochemical Biosensor

A phage-based electrochemical biosensor for the quick and accurate detection of *Yersinia pseudotuberculosis* is described in a study where the electrode's surface is modified using the conductive poly ("indole-5-carboxylic acid"), reduced graphene oxide, as well as using gold nanoparticles. On modified electrodes, the particular "bacteriophages vB YepM ZN18" were immobilized by an Au-NH2 link between gold nanoparticles and the phages[129]. The phage incubation duration and the reaction time for detection are crucial elements that must be tuned to maximize the efficacy of the phage-based biosensor[129]. In order for sensors to be successfully commercialized, qualities like reproducibility and storage stability are of the utmost importance[130]. Furthermore, the phage-based biosensor is more effective due to its low detection limit, rapid testing, and ability to distinguish between living and dead *Y. pseudotuberculosis* cells[129]. Furthermore, optimizing the density of phage immobilization can help the phage-based biosensor operate even better[129]. However, it is not possible to reuse the created "PI-5-CA/rGO/AuNPs/phage electrochemical biosensor", nonetheless it is quick, precise, sensitive, and reasonably priced, making it a potential tool for clinical applications of *Y. pseudotuberculosis* detection[129].

8.4 Surface Plasmon Resonance Biosensors

It has been demonstrated that methods based on "phase imaging" or methods based on "surface plasmon resonance imaging (SPRi)" are possibilities for rapid (less than two hours) phage susceptibility testing in the broth phase. Covalently immobilized arrays of the "phage 44AHJD, "phage P68", and "phage gh-1" were used to create biosensing layers, which were then subjected to liquid cultures of either *Pseudomonas putida* or methicillin-resistant *Staphylococcus aureus* (MRSA)[131]. The targeted, addressable immobilization of phages on the sensor surface is required to apply surface plasmon resonance for phage susceptibility testing, and this task is far from simple[132,133]. Under prior findings of earlier studies, the purification and immobilization process utilized in this work consistently produces homogenous, high-purity and high-density phage monolayers from suspensions of the "phage gh-1" and "phage44AHJD"[131]. In another study, using the full-length Det7 phage tail protein (Det7T), a surface plasmon resonance biosensor can quickly and accurately identify "*Salmonella enterica serovar Typhimurium*" (*S. Typhimurium*)[134]. Surface plasmon resonance-based biosensors have long been acknowledged as the most effective method for evaluating how a solution species interacts with a surface-immobilized species[135]. In addition to concentrating on the many formulations of phages that are now accessible, the study will directly address inhaled phages as the most recent type of treatment.

9. INHALED PHAGE THERAPY USHERS IN A NEW ERA OF THERAPEUTICS

Studies involving the use of bacteriophages to treat a variety of pulmonary pathogens, including *E. coli*, *Klebsiella* sp., *Streptococci* sp., *Staphylococci* sp., and *Pseudomonas* sp., have been the subject of various research conducted since 1936. Many of these investigations have demonstrated an effectiveness of 80 to 100 per cent. However, some have failed due to a lack of understanding regarding the selectivity, quality management, and longevity of phages[136-138]. Although numerous incidences of respiratory infections have been reported in recent years, the first two-arm, open-label trial conducted involves four critically ill coronavirus 2019 (COVID-19) patients, assesses the efficacy of an inhaled phage-based therapeutic intervention to stop the pathogenesis of a secondary infection caused due to *Acinetobacter baumanii*[139]. In addition, the safety of a phage cocktail involving three phages (AB-SA01) as adjunctive therapy for the management of severe *Staphylococcus aureus* infection is being evaluated in another study[140]. Moreover, the relationship between phages and the immune system can aid in the prevention of infections[141]. Majorly, phages have been utilized in place of antibiotics. The benefits of direct pulmonary administration include increased lung phage density and quick contact with the target pathogen[142,143]. The same group used the "Mycobacteriophage D29" to examine the titer reduction and phage delivery rates of three inhalation devices (Vibrating Mesh Nebulizer, Soft Mist Inhaler, and Jet Nebulizer), which demonstrated this method appropriate for administration is suitable for delivering phage to lung tissue[144].

9.1 Nebulization

Nebulization moves a particular nozzle into a liquid to create a thin mist of active component solution[145]. A substance to be administered by nebulization must first be dissolved in a medium that is usually water-based. Then, following the application of ultrasonic waves or a gas jet for dispersion, the drug particles are encapsulated in the aerosol droplets, which are subsequently inhaled[146]. There are several varieties of nebulizers, such as Vibrating mesh nebulizers, Jet nebulizers, etc. A significant advantage of Nebulizers is their ability to deliver filtered phage lysate without further processing, in contrast to metered-dose inhalers or dry powder inhalers, or nebulizers can more easily be connected to animal exposure devices and can continuously deliver enormous amounts of aerosol, even to people who are unable to coordinate the breathing manoeuvre required for inhalers[147]. Furthermore, according to animal studies, the vibrating mesh nebulizer is the best option for delivering anti-TB "phage D29" because of its high active phage delivery rate and barely any titer reduction of aerosolization[144]. Subsequently, in another study, two phage-ciprofloxacin combinations were aerosolized using air-jet and vibrating mesh nebulizers. The synergistic antibacterial activity was maintained after nebulization, and the two combinations contained 1/4 and 1/2 of the minimum inhibitory concentration (MIC) of ciprofloxacin against "*P. aeruginosa* 2 FADD1-PA001 (A)" and "*P. aeruginosa* JIP865", respectively. The study observed that the combination of the drug ciprofloxacin and the nebulized "phage PEV20" exhibits promising antimicrobial and aerosol characteristics for potentially treating infections associated with the respiratory tract caused by the pathogenesis of drug-resistant *P. aeruginosa*[79].

9.2 Soft Mist Inhalation

Soft Mist Inhalers, also known as Respimat inhalers, have been developed in recent years; when inhaled, they softly spray a little mist of medicine[145]. Researchers compared the titer

reduction and delivery rate of anti-tuberculosis "phage D29" while using a soft mist nebulizer versus those using a jet nebulizer or vibrating mesh nebulizer. The findings demonstrated that the vibrating mesh nebulizer performed better than the jet nebulizer and that the soft mist inhaler might be beneficial for self-administered phage therapy because of its mobility and ability to deliver "phage D29" at high titers rapidly and conveniently[144]. Since the various methods of using inhaled phages have already been discussed, it is of the utmost importance to comprehend the multiple innovations available for the inhaled use of diverse bacteriophages to minimize the decrease in phage titer and maximize the delivery of stable phages while minimizing the inevitable titer drop that inevitably occurs. The following section describes the many novelties.

10. INNOVATIONS IN INHALED PHAGE THERAPY FORMULATION AND DELIVERY

Different improvements have been made in the administration and formulation of inhaled phage treatment. The following are examples of recent developments:

10.1 High-Frequency and Surface Acoustic Wave Nebulization Improves Pulmonary Delivery

A nebulizer can effectively treat bacterial lung infections, but the aerosolization process can be very taxing on proteins and bacteriophages, leading to severe structural and functional degradation[147]. Myoviridae bacteriophage "phage K" and "lysostaphin", a lytic enzyme that targets Staphylococcus aureus, were used in an experiment to demonstrate that they may be nebulized utilizing a revolutionary low-cost and portable hybrid surface and bulk acoustic wave platform (HYDRA)[147]. Since nebulizers don't require specialized patient coordination training, they may be utilized with many patients. The experiment shows that the HYDRA device, which uses relatively lower powers and higher frequencies than its bulk ultrasonic counterparts[147], successfully nebulizes a phage (a mycovirus "phage K") and lytic enzyme (a "lysostaphin") specific to *S. aureus* within a specific aerosol size range between 1 and 5m for optimal deep lung delivery, with little loss in structural and functional viability. Surface acoustic waves (SAW) are another method, and they work at much higher frequencies (>10 MHz) than ultrasonic nebulizers (usually kHz to 1 MHz)[148-151]. Additionally, SAWs are more efficient than their bulk analogues, needing just one to two magnitudes less power for nebulization[152]. However, the most significant limitation of nebulizers, particularly SAW nebulizers, is that it takes a long time to provide an effective dosage to more distant parts of the body[145].

10.2 Electrospray: Inhalation of Controlled, Targeted Doses of Drugs

The most common way of administering medications by inhalation is nebulization, although this technique is not without its downsides. Nebulization creates ultrafine pollutant particles from dry solutes plus biological fragments inside the nebulizer fluid. These contaminants reduce process efficiency by masking the size distribution of virus particles roughly the same size[153,154]. In addition, it is not desirable for the nebulized solution to become increasingly concentrated as a result of some of it flowing back to the nebulizer reservoir and a portion evaporating over time[155]. Low spray-to-target ratios (>20%) are familiar not only with nebulizers but also with inhalation of dry powdered formulations, or inhaling pressurized metered doses of phages, due to the fact that most of the drug particles become deposited in the upper airway. Particles of varying sizes are also generated. Less of the provided dose (or phage titers) reaches the desired location for action due to the bigger particle's tendency to lodge in upper airways rather than lungs[156,157]. "Electrospray (ES)" or "Electrohydrodynamic atomization (EHDA)" produces a spray uniform in particle size. It's a form of atomization in which electrohydrodynamic forces break liquid into very fine droplets[158,159]. Unfortunately, the aerosol size distribution produced by electrospray is somewhat narrow. The resultant aerosolized product is free of aggregates and specific other contaminants resulting from the drying process of the solute molecules[160]. For instance, in a study, airborne "MS2 bacteriophage" particles 30 nm in size were characterized using charge-reduced electrospray. Using a "cone-jet electrospray" apparatus, phage suspension was sprayed. In contrast to nebulized particulates, electro-sprayed MS2 particles showed consistent size distribution, excellent stability, as well as homogeneity. The researchers discovered that electrospray might produce uniform-sized, non-agglomerated particulates[161].

10.3 Encapsulating Phages in Liposomes Facilitating Enhanced Pulmonary Delivery

Liposomes are nano-vesicles made self-assemble into lipid microspheres. They are ideally suited for administering phages because of their compatibility with a wide range of phage compositions. Therefore, the enclosed phages remain protected from the action of enzymes, bodily fluids, or neutralizing antibodies[162]. Since Liposomes exhibit the appearance of biomembranes, they can undergo structural changes that allow them to get through the defences of the living tissues and access deeper places. As free phages cannot infiltrate eukaryotic cells, liposome encapsulation may allow them entry into the cell to tackle intracellular infections[163]. Recent research has documented the efficacy of using liposome encapsulation technologies to deliver phages and different medicines against a wide variety of lung infections. For instance, in a study effective encapsulation of the *Klebsiella pneumonia*-specific "phage KPO1K2" in cationic liposomes was reported, with the encapsulation yielding 92 percentage of efficacy and substantial structural as well as functional stability for a period of nine weeks at temperatures ranging from 4°C to room temperature. Absolute eradication of specific pathogens from the lungs occurred within 72 hours following treatment with the liposomal preparations, protecting all assessed mice against pneumonia-induced mortality even when treatments have been deferred by a period of 3 days after initiation of infections by *K. pneumoniae*[164]. However, aerosolized phage administration via liposomes may provide its own unique set of difficulties. To begin, considerable losses and limited encapsulation effectiveness may result from exposing phages to heat while hydration and solid mechanical stress while ejection[96,165]. Secondly, maintaining the integrity of liposomal vesicles throughout nebulization is extremely difficult. It is possible that the encapsulated bacteriophage will be lost if the liposome vesicles are nebulized into tiny aerosol particles due to the shearing stress involved[159].

10.4 Advanced Inhalation Control with a Centralized Processing Unit and User-Friendly Software

"Individualized Controlled Inhalation Technology (ICIT)" can be defined as a novel approach that promotes increased drug targeting, decreased lung dosage variability, and being one-of-a-kind integrated software-based regulation, end up making it one of the most enticing innovative techniques for enhancing respiratory aerosol accumulation[166]. "AKITA®" is the most cutting-edge aerosol delivery technique based on ICIT since it regulates the patient's whole breathing motion, allowing for more targeted medication administration. It works well with ultrasonic mesh nebulizers. At the same time, the updated version exhibits complete compatibility with the "vibrating mesh nebulizers", delivering up to 98% of the loaded dosage as aerosol particulates with a "Median Mass aerodynamic diameter" of less than 4m[167,168]. With the ability to constantly monitor parameters and notice undesirable effects, these cutting-edge devices also give clinicians a more significant say over the therapy and when to make adjustments[162,169]. In patients with diseases such as "chronic obstructive pulmonary disease"[170] or "cystic fibrosis", for instance, the presence of the endotoxin lipopolysaccharide in medication formulations has been linked to the initiation of inflammation of airways and deleterious consequences[171,172]. However, this may be effectively handled by employing ICIT systems equipped with cutting-edge features, including continuous scanning of data concerning nebulized medicine dose, therapy time, and any unwanted effects. This is especially helpful for the preparation of phage-based formulations because endotoxin might potentially contaminate the formulation due to flaws in high titer phage manufacturing and filtration if present at all[172]. Phage treatment and other ICIT-based nebulizer research are still in the early stages of development. Therefore, additional targeted studies are needed to improve phage administration, particularly regarding the volume of dosages, the time between dosages, and the rate of aerosolization. This will help in achieving elevated success levels concerning the outcomes of phage therapy[145].

11. REGULATIVE MEASURES APPLICABLE FOR PHAGE-BASED THERAPEUTIC INTERVENTIONS

With renewed interest in phage treatment, it's more crucial than ever to have strict laws governing phage-based goods. Because of its peculiar pharmacokinetics and evolutionary concerns, phage treatment is sometimes classified as a kind of evolutionary or customized medicine, making it difficult for regulators to apply existing pharmaceutical law to the field[153]. Nevertheless, countries such as Georgia[173] or Russia[174] have supported this alternative intervention. This has led countries from the west to form new regulations for properly implementing bacteriophages as a potential therapeutic intervention[175]. For instance, the initial steps toward regulating phage treatment are being conducted in Belgium, with the establishment of two pillars: the availability of recognized laboratory facilities with phage stock and the compilation of complete information on the bacteriophage to be used in the phage-based product to assure its quality[176]. Contrarily, in France, a specialist committee offered recommendations for using phage-based products in an order with the "Temporary Authorization for Use". Due to the lack of suitable alternatives, this rare method permits the unapproved use of a pharmaceutical medication[176]. The "Food and Drug Administration (FDA)" regulated by the United States, authorized the emergency use of phage therapy for patients afflicted by the global pandemic of COVID-19 due to the absence of adequate clinical studies[177]. This is because of the increased attention paid to bacteriophages to treat the problem of drug resistance among several bacterial pathogens, which emerged in hospital wards during the pandemic[178]. A significant barrier exists for using bacteriophage in animals since they do not readily comply with current European Union rules concerning food additives and food manufacturing aids[178]. Therefore, phage-based treatment is hindered by a lack of clear restrictions, which does nothing to pique the attention of the powers in drafting such rules—because of this, doing more studies and clinical testing is the need of the hour[175].

12. LIMITATIONS AND FUTURE ASPECTS

Bacteriophages have been used therapeutically to treat various infections since the early 1920s. However, varied results from phage studies reported during the 1930s raised significant concerns regarding the safety and effectiveness of this medical procedure[176,177]. The lack of regulation and the inappropriate synthesis, characterization, and refinement of phage preparations prompted these inquiries. Despite its inherent limitations, phage treatment has proven a successful alternative to antibiotics in the fight against multidrug resistance. In 85% of the instances, a single bacteriophage or phage cocktail was given as part of the medical treatment. One of the critical factors in this achievement is the employment of specific bacteriophages for each species of bacteria. Even though all studies reported performing in vitro assays of bacteriophage activity before the therapy, the infection persisted in 15% of cases after the therapy ended[178]. Phages have a variety of traits that are distinct from those of antibiotics and obstruct the development of phages as pharmaceuticals and therapeutic uses. They are typically picky about the bacteria they infect, to start with. At most, they will only infect a few strains of that species[179], but at worst, they will concentrate on a substantial section of that species. One of the main issues with phages is their slow availability, which is understandable given how difficult it is to develop therapeutic phages. The encapsulation method is rarely a solution because few phages are unstable. However, phages can be incorporated inside a material to maintain its integrity for extended periods, which also holds the potential for targeted delivery[179]. Lyophilization is a practical method for long-term phage preservation even if not all phages can be done so. Each phage requires extensive research and empirical testing, and over the past 70 years, several cutting-edge strategies have been produced. Phage resistance is both undesired and inevitable, given the wide range of mechanisms discovered in bacteria. Researchers may follow the development of bacterial resistance to phage[180] in a way comparable to that of antibiotic resistance using standard laboratory culture techniques[181]. Resistance development has been shown in animal models with phage treatment and infections[51]. Fortunately, the majority of these research findings show that phage resistance decreases the pathogenicity of bacteria[99,182]. Through a specific health approach, phage treatment may be used to combat multidrug resistance. Phage use as a natural tool can save lives, enhance health, and lessen the number of drugs in the environment over the long term and for future generations[183]. Phages should be used instead of or in addition to antimicrobial medications whenever possible and when necessary. The sustainable One-Health strategy will begin with animal husbandry since this is where the environment and the human microbiome converge, even though it may be studied piecemeal. Antibiotics used to treat phages may work well together in a clinical context. Antibiotics used to treat phages may work well together in a

clinical context[184]. However, the underlying mechanical principles of these synergies could be more straightforward and speculative. Few inferences have been made thus far, partly because some phages may have unique effects when taken with medications with similar mechanisms of action, such as inhibiting cell wall synthesis. Further study is required to examine these divergent findings. Future studies should concentrate on how the immune system interacts with phage treatment and if this has a positive or negative impact[184,185]. Despite being in its infancy, research on the connections between phage treatment and innate and adaptive immunity is essential. Phage treatment is becoming increasingly viable for use as an antibacterial agent to combat illness as more thorough studies are released by leading Western scientists and corporations[186,187]. The public and the medical community may become more interested in phage treatment, particularly in countries like the US, where the regulatory framework is less conducive to its implementation in the near future[188,189]. Shortly, a wide range of illnesses will be treated with the help of phage treatment, a well-researched and well-established therapy that has effectively addressed multidrug resistance[190-193].

13. CONCLUSION

This review demonstrates the success of phage therapy against multidrug-resistant bacteria and the numerous formulations used in conjunction with phages to treat it. The multiple phage combinations show that phage therapy could be used successfully in conjunction with nanoparticles, phage cocktails, and antibiotics against various pathogens. In phage therapy, diverse phages are typical about infecting specific bacteria, and a majority of the phages can decrease the pathogenicity of the bacteria and have shown remarkable outcomes. Commercial phage products have proved useful in the fight against antibiotic resistance in livestock and in improving the health of everyone in the world. Bacteriophages are naturally occurring antibacterial substances with antimicrobial properties that help fight pathogens and are a good substitute for antibiotics. In the current years, phage therapy has had a lot of advantages, and it is seen that phage cocktails and antibiotic-phage formulations have been a part of medical treatment, along with hydrogels and phage together showing their antimicrobial properties and phages in combination with nanoparticles reducing bacterial diseases. Through recent research, the successful and emerging results of phage therapy have been observed. Shortly, phage therapy with various combinations and more emerging effects will be established to fight multidrug resistance.

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15. AUTHORS CONTRIBUTION STATEMENT

Ms Punam Kumari and Mr Sutripto Ghosh collaborated on the data collection and writing of the manuscript. Ms Tamalika Chakraborty proposed the idea of writing this particular manuscript on this topic and guided me during the preparation of this manuscript. All authors have contributed equally to the final manuscript.

16. CONFLICT OF INTEREST

Conflict of interest declared none.

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