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Review article

Bacteriophage therapy in diabetic foot ulcer caused by *Pseudomonas aeruginosa*

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ABSTRACT

Background: Bacteriophages are viruses that are used to destroy bacteria without harming host cells. As a result, it is thought that they can be used to treat bacterial infections alone or in conjunction with antibiotics. *Pseudomonas aeruginosa*, a multidrug-resistant bacterial pathogen, is one of the most prevalent infections in diabetic foot ulcers and has a high fatality rate. This bacterium produces a biofilm that causes recurring infections that are antibiotic-resistant and causes severe tissue damage with varying levels of severity. In this review, the role of bacteriophage therapy in diabetic foot ulcers, their advantages, limitation, and future perspectives will be discussed. The bacteriophage therapy to combat antibiotic-resistant bacteria is quite appealing, and some study data suggests that it could be a reasonable solution to overcome severe bacterial infections which cannot be treated by antibiotics. Some studies revealed that bacteriophage can be combined with antibiotics and used for effective treatment. However, phage therapy application in human treatment is scanty because the concurrent and underlying mechanism of this strategy is ambiguous among clinicians. Additionally, there are still problems with how to create formulas for standardized and therapeutic use in bacterial control. More study that is specifically devoted to resolving these problems is necessary before bacteriophages can be used in humans. Bacteriophage therapy will be a better choice for treating diabetic foot ulcers once everything has been cleaned up, and the incidence of amputation can be decreased by bacteriophage therapy.

Introduction

Diabetic foot ulcers are one of the most common consequences for those with poorly managed diabetes mellitus. The most frequent reasons include poor foot care, inadequate diabetic control, underlying neuropathy, and peripheral vascular disease[1]. Worldwide diabetes has reached epidemic proportions. According to the International Diabetes Federation (IDF), 425 million people globally had diabetes in 2017, there would be 536.6 million adults with diabetes in 215 nations and territories in 2021 with that number expected to climb to 783.2 million by 2045[2,3]. According to a comprehensive report that was released in 2017 found that 3% to 13% of people with diabetes globally had foot ulcers[1,4]. Compared to non-diabetic individuals, patients with diabetic foot ulcers are more likely to lose their legs, and also people with diabetes are more prone than non-diabetic patients to sustain foot injuries [5].

Several factors, including poor glycemic control, foot deformities, improper foot care, poorly fitting footwear, underlying peripheral neuropathy, poor circulation, dry skin, etc., are common underlying causes of diabetic foot ulcers. An ulcerated foot eventually develops in about 60% of diabetics who have neuropathy. Individuals with a flat foot are more likely to develop a foot ulcer because they experience excessive stress across the foot, which causes tissue inflammation in high-risk areas [1].

A diabetic ulcer normally progresses in three phases. The formation of a callus is the first step. Neuropathy is the cause of the callus. Sensory neuropathy produces sensory loss, which leads to continuing trauma, whereas motor neuropathy causes physical deformities of the foot. Another significant reason is skin drying caused by autonomic neuropathy. Finally, repeated stress to the callus causes subcutaneous bleeding, which leads to the callus eroding and becoming an ulcer [6].

There have been 12 indications of diabetic foot ulcers clinically reported. Inflammation causes heat, erythema, edema, and pain. Inflammation and purulent discharge are recognized as classic indicators of infection, which have historically been linked to wound infection. Seriously delayed healing, exudate, discolored granulation tissue, friable granulation tissue, bad odor, pocketing of the

wound base, and disintegration are all considered signs of diabetic foot ulcer [7].

The following are some of the diabetic foot ulcer risk factors: Peripheral vascular disease, diabetic nephropathy in patients receiving dialysis, history of a foot ulcer, anatomic foot deformity, poor glycemic control, smoking, and previous lower extremity amputation [8].

Those with foot ulcers had a 2.5-fold increased chance of passing away within five years than those without foot ulcers [9].

Diabetes patients who develop foot ulcers will have a severe problems because there aren't any new medicines being created due to the rise in antimicrobial resistance. This could result in mortality or catastrophic morbidity like an amputation. To overcome this issue, we need a new antibiotic or any other therapy [10]. Due to antimicrobial resistance, some researchers suggest bacteriophage therapy to treat diabetic foot ulcers [11]. Phage therapy can be utilized to prevent the biofilm formation of *Pseudomonas aeruginosa* (*P. aeruginosa*) in diabetic foot ulcers [12]. The prevalence of *P. aeruginosa* in diabetic foot ulcers according to two studies is 18.79% [4,13].

A virus known as a bacteriophage or phage infects and reproduces within bacteria and archaea. Bacteriophages are among the biosphere's most abundant and varied organisms [14,15].

In former Central Europe, the Soviet Union, and France, phages have been employed as an alternative to antibiotics since the late twentieth century [16].

Phage therapy is used first for the treatment of bacterial dysentery by Felix d herelle, but he did not publish his results. For this achievement, he is known as the father of bacteriophage [17]. The first published application of phage therapy by Richard Bruynoghe and Joseph Maison for treating *Staphylococcal* skin disease [18]. With the success of bacteriophage, preclinical studies in animals [19,21] researchers are prompted to investigate bacteriophage therapy on antimicrobial-resistant bacteria [22].

In addition, various new non-antibiotic treatment techniques for killing antibiotic-resistant *P. aeruginosa* strains have been identified in recent investigations. Some of the methods include quorum sensing inhibition, bacterial lectin treatment, phage therapy, nanoparticles, immunization approach, iron chelation, antimicrobial peptides, and

electrochemical scaffolds. These therapeutic techniques can be employed as a stand-alone treatment or in conjunction with traditional antibiotics [23].

The resistance of bacteria in diabetic foot ulcers increasing day by day due to genetic changes and improper treatment. Many people with diabetes develop diabetic foot ulcers with MDR or PDR infections and are unable to be treated with antibiotics, these sores can become serious and necessitate amputation. I am writing this review in the sense aware patients and clinicians improperly treating DFU (Diabetic foot ulcer) and the bacteriophage therapy importance in diabetic foot ulcers. There are lots of microorganisms that play a major role in diabetic foot ulcers. *Pseudomonas aeruginosa* is one of the pathogens included in the ESKAPE group, so I have chosen it for this review. The bacteriophage treatment for *P. aeruginosa* in diabetic foot ulcers is the main theme of this review.

Role of *Pseudomonas aeruginosa* in diabetic foot ulcer

Over the last 25 years, several research has been published on the bacteriology of diabetic foot infection, but the results have been mixed and often contradictory [24]. In the last few years, the prevalence of ESBL (Extended-spectrum beta-lactamase) and carbapenems-producing bacteria are increased in diabetic foot ulcers [25].

Pseudomonas aeruginosa causes frequent tissue damage in diabetic foot ulcers and it may lead to severe infections because of its highly resistant to broad-spectrum antibiotics and its resistance leads to severe infections. The pathogenicity of *Pseudomonas aeruginosa* is identified by toxins that produce an ability to resist phagocytosis [26].

In one of the studies, researchers said that the gram-negative organism *P. aeruginosa* (22%) is highly prevalent in diabetic foot ulcers compared to other organisms [9].

Another study revealed that the common isolate was *P. aeruginosa* (16%) [27].

In 2017 study revealed that 19% of *P. aeruginosa* prevalent in diabetic foot ulcer patients [28]. There is also another study conducted in 2017, in this study the prevalence of *Pseudomonas* they reported as 11.3% [29].

Antibiotic resistance of *Pseudomonas aeruginosa*

Many antibiotics are ineffective against *P. aeruginosa*, although meropenem, ceftazidime, imipenem, amikacin, gentamicin, ciprofloxacin,

piperacillin coupled with tazobactam and tobramycin are effective. Resistance patterns for important antimicrobial drugs remained largely unchanged between 2015 and 2016, with slight declines in resistance to gentamycin (4 percent to 3 percent) and tobramycin (4 percent to 3 percent). Over the same period, resistance to imipenem (9 percent to 11 percent), amikacin (1 percent to 2 percent), and piperacillin-tazobactam (6 percent to 7 percent) increased [5,13,30].

The three main ways that *P. aeruginosa* evades antibiotic attack are acquired, intrinsic, and adaptive resistance. *Pseudomonas aeruginosa*'s intrinsic resistance is characterized by low outer membrane permeability, the evolution of efflux pumps that expel drugs from the cell, and the creation of enzymes that inhibit antibiotic action. Resistance genes can be transferred horizontally or mutationally to create *P. aeruginosa*-acquired resistance [31]. *Pseudomonas aeruginosa* adaptive resistance includes the production of biofilm in the lungs of infected people, which acts as a diffusion barrier, limiting antibiotic access to the bacterial cells [32]. As a result, biofilms play an important role in antibiotic resistance of *P. aeruginosa*. Intrinsic and acquired antibiotic resistance mechanisms have elevated the frequency of MDR (Multi-drug resistant) strains in recent years, although there are no completely effective medicines available to combat this bacterium. The researchers are seeking innovative strategies to stop *P. aeruginosa* biofilms from growing on diabetic feet. One of the most important approaches for inhibiting *P. aeruginosa* biofilm formation is phage therapy [33].

Biofilm formation by *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is one of the crucial pathogens which can produce biofilm. Biofilm formation aids microorganisms in evading the immune system of the patient and increases drug resistance. A biofilm is a group of microorganisms established on a biotic or abiotic surface that consists of bacterial cells in an extracellular polymeric substance (EPS) comprised of teichoic acids, polysaccharides, extracellular DNA, and proteins [20]. Extracellular polymeric substance creates a thick, tissue-like substance that is challenging to remove from wounds within a biofilm. Biofilm-producing bacteria are considerably more resistant to antibiotics than planktonic bacteria complicating the treatment of biofilm-associated illnesses [34,35].

The bacterial biofilm produces resistance to antibiotic therapy and is a significant barrier to treating biofilm-associated infections, which can hamper wound healing and result in chronic wounds that can lead to amputation or death. Because biofilm formation makes treating wound infections more difficult, numerous methods for controlling biofilm formation have been developed. These techniques render the biofilm-bound planktonic bacteria more susceptible to antibiotic treatment rather than killing the bacteria within the biofilm. One strategy for combating antibiotic-resistant biofilm-associated infections like those brought on by *P. aeruginosa* is the use of lytic phages, which have been demonstrated to efficiently infect and lyse both planktonic and biofilm forms of host bacteria [36].

Structure of phage

Phages differ in size, intricacy, genetic material, and shape [37]. DNA that is either single or double-stranded makes up the genome. RNA organized in a circular or linear pattern and of extremely varying lengths. In G phage, the biggest phage sequenced to date, its size ranges from a few thousand base pairs to 498 kilobases (Kb) pairs [38,39].

The majority of bacteriophages consist of a protein tail with helical symmetry and an icosahedral head [39]. The capsid, which is made up of repetitive structural protein components and encloses the nucleic acid, forms the head. The nucleic acid is shielded by the capsid, which also has proteins that give identity to particular bacterial organisms [40]. The heterooligomer that links the head and tail and ensures genome release when the virion is attached to the recipient cell is called the neck.

There is a basal plate at the distal end of the tail, to which tail fibers and spicules with proteins that can adhere to the membrane receptors of specific bacteria are connected [39]. The tail may display accessory structures like spicules, collars, lipid envelopes, or even a lack of an envelope, depending on its contractility and proportions [39].

Interactions between the host and the phage

Through the process of adhesion, phages cling to the bacterium's surface. Bacteria have defense systems that prevent the phage from adhering to the surface; however, phages can modify their binding proteins to conform to the receptor [41].

Due to a variety of polysaccharides, extracellular components, and other biofilm-forming elements, *Pseudomonas* species have a high potential for biofilm development. Phages can control the expression of cell receptors to avoid superinfection with other phages after an infection. Phages adhere to their target and then transfer their DNA into the recipient cell. Many bacteria do, however, produce restriction endonucleases to sever foreign DNA and stop phage invasion. To protect their DNA from breakage by restriction endonucleases, the phages in turn evolved anti-restriction methods [42].

The CRISPR-Cas System, which functions as an adaptive bacterial defense system, is an alternative antiviral mechanism that defends the host by degrading alien DNA.

When a virus infects a bacteria, the CRISPR-Cas system is triggered. Enzymes (Cas) recognise the exogenous DNA and break up parts of it to introduce into a particular genomic area of the organism known as the CRISPR locus. The bacteria that have these fragments of virus DNA introduced into the CRISPR locus produce an RNA from this sequence during subsequent viral infections. This RNA will join the Cas enzyme and travel to the viral DNA, where it will break and inactivate the virus [43].

Isolation of bacteriophage

The bacteriophage preparation was done by taking three sewer samples from waste water and examined for phage prevalence. After the samples were centrifuged and cleaned, they were put into a conical flask filled with nutrient broth and contaminated with the test bacteria, which included *Staphylococcus aureus*, *P.aeruginosa*, *Klebsiella variicola*, *Escherichia coli*, and *Proteus mirabilis* (OD₆₀₀=0.4-0.6). Flasks were kept for 24 hours at 37 °C with 100 rpm stirring before being centrifuged at 6000 g for 10 minutes. To eliminate any potential bacterial organisms, the supernatant of each sample was filtered through a 0.45-µm cellulose acetate syringe filter and kept at 4 °C. The phages' obtained crude lysate was assessed subjectively by spot test and numerically by plaque assay and represented as plaque-forming units (PFU/ml). Transmission microscopy will be done to test the morphological properties of the phage at 80KV [44].

The therapeutic ability of phage towards MDR pathogens was done by using treat the infected foot of mice with the prepared phage [44].

Bacteriophage therapy on diabetic foot ulcer

Bacteriophage (phage) treatment has recently been proposed as a possible alternative to antibiotics against MDR and has fewer adverse effects [45]. Phages will act to prevent the production of biofilms in addition to lysing bacterial pathogens. Furthermore, phages can infiltrate the deeper levels of the biofilm and disintegrate it by inducing or generating polysaccharide depolymerase [46]. Because phage has a preventive effect against biofilm, we can use antibiotics in combination with phage treatment when biofilm development prevents phage, an antibiotic can enter the infected location readily.

Bacteriophages are under two categories temperate and lytic [47]. The phages attached to the targeted host in the case of lytic phages release their genetic material into the host and it along replicates with the host DNA and subsequently disseminates through host lysis to infect more hosts, repeating the infection cycle. Mostly lytic bacteriophages are used for therapeutical purpose because it leads to killing more rapidly than temperate phage[48].

A combination of two or more bacteriophages can successfully treat the MDR *Pseudomonas spp* and these types of combinations of bacteriophages are termed bacteriophage cocktails. In one research, they used various mixtures of phages either concurrently or consecutively to test single- and multiphage therapy against *P. aeruginosa* PAO1 in vitro. In terms of lowering bacterial population density, simultaneous application reliably outperformed sequential application across a range of phage combinations, and there was no significant difference (on average) in terms of decreasing resistance. In all experimental conditions, phage-resistant bacteria developed and suffered substantial fitness costs, as shown by their slower growth rate in the lack of phages. Finally, phage therapy prolonged the lives of *P. aeruginosa*-infected wax moth caterpillars, with a phage cocktail being the most successful short-term remedy. Throughout the study, 96-well microplates containing *P. aeruginosa* PAO1 were maintained at 37°C in 200 l of liquid King's medium B (KB). Using a set amount of phages each time, they added 14/1, KZ, PNM, and PT7 phages to bacterial cultures. By growing wild-type PAO1 from frozen stock for an entire night in liquid KB medium, phage stock solutions were created. Phages were then chloroformed, centrifuged, and stored at 4°C after being purified from bacteria. Because of this, phage

stock solutions were roughly at the same abundance as they would be in a stationary-phase culture of ancestor bacteria. By dilution and plating three separate stock solutions of each phage onto lawns of PAO1, the following PFU per ml amounts for each phage were calculated (means \pm standard deviations [SE]): 14/1, $5.9 \times 10^8 \pm 1.8 \times 10^7$; Φ KZ, $3.1 \times 10^8 \pm 9 \times 10^6$; PNM, $2.1 \times 10^8 \pm 7.1 \times 10^7$; and PT7, $1.1 \times 10^9 \pm 1.5 \times 10^8$ [49].

By promoting the creation of enzymes such as polysaccharide depolymerase, bacteriophage cocktails may readily infiltrate the *P. aeruginosa* biofilm and disrupt its structure[50,51] Polysaccharide depolymerase, a bacteriophage-encoded polysaccharide hydrolase, may specifically destroy the host bacterial envelopes of macromolecule carbohydrates. This enzyme aids bacteriophage adsorption, invasion, and disintegration of the host bacterium [52]. At the end of the lytic cycle, bacteriophages produce endolysins a peptidoglycan hydrolases enzyme. They help to create new offspring phages to release from the cell by decomposing peptidoglycan from inside [53]. Because of their significant antibacterial activity, endolysins are frequently recommended as antibacterial agents. Specific antibacterial activity and a one-of-a-kind method of action of endolysin activity are unaffected by antibiotic susceptibility patterns [54,55].

According to one study, bacteriophages can genetically modify cells to produce quorum quenching (QQ) lactonase, which prevents *P. aeruginosa* from forming biofilms by hydrolyzing acyl-homoserine lactones (AHL) and suppressing quorum sensing (QS) activity [56]. This will aid in the avoidance of antimicrobial resistance and appropriate treatment.

Bacteriophage therapy on medical importance

Several papers on phage treatment in humans have been published in the worldwide literature, with the bulk of recent publications coming from researchers in the former Soviet Union and eastern Europe, and only a few reports [57,58] coming from other nations.

Researchers looked at a group of virulent *Staphylococcal* phages that attacked practically all strains of *Staphylococcus aureus*, including the majority of Methicillin-resistant *Staphylococcus aureus* (MRSA) strains. They provide a case series evaluating the efficacy of utilizing topically applied *Staphylococcus aureus*- specific phage to treat

infected and poorly vascularized toe ulcers with exposed bone following the failure of conventional antibiotic treatment. As a result of this study the infected bone debridement, all infections responded to the phage treatments, and the ulcers healed in an average of seven weeks. Finally, they concluded their study as poor vascularity and antibiotic failure, topical administration of a staph mono-phage preparation can be used effectively to treat infected toe ulcers with bone involvement [59].

One of the studies showed how well phages (anti-k1) could treat mice with *E. coli* infections in an experimental setting. In vivo and in vitro experiments with *E. coli*, anti-K1 phages are more active. A single anti-k 1 phage dosage has been demonstrated to be more effective than many intramuscular doses of tetracycline, ampicillin, chloramphenicol, or trimethoprim with sulphafurazole. All animals are given phage therapy to recover from the bacterial infection, and the treatment also stopped the associated fluid loss [60].

Bacteriophage therapy against *P.aeruginosa*

Recent in vitro research has evaluated the effectiveness of phages against *P. aeruginosa*, including multiresistant isolates [61].

Because of the microorganism's significant role in employing bacteriophages to suppress *Pseudomonas aeruginosa* in nosocomial infections with great antibiotic resistance, bacteriophages that target the *Pseudomonas* species were first discovered in the middle of the 20th century [62,63].

The majority of the 137 sequenced phages that have been found to target *P. aeruginosa* are members of the class Caudovirales [64].

The outcome of the first clinical trial of *P. aeruginosa*-specific phage treatment was announced at the start of 2019. In this research, burn victims were treated with a cocktail of anti-*P. aeruginosa* phages. The results of the test suggested that using pre-selected stutterers might not be the best option. Some *P. aeruginosa* strains won't be vulnerable to the phages used or will develop resistance to them very rapidly. Phage cocktail formulation must be personalized in order for phage treatment to be successful [65].

In one trial, nine patients with severe burns colonised by multidrug-resistant *P.aeruginosa* and *Staphylococcus aureus* were treated with a BFC-1 phage cocktail and get cured [66] .

In mice and Guinea pigs infected with *P. aeruginosa* and *Acinetobacter*, several investigations revealed that phages were successful in preventing and treating experimental disease. They also suggested that phages may help to prevent infections of skin grafts used to heal burn patients. A scaled-up version of Adam's enrichment approach was used to extract phages in -vitro activity and was measured in shaken broth cultures using a technique developed by smiths [67,69].

Staphylococcal lung infections [70] *P. aeruginosa* infections in cystic fibrosis patients [71] eye infections [72] newborn sepsis [73] urinary tract infections [74] and surgical site infections [75] have all been reported to be treated successfully using phages.

Engineered bacteriophages are useful tools for diagnosing, controlling, and treating pathogens. Synthetic genome rebooting enables quick, non-selection-based phage engineering and manufacture. This method allows for comprehensive and unlimited genome creation and editing, including large rearrangements, hybrid phages, and even completely customized genomes. Rev2L L-forms are used to reactivate viruses infecting *Bacillus*, *Staphylococcus*, and presumably other Gram-positive hosts in addition to rebooting *Listeria* phage genomes. As a result, the synthetic platform is universally applicable and does not necessitate the use of any extra specialist strains for the creation of recombinant phages[76,77].

This study showed that the combination of bacteriophages and antibiotics helped to limit MDR *P. aeruginosa* biofilm in individuals with type 2 diabetes mellitus who were also diagnosed with recurrent right knee periprosthetic joint infection and chronic osteomyelitis. In this patient, bacteriophage was given locally after surgery and then applied once for eight hours for five days. According to findings from isothermal microcalorimetry, bacteriophages can help destroy biofilm. Pre-treating *P. aeruginosa* with phages eight hours before being exposed to colistin showed the most notable reduction of biofilm biomass. The sickness in question was effectively treated in this case by combining the use of phage, surgery, and conventional antibiotics[78].

Studies have demonstrated that the use of bacteriophages like M-1, which were isolated from wastewater to remove biofilms formed by MDR isolates of *P. aeruginosa*, is a potential method for

removing biofilms. After 6 hours of treatment, the findings demonstrated that the bacteriophage MA-1 lowered biofilms and slowed *P. aeruginosa* development. The fact that bacteriophage may break down alginate polymers through an enzymatic process and even remove the 20-day biofilm created by *P.aeruginosa* [79].

Another investigation examined how the phages PB1, PhiKZ and LUZ24 impacted MDR *P. aeruginosa* under various growth conditions. The findings showed that each phage could suppress both the planktonic and biofilm forms of MDR isolates. The most effective phages at suppressing planktonic form were those that resembled phiKZ viruses. Additionally, the LUZ24-like phage was the most successful phage in destroying the biofilm of isolates that were resistant to antibiotics. Additionally, the combined effect of the three phages or cocktails was stronger than the effects of the individual phages. However, a high-volume phiKZ-like phage had the least detrimental effects on the biofilm. The LUZ24-like phage, on the other hand, had a considerable impact on biofilm disintegration. Additionally, it has been suggested that phages may not have a significant effect on high-density biofilms. However, they can prevent the accumulation and subsequent spread of biofilms by inhibiting migratory microorganisms [80].

In one study, *P. aeruginosa* biofilms from patients with chronic rhinosinusitis were removed using the bacteriophage Pa193, Pa204, Pa222, and Pa223. After 24 and 48 hours of treatment, they found that a single dosage of these phages, both by themselves and in cocktail form, greatly decreased the rate of biofilm development. The biofilms of the isolates were decreased by individual phages by 53%-73%, however the phage cocktail boosted its effectiveness on the biofilms by 89%. They also suggested that the use of cocktail phages increased activity by broadening the host range and prevented the emergence of mutant bacteria that were resistant to bacteriophages [81].

In a 2017 study, they saw the isolation of bacteriophage AZ1 and testing of its anti-biofilm functionality against MDR *P. aeruginosa*. The outcomes supported the suppressive and damaging activity of phage AZ1 against planktonic and biofilm cells of *P. aeruginosa*. Researchers hypothesized that natural phages' mode of action was to pierce the biofilm; however, full elimination might call for a mix of phages [82].

Another research looked into how bacteriophages vB-Pa4 and vB-Pa5 affected MDR *P. aeruginosa* biofilm development. The findings indicated that bacteriophages almost completely stopped biofilm formation and also partly destroyed pre-formed biofilms. In an in vitro research, Ahiwale et al. looked into the use of native BVPaP-3 phage to manage biofilm generated by antibiotic-resistant *P. aeruginosa*. It was discovered that the T7-like lytic phage (BVPaP-3) could prevent *P. aeruginosa* hospital strains from forming biofilms. Additionally, it was successful in dispersing all isolates' pre-made biofilms after 24 hours [83].

Acinetobacter baumannii, *Klebsiella pneumoniae*, *P. aeruginosa*, and *E.coli* were among the MDR gram-negative bacteria that were significantly affected by the phage endolysin, LysPA26 in another study that tested it against planktonic form and *P. aeruginosa* biofilm. The phages also successfully eliminated *P.aeruginosa* biofilm. Interestingly, the results demonstrated that LysPA26, by altering the outer membrane under 100°C heat treatment, has strong antibacterial activity against isolates of *P. aeruginosa*. LysPA26 degrades biofilms in a concentration-dependent way, although the mechanism by which it does so is yet unknown [84].

Based on the aforementioned findings, it can be said that discovering novel phages can be a great antimicrobial agent substitute for treating MDR *P.aeruginosa* biofilm and may even completely remove infections brought on by *P.aeruginosa*. The results of invitro studies can help phages be used more frequently to treat nosocomial MDR *P. aeruginosa* infections. The use of bacteriophage mixtures or cocktails can improve antibiofilm effectiveness and reduce bacteriophage resistance [85].

Bacteriophage and antibiotics combination therapy

There has been a lot of interest in combining other compounds with antibiotics to improve their efficiency against MDR bacteria [85]. In order to increase the permeability of the biofilm and eliminate the EPS, several chemicals can be used. Additionally, bacteriophages have been shown in earlier research to improve antibiotic performance in biofilms [78].

In a study, *P. aeruginosa* biofilms isolated from wounds and cystic fibrosis patients were inhibited using phage PEV20 and ciprofloxacin. In

comparison to a single dose of ciprofloxacin, the results demonstrated that a combination of antibiotics and phage therapy improved biofilm eradication. Because they have little metabolic activity and little of this antibiotic penetrates the biofilm, the bacteria on the inner layers of the biofilm may be the source of this phenomenon. When bacteriophages break down the extracellular matrix, the bacteria in the inner layers of the biofilm become subjected to nutrients and oxygen and are metabolically active. The antibacterial properties of ciprofloxacin and phage may result from this [86].

In a similar investigation, the biofilm of several isolates of *P. aeruginosa* was eliminated with a combination of ciprofloxacin and the bacteriophages 175-B1 (Pa1), ATCC 14, 203-B1(Pa2), ATCC 12, and 205- B1(Pa11), ATCC 14. The findings demonstrated that bacteriophages functioned better when combined with antibiotics than when used alone because they had a more inhibitory effect on biofilm. Because it appears that phages can proliferate to a higher extent in the vast bacterial population when added before antibiotic therapy than when added after, using phages before antibiotics has the best inhibitory impact on the biofilm [87].

Advantages and limitations of bacteriophage therapy

Antibiotics are substantially less effective than bacteriophages because, while certain antimicrobial medications have a broad spectrum of activity and kill all bacterial species. The most appealing feature of bacteriophages is the specificity of action or their ability to destroy the suspected pathogens that they identify and it will multiply only in the target bacteria and cannot infect mammalian cells; bacteriophages are much safer and better tolerated [88,89].

Furthermore, the administration is simpler since bacteriophages do not require many doses over several days, as is customary with antibiotics, because they may stay in the human body for quite extended periods, up to several times [90].

Limitations of antibiotic treatment can be overcome by engineered bacteriophages. The evidence is that bacteriophages can disperse biofilm, an extracellular polymeric substance that makes infections difficult to remove with normal antibiotic therapy even when bacteria are responsive to the delivered medicine. **Lu and Collins** designed a bacteriophage against an *E. coli*-generating biofilm

to express a biofilm-degrading enzyme in in-vitro research [91].

There are very little data on the use of bacteriophages to treat bacterial illness in humans. It is extremely difficult to find a therapeutic bacteriophage. The first is to isolate bacteriophages, which are usually done from wastewater or sewage. This is a reasonably simple process, but there are some variances between the various bacterial pathogens [92].

Furthermore, the bacteriophage genome must be sequenced and must not contain integrase genes, antibiotic-resistant genes, genes for phage-encoded toxins, or other bacterial virulence factors, as in the lysogenic type. Finally, issues relating to the formulation and stability of pharmacological formulations for clinical use are still a work in progress. In this regard, investigations appear to imply that the stability of therapeutic preparations is strictly bacteriophage dependent, and stabilization procedures should be tailored for each bacteriophage independently. This might result in expensive and time-consuming clinical studies, discouraging the pharmaceutical sector from beginning research and development of human-use medicines [12,93].

Future perspectives

Phages can be used for a variety of purposes including disease diagnosis through phage typing, prevention by phage vaccination, and treatment by phage therapy. Humans also get benefit from phages in a variety of ways. It would be simple to cure a wide range of bacterial diseases that are resistant to all-generation antibiotics by creating a cocktail of phages.

In a time of rising multidrug resistance and a declining supply of new chemical antibiotics, bacteriophage therapy is a rapidly developing field with unproven potential for treating infections caused by resistant bacteria. To properly assess the potential of phage medications, the scientific community's discoveries, as well as long-term experience with phage treatment in some areas of the world, must be used to develop and test novel phage therapies that best answer for the treatment of antibiotic-resistant diabetic foot ulcers. Through this proper and better treatment can give to patients and can avoid disastrous morbidity like amputation.

Conclusion

Biofilm formation in the infected location is the primary source of antimicrobial resistance.

Antibiotics cannot reach the affected region because of the biofilm. Bacteriophages can be utilized to get rid of the biofilm, making therapy more successful. In diabetic foot ulcers, *P. aeruginosa* is an invasive bacterium that commonly causes serious tissue damage and severe infections. Due to the emergence of multi-drug-resistant *P. aeruginosa*, foot ulcer treatment became tough. In recent research, they identified as bacteriophage treatment can destruct *P. aeruginosa*. The use of bacteriophages to combat antibiotic resistance is appealing, and some study data suggests that it could be a reasonable solution. However, current information does not appear to be adequate to permit the use of bacteriophages for the treatment of diabetic foot ulcers in humans. To present, there are just a few well-designed clinical trials particularly designed to test bacteriophages' effectiveness. Furthermore, the issues of how to develop formulations for standardized and clinical usage in bacterial control remain unsolved. Before bacteriophages may be utilized in people, more research especially committed to solving these issues is required.

Once the shortcomings of the therapeutic uses of bacteriophage have been successfully addressed, MDR, XDR, or PDR pathogens can be readily eliminated through bacteriophage therapy in diabetic foot ulcer patients.

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